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Chromosome Analysis Suite (ChAS) v4.6

USER GUIDE

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Revision	Date	Description
W	15 May 2026	Changed user guide version from v4.5 to 4.6. • Cybersecurity updates including updated components, self-signed certificate upon installation, https communication. • ISCN 2024 Nomenclature update • Inclusion of MANE in the annotations • Usability updates Changed address of Manufacturer from “3450 Central Expressway, Santa Clara, CA 95051” to “6055 Sunol Blvd, Pleasanton, CA 94566”.
19	29 Sept 2023	Changed user guide version from v4.4 to v4.5 Documented the following new features in ChAS 4.5: • Automatic upload of CytoScan and OncoScan data files from scanner to ChAS and Franklin™ by Genoox™. • Support Auto CEL Analysis for CytoScan HD Accel array. • Enhanced cybersecurity protection with ChAS DB administrator password change. • Option to display OMIM™ Gene with Phenotype map key of 3 only. • Easier scrolling within the Detail View.
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1 Introduction

The Chromosome Analysis Suite (ChAS) software for cytogenetic analysis enables you to view and summarize chromosomal aberrations across the genome.

Chromosomal aberrations may include copy number gain or loss, mosaicism, or loss of heterozygosity (LOH).

ChAS provides tools to:

- Perform single sample analysis of CEL files from CytoScan™ arrays and OncoScan™ arrays.
- Automatic workflow from scanner to results file with no manual intervention required.
- Seamless integration with Franklin by Genoox™.
- Analyze segment data at different levels of resolution.
- View results data (CYCHP, XNCHP, and OSCHP files) that summarize chromosomal aberrations in table and graphical formats.
- Display CNCHP data from Genome-Wide Human SNP 6.0 Array.
- Customize and load your own annotations and regions for focused analysis.
- Display ReproSeq™ Aneuploidy data from Ion Reporter™ Software.
- Apply separate filters to the entire genome and user-specified regions of interest to remove irrelevant information such as segments in areas that are not of interest.
- Perform detailed comparisons between different samples.
- Directly access external databases such as UCSC Genome Browser, NCBI, DECIPHER, ClinVar, and ClinGen via https.
- Export user-selected data in graphical and tabular formats.
- Store and query segment data for streamlined analysis.
- Check for Mendelian Inheritance errors across related samples.
- Combine gene expression and copy number data in both tabular and graphical formats.
- Export data for viewing in Integrative Genome Viewer (IGV).
- Directly access the TaqMan™ website for follow up analysis.

IMPORTANT! The results from ChAS are for Research Use Only and not for use in diagnostic procedures.

Chromosome Analysis Suite is not a secondary analysis package. However, it does create CYCHP, OSCHP, XNCHP, and tab-delimited text files required for secondary analysis packages.

Features in v4.6

- Mosaic LOH segmentation
- Ability to filter segments based on mosaic percentage
- ISCN 2024 Nomenclature update
- New Estimated Mosiac Percentage Column in the Segment Table
- Annotation addition of the MANE track
- Ability to perform a Mendelian Error Check between a proband and parents run on different CytoScan™ arrays
- Ability to hide Chromosome X in the Karyoview Export
- Ability to select/unselect all chp files in a single action
- Ability to export the Selected Chromosome View as .png
- Inclusion of column headers when using the clipboard function in tables
- Ability to export Microarray Nomenclature for a sample with no segments
- Cybersecurity Updates: ChAS database password storage in Windows Credential Manager, Admin password change after installation, ChAS database tools log out after 30 min of inactivity, self-signed certificate upon installation, https communication with ChAS DB and external DB, updated backend components to recent versions.
- Standardized Log2 Ratio in Chromosome Summary Data

About this user guide

This user guide provides step-by-step instructions for performing the procedures required to use ChAS and can also be accessed from the software by clicking the top menu bar's **Help** dropdown list.

The steps outlining procedures through out this user guide are frequently supplemented with screen captures to further illustrate the instructions.

Note: The screens that were captured for this user guide may not exactly match the windows displayed on your screen.

Customer support

Visit thermofisher.com/support for the latest in service and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols

2 Install ChAS

ChAS is a stand-alone application that supports the analysis and/or visualization of the following results data files:

- CytoScan™ (CYCHP)
- OncoScan™ (OSCHP)
- CytoScan™ XON (XNCHP)
- Genome-Wide Human SNP 6.0 (CNCHP)
- ReproSeq™ Aneuploidy (.zip)
- BED/AED files
- VCF files

IMPORTANT! Due to the amount of memory that ChAS requires to operate, Thermo Fisher Scientific *very strongly* recommends that you *do not* install the ChAS software on instrumentation computers being used for scanning and operating fluidics systems.

Recommended and minimum requirements

Note: The full Database and Browser software must be installed on at least 1 or more 64-bit analysis workstations to create results data files.

Table 1 Software.

System properties	Recommended system requirements	Minimum system requirements
Processor	3 GHz (or greater) Pentium™ Quad Core Processor	3 GHz (or greater) Pentium™ Dual Core Processor
64-bit Windows™ operating system and web browser	Windows™ 11 Windows Edge™ browser	Windows™ 11 Windows Edge™ browser
Available disk space	250 GB HD + data storage	150 GB HD + data storage
Free disk space required at installation	≥5 GB	≥5 GB
RAM	32 GB	16 GB

Table 2 Server.

System properties	Recommended system requirements	Minimum system requirements
Processor	3.1 or 3.3 GHz Quad Core Processor	2.7GHz Quad core processor
Windows™ operating system	Windows™ Server 2025 Standard 64-bit	Windows™ Server 2025 Standard 64-bit
Available disk space	1 TB HD + data storage	512 GB HD + data storage
Free disk space required at installation	≥5 GB	≥5 GB
RAM	≥24 GB	16 GB

Requirements and prerequisites for arrays

IMPORTANT! A Windows™ 64-bit operating system is required for all array types.

IMPORTANT! Chromosome Analysis Suite requires GCDC 1.0/GCC 6.1 or higher to produce CytoScan™ and OncoScan™ CEL files.

IMPORTANT! The larger file sizes associated with the CytoScan™ HD Array should be taken into account when calculating the necessary free space requirement. A CytoScan™ HD Array CYCHP file is ~155 MB. A CytoScan™ XON Array XNCHP files is ~174MB.

IMPORTANT! The ChAS software has been verified for use on a Windows™ 64-bit operating system. ChAS may work on other Windows™ operating systems, but only the 64-bit version has been verified.

ZIP file contents

Go to thermofisher.com ChAS product page to downloaded the ZIP files.

Before performing an analysis, you must download the appropriate ZIP file package(s) listed in the following table.

Table 3 Available library file packages for human genome build hg19.

Array name	hg19
CytoScan™ HD	CytoScanHD_Array_Analysis_Files_hg19_NA33.r11.zip
CytoScan™ 750K	CytoScan750K_Array_Analysis_Files_hg19_NA33.11.zip
CytoScan™ HD Accel	CytoScanHD_Accel_Array_Analysis_Files_hg19_NA33.r2.zip
CytoScan 750K Accel	CytoScan750K_Accel_Array_Analysis_Files_hg19_NA33.r2.zip
CytoScan™ Optima	CytoScanOptima_Array_Analysis_Files_hg_19_NA33.r10.zip
CytoScan™ XON	CytoScan_XON_Array_Analysis_files_hg19_NA33.r8-Part1.zip CytoScan_XON_Array_Analysis_files_hg19_NA33.r8-Part2.zip
OncoScan™ CNV Plus	OncoScan_Array_Analysis_Files_hg_19_NA33.r9.zip

Table 3 Available library file packages for human genome build hg19. (Continued)

Array name	hg19
OncoScan™ CNV	OncoScan_CNV_Analysis_Files_hg_19_NA33.r7.zip
CytoScan™ HTCMA	CytoScan_HTCMA_96_ChAS_Files_hg19.r3.zip

Table 4 Available library file packages for human genome build hg38.

Array name	Library file package
CytoScan™ HD	CytoScanHD_Array_Analysis_Files_hg38_NA36.r8.zip
CytoScan™ 750K	CytoScan750K_Array_Analysis_Files__hg38_NA36.r8.zip
CytoScan™ HD Accel	CytoScanHD_Accel_Array_Analysis_Files_hg38_NA36.r2.zip
CytoScan 750K Accel	CytoScan750K_Accel_Array_Analysis_Files_hg38_NA36.r2.zip
CytoScan™ Optima	CytoScanOptima_Array_Analysis_Files_hg_38_NA36.r7.zip
CytoScan™ XON	CytoScan_XON_Array_Analysis_Files_hg38_NA36.r8-Part1.zip CytoScan_XON_Array_Analysis_Files_hg38_NA36.r8-Part2.zip
OncoScan™ CNV Plus	OncoScan_Array_Analysis_Files_hg38_NA36.r7.zip
OncoScan™ CNV	OncoScan_CNV_Analysis_Files_hg38_NA36.r7.zip
CytoScan™ HTCMA	CytoScan_HTCMA_96_ChAS_Files_hg38.r3.zip

After downloading and extracting the **Chromosome_Analysis_Suite_4.6zip** file, a **Chromosome Analysis Suite 4.6** folder appears containing the following files:

- ChAS4.6_setup.exe
- ChAS_4.6_Manual.pdf
- ChAS_4.6_Release_Notes.pdf
- ChASDB_adgv_hg19_Gold.backup
- ChASDB_adgv_hg38_Gold.backup
- ChASDB_adgv_hg19_Gold_HD.backup
- ChASDB_adgv_hg38_Gold_HD.backup
- ChASDB_adgv_hg19_Gold_XON.backup
- ChASDB_adgv_hg38_Gold_XON.backup

Install ChAS

Note: The installation process also installs additional required components, which includes Java™ components and Visual C++ runtime.

New installation

1. Double-click the **ChAS4.6_setup.exe** file from the "Chromosome Analysis Suite 4.6" folder.

   The Install Shield Wizard for Chromosome Analysis Suite begins.

2. At the Welcome window, click **Next**.

   The License Agreement window appears.

3. Read the license agreement carefully, click **I accept the terms of the license agreement**, then click **Next**.

   The Setup Type window appears.

4. Select the appropriate drive's checkbox, then click **Next**.

   The PostgreSQL Database Server Installation window appears.

   Note: Ensure that you select a drive with the most available space. Consider 1 GB of space is required for every 1,000 samples you add to the database.

   The installer auto-detects and displays a default **Port** number. It is recommended that you do not change this number.

5. Click **Next**.

   The Start Copying Files window appears.

6. Click **Next**, then follow the on-screen instructions to complete the installation.

7. After all software installation is complete, you must download the new analysis files from the NetAffx™ Analysis Center or copy them into your ChAS Library folder. If you are unable to connect to the internet, see "Copy analysis files" on page 23.

IMPORTANT! If your Windows™ Firewall is enabled during the installation of ChAS and you want to Backup the ChAS Database and Restore it to your local ChAS DB (see "Use a shared ChAS Database while off-line" on page 425) a message may appear indicating that you cannot connect to the shared folder.

If this message appears, contact your IT department for help in allowing file sharing through the Windows™ Firewall.

Upgrade installation

IMPORTANT! The ChAS 4.6 Installer does NOT support upgrade from previous versions. Due to an updated version of the ChAS DB, previous versions of ChAS must be uninstalled using add/remove programs prior to running the ChAS 4.6 installer. Be sure to make a backup of your ChAS DB prior to uninstalling your previous version of ChAS.

To keep current preferences, see "Export and import preferences" on page 413.

Copy analysis files

The CytoScan Analysis Library Files zip package download contains the Analysis Files required to process their respective CytoScan Array CEL files into CYCHP files.

The OncoScan Analysis Library Files.zip package download contains the Analysis Files required to process their respective OncoScan Array CEL files into OSCHP files.

The CytoScan XON Analysis Library Files.zip package download contains the Analysis Files required to process their respective CytoScan XON Array CEL files into XNCHP files.

Also included in the CytoScan HD Analysis Library Files.zip are the files for GenomewideSNP_6 files (required in ChAS to view GenomeWideSNP_6 CNCHP files).

If you are unable to download library files through the software, you can download the zipped library files from the ChAS product page at www.thermofisher.com, then extract (unzip) the files into the following location: C:\Affymetrix\ChAS\Library

Analysis file locations in Windows™ 11

- Library: C:\Affymetrix\ChAS\Library
- Preference file: C:\ProgramData\Affymetrix\ChAS\preferences.xml
- All other user profile related preference files and saved settings: C:\ProgramData\Affymetrix\ChAS\users

View hidden files and folders

The ChAS preference files may be placed in folders and files that are normally hidden from the user in Windows.

Display hidden files and folders in Windows™ 11

1. In the taskbar at the bottom of the desktop, type Control Panel in the **Search** box.

   The Control Panel window opens.

2. Click **Appearance and Personalization** in Control Panel. Under Folder Option, click "Show hidden files and folders".

3. In the Folder Options window that appears, click the View tab. Under Hidden files and folders, click **Show hidden files and folders.**

   Hidden files and folders are dimmed to indicate they are not typical items. If you know the name of a hidden file or folder, you can search for it.

4. Click **OK**.

5. Close all open windows.

Analysis file download

When you start ChAS for the first time, you are prompted to create a user profile. (See "Create and use user profiles" on page 410.)

Note: To process the CytoScan™ Arrays in GCDC/GCC, you must install the appropriate library files for the instrument software on the instrument workstation. See the specific array product page at www.thermofisher.com for details.

You can download the ChAS analysis files from NetAffx™ Analysis Center using either the ChAS Browser or the Analysis Workflow. The files are saved into the same folder whether downloading through the ChAS Browser or the Analysis Workflow.

Download ChAS analysis files from NetAffx™ Analysis Center for use with the ChAS Browser

1. Start ChAS.

   If no annotations are installed, a Download Annotations notice appears. (Figure 1)



Figure 1 Download Annotations notice.

2. Click **OK**.

   The Library File Download Service window opens.

   Note: You can also open the Library File Download Service window by selecting **Update Library and Annotation Files** from the Help menu.

3. From the Library File Download Service window, click **OK** to view available Library Files for download.
4. Select the library and annotation files you want to download.
5. Click **Download**.

   The Download Progress window displays the progress of the downloading and unpacking of the files.
6. Click **OK** when the download is complete.
7. The NetAffx™ Authentication window remains open, click **Close** when finished downloading the library files.

Update NetAffx™ Genomic Annotation files

The publicly available annotations in the NetAffx™ Genomic Annotation file are updated quarterly. When launching the ChAS Browser or Analysis Workflow, the software checks for the availability of new NetAffx™ Genomic Annotation files.

If more current files are available, you are prompted to download the files from the NetAffx™ Analysis Center using the instructions from either of the previous 2 sections.

If you are unable to download from the NetAffx™ Analysis Center through the software, contact Technical Support for access to the current NetAffx™ Genomic Annotation file.

Set up proxy server access

Note: If you do not know what the proxy settings are, contact your IT department.

Follow the steps below if your system has to pass through a Proxy Server before it can access the NetAffx™ server.

To change from a Local Disk (C: or D:) Database to a dedicated remote Database Server, see "Access a remote ChAS Database server from the ChAS Browser" on page 26.

Access NetAffx™ Analysis Center from the analysis workflow

1. Launch the Analysis Workflow by selecting **Analysis → Perform Analysis Set Up in the ChAS Browser**.
2. Click **Utility Actions → Download Library Files**.

   The Library File Download Service window appears.
3. Click the **Proxy Settings** tab. (Figure 2)
4. Select the **Enable Custom Proxy Server** checkbox.
5. Enter the Host Address, Port (if not listed), User and Password.

IMPORTANT! This proxy user ID and password is NOT the same ID and password used to connect to the NetAffx™ Analysis Center.

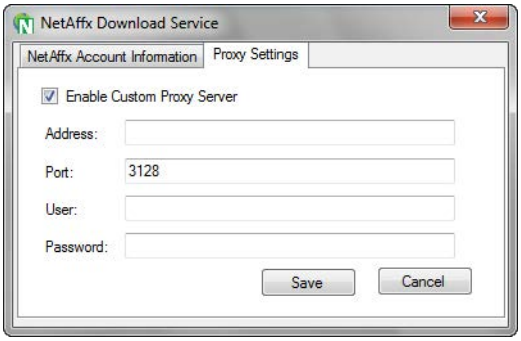


Figure 2 Proxy Settings window.

6. Click **Save**.

Access NetAffx™ Analysis Center from the ChAS Browser

1. From the **Help** dropdown list, click **Update Library and Annotation Files…**

   The Library File Download Service window appears.
2. Click the **Proxy Settings** tab.
3. Select the **Enable Custom Proxy Server** checkbox.
4. Enter the Host Address and Port information, then enter your user name and password.

   IMPORTANT! This proxy user ID and password is NOT the same ID and password used to connect to NetAffx™ Analysis Center.
5. Click **Save**.

Access a remote ChAS Database server from the ChAS Browser

1. From the **Preferences** dropdown list, click **Edit Application Configuration…**

   The Configuration window appears. (Figure 3)

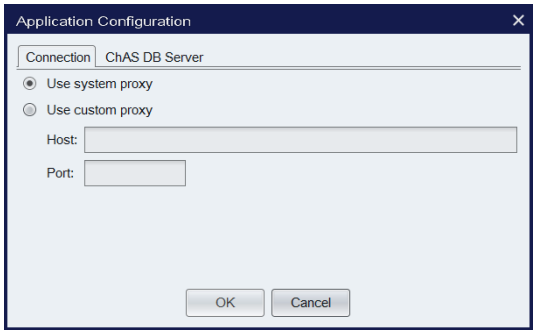


Figure 3 Proxy Settings window.

2. Click the **Use custom proxy** button.
3. Contact your IT department for help with entering the Host and Port information.
4. After you have completed the appropriate fields, click **OK**.

Uninstall ChAS

IMPORTANT! It is strongly recommended you backup your database *before* uninstalling ChAS.

1. Navigate to the Windows™ Control Panel.
2. Select **Programs → Uninstall a program**.
3. Locate the **Chromosome Analysis Suite** application, then perform the uninstall as you normally would.
4. Click **OK** to acknowledge the message box that warns the ChAS application must be closed (before removing it).

3 Getting started

Start ChAS

1. Double-click the desktop ChAS 

icon.

   The **Select User** window appears. (Figure 4)

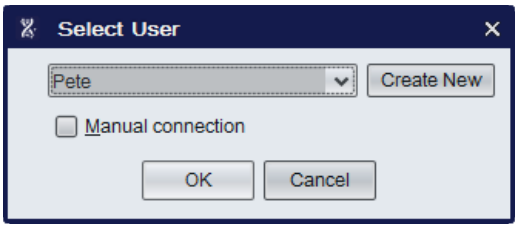


Figure 4 Select User window.

2. Use the dropdown list to select a user or click **Create New** to create a new user profile. For more information, see "Create and use user profiles" on page 410.
3. Optional: Select the **Manual connection** checkbox. For information on manual connections, see "Manual or automatic connection mode" on page 379.
4. Click **OK**.

   The Chromosome Analysis Suite application opens. as shown in Figure 5 on page 28 after logging into the ChAS DB. To login, see "Log into the ChAS Database".

Note: A message may appear indicating a more current version of the NetAffx™ Genomic Annotation file is available for download. To download the newer version of the file, see "Analysis file download" on page 24. If you are unable to download the files via the NetAffx™ dialog, contact Technical Support for alternative downloading options.

Log into the ChAS Database

See Chapter 21, "Database tools" on page 416 for steps on how to log into the ChAS Database.

IMPORTANT! After installation and before you can access the ChAS Database, the default **admin** password must be changed from **admin** to a more secure password. To do this and to add additional user profiles, see "Administration" on page 423.

Note: Your login is retained throughout a working session, however if you close the ChAS Browser window, then re-open it, you must login again.

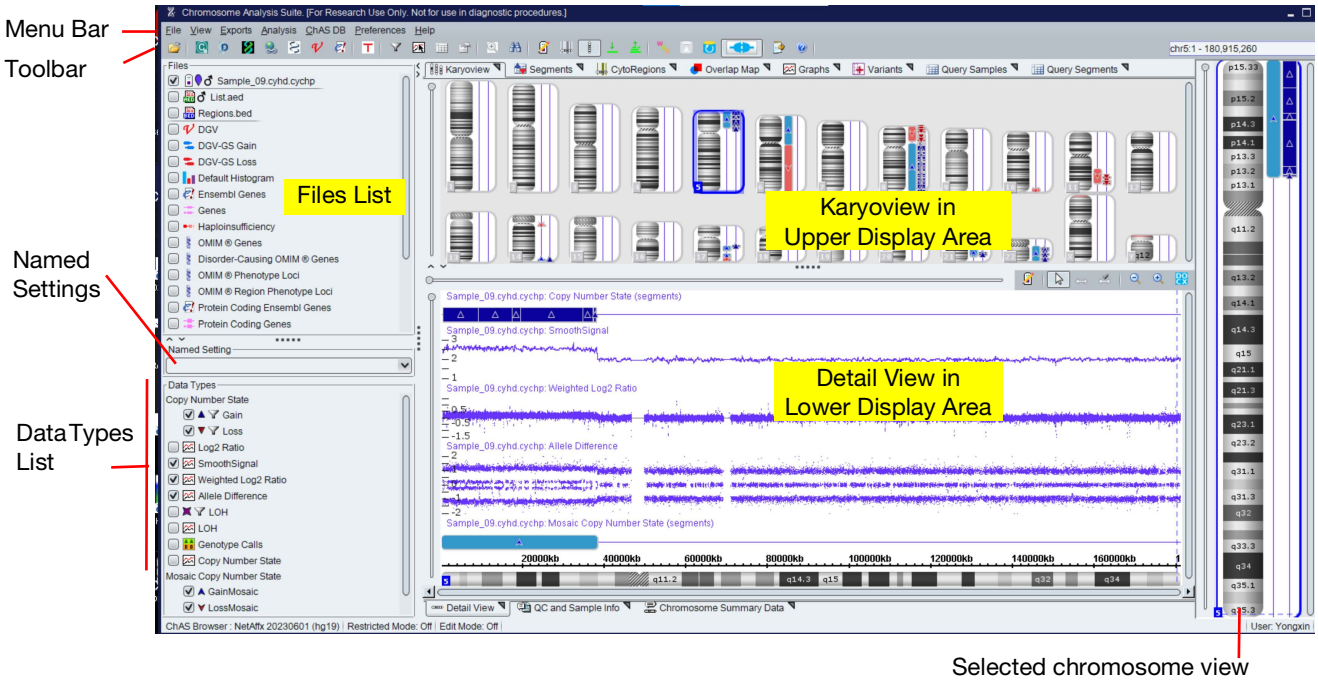


Figure 5 Chromosome Analysis Suite with CYCHP file loaded.

The ChAS Browser window has the following components:

- **Menu Bar** - Provides access to the functions of the software.
- **Toolbar** - Provides quick access to commonly used functions. **Note:** Some features that were previously in the toolbar (such as Dark/Light Schema) have been removed, but continue to be available under the View menu item.
- **Files List** - Shows the data and annotation files that are available for display. See "Files list" on page 136.
- **Data Types List** - Displays the type of data available in the files. See "Data Types list" on page 137.
- **Named Settings** - Displays a list of the previously saved display settings for ChAS. See "Named Settings" on page 138.
- **Status Bar** - Displays information on the status of the software, the ChAS Browser NetAffx™ Genomic Annotation file version, the hg version, information about the annotation or probe that the mouse pointer is nearest to in the Detail View, and the user profile name. See "Status bar" on page 139.

- **Display Area** - Displays the following data in graphical and table formats:
  - CYCHP, CNCHP, XNCHP, OSCHP, RHCHP, and/or ReproSeq™ Aneuploidy data
  - Detected segments
  - Region information file data
  - Histogram data (representing segments uploaded to the database)
  - Reference annotations

For more display area information, see "View data" on page 134.

Analysis workflow module

The Analysis Workflow generates xxCHP files from CEL files and tracks ongoing ChAS analysis tasks.

You can access the Analysis Workflow at any time by clicking its AW icon.

Setup for the first time

After installation, you must configure your data paths.

The Analysis Workflow requires the following steps:

1. From the Analysis menu, select **Perform Analysis Setup.** (Figure 6)

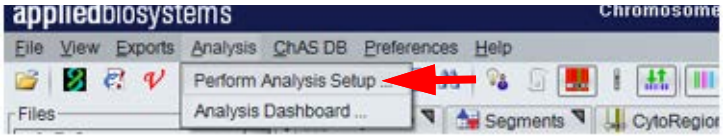


Figure 6 Analysis dropdown list.

The Analysis Workflow Configuration window tab appears. (Figure 7)

If it does not appear, click **Utility Actions → Configuration**.

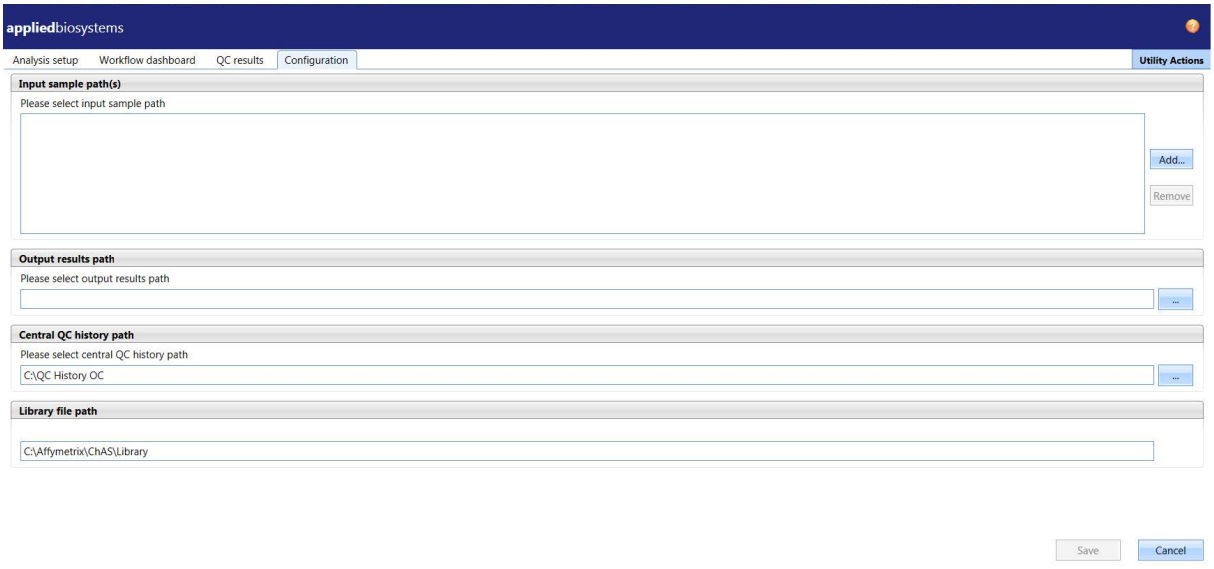


Figure 7 Analysis Workflow Module - Setup example.

Assign an Input sample path(s)

A minimum of 2 sample paths is recommended.

1. Click **Add**.

   The Add Input sample files window appears. (Figure 8)

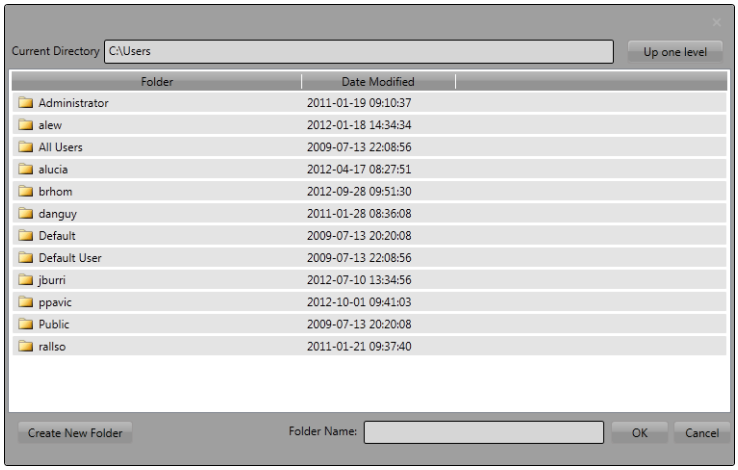


Figure 8 Add Input sample files window.

2. Click the **Up one level** button to navigate to the recommended C:\Users directory, then click the **Create new folder** button to label a new input sample folder.
   (Example: C:\Users\YourUserName\CytoScan Data)
3. Repeat **Step 2** to create a second input folder in an easy to access area.
   (Example: C:\Users\YourUserName\Collaborator_OncoScan Data)

Assign an Output results path

1. Click the **Browse** button.

   A good practice is to navigate to your current ChAS data folder location, then click **Create New Folder** to create an output results path folder inside this folder.
   (Example: C:\Users\YourUserName\CytoScan Data\CytoScan_Results_Files)
2. Click **Save**.

Assign a Central QC history path

1. Click the **Browse** button, then navigate to a folder in which to store the QC history file. (Example: C:\Cytoscan_data\)
2. Click **Create New Folder** to create a central QC history path folder. (Example: QC_History)

File types and data organization in ChAS

To fully use the capabilities of ChAS, you need to understand the ChAS file types and data organization in ChAS.

ChAS file types

ChAS uses the following types of files:

- Data files
- Region Information files
- Support files

File Types Supported in ChAS

Some data files that ChAS uses are generated by other Thermo Fisher Scientific software, as shown in Table 5.

Table 5 Supported file types.

File type	Created in	ChAS...
Sample file (ARR)	GCC	Uses this information to associate sample attribute information with CEL and xxCHP or CNCHP files.
Intensity Data file (CEL)	GCDC/GCC	Analyzes the intensity data in the CEL file, then generates a xxCHP files. **Note:** A 64-bit system is required to analyze intensity data.
Analysis Results (CYCHP) CytoScan™ array: CYCHP contains copy number, LOH, mosaicism, and genotype call information	ChAS	Displays results in graphical and tabular formats.
Analysis Results (RHCHP from HTCMA) contains copy number, LOH, Carrier Variant, and SMN results	RHAS	Displays results in graphical and tabular formats.
Analysis Results (OSCHP) OncoScan™ FFPE and OncoScan™ CNV arrays, OSCHP contains copy number/LOH and somatic mutation information (FFPE Assay only)	ChAS	Displays the probe-level analysis, segment level data, and somatic mutation data.
Analysis Results (XNCHP) CytoScan™ XON array: XNCHP contains LOH, Exon Region, and Genotype information.	ChAS	Displays probe-level analysis, segment level data in graphical and tabular formats.
Analysis Results (CNCHP) GenomeWide SNP 6.0 array contains copy number and LOH segments	GTC	Displays probe-level analysis data and generates segment data on-the-fly.

Table 5 Supported file types. (Continued)

File type	Created in	ChAS…
CHP Change Archive (CHPCAR)	ChAS	Stores user-annotated segment and sample annotations as well as modifications made to the segment data.
Analysis Results (.zip) ReproSeq™ Aneuploidy contains copy number and tiling information	Ion Reporter™ Software	Displays copy number segment data and sequence tile information.
Region Information File (BED or AED)	ChAS or Text Editor	Allows users to display their own custom data and optionally use the information to define CytoRegions or an Overlap Map. ChAS can export data in BED format for use with the UCSC Browser and other programs which understand this format.
Tab-separated values (TSV, TXT, and DOCX)	ChAS	Exports data in this format for use in a spreadsheet program or other user-defined uses. This format is for export only. ChAS does not import TSV or TXT files.
VCF Files	Other software packages	Allows users to view genotype and indel data in the Detail View.

Region information files

The region information files in Browser Extensible Data (BED) and Affymetrix Extensible Data (AED) format provide lists of regions in the genome with position information and other annotations. To open a BED or AED file, click the icon or select **File** → **Open** on the menu bar. All BED or AED files that are opened during a session reload when you start a new session with the same user profile.

Note: You can use the reference annotations to provide region information or use the Export feature to export data in existing BED files to an AED file. See "Export information in AED or BED format" on page 296.

Analysis and visualization library files

IMPORTANT! Every feature in ChAS requires support files.

- Array-type specific Library file sets with files for running Copy Number/LOH/Mosaicism analysis and Reference Creation workflows (Analysis files)
- Files for visualizing and exporting data from xxCHP results data files.
- Reference Annotation files
  - Browser Annotation files are named using the following format: <NetAffxGenomicAnnotations.Homo_sapiens.hgXX.naYYYYMMDD.db>

Data organization in ChAS

ChAS enables you to keep your CEL and Analysis Results files in any folder on your computer. As long as you know where the files are, you can load them from anywhere and move them around at your convenience.

IMPORTANT! It is recommended that you perform analysis operations with all analysis files stored on a local disk drive.

Basic workflow for cytogenetics analysis

Note: xxCHP is used when referring to CYCHP, CNCHP, XNCHP, and OSCHP files.

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

ChAS can be used to:

- Perform probe-level analysis of CEL file data for CytoScan™ and OncoScan™ arrays.
- Display probe-level analysis data (xxCHP) from:
  - CytoScan™ arrays (CYCHP)
  - Genome-Wide Human SNP Array 6.0 (CNCHP)
  - OncoScan™ arrays (OSCHP)
  - CytoScan™ XON Arrays (XNCHP)
  - CytoScan™ HTCMA Arrays (RHCHP)

  Note: There are some differences in the way the ChAS handles these different types of arrays and how it treats the data from these 4 types of files. The basic cytogenetic analysis workflow includes the following steps:
  - "Array processing workflow (using instrument control software)" on page 34.
  - "Probe-level analysis of CEL file data" on page 34.

  - For CytoScan™ arrays, this analysis is performed in CHAS and produces CYCHP or XNCHP files depending on array type. See "CN/LOH/Mosaicism analysis" on page 41.
  - For CytoScan™ HTCMA Arrays, analysis is performed in the RHAS and produces RHCHP files.
  - For the Genome-Wide Human SNP Array 6.0, this analysis is performed in Genotyping Console (GTC) software and produces CNCHP files. For more details, see the **GTC User Guide**.
  - For the OncoScan™ files, this analysis can be performed in ChAS and produces OSCHP files.
- "View data and features of interest using the ChAS display controls" on page 38.

Array processing workflow (using instrument control software)

Array processing is performed in GCDC 1.0/GCC 6.1 or higher for the CytoScan™ arrays, OncoScan™ arrays, and the Genome-Wide Human SNP 6.0 Array.

Note: You need to have the appropriate library files installed on the instrument control workstation to perform these analyses for the different array types.

The array processing includes the following steps:

1. Registering samples and arrays.
2. Washing and staining the arrays.
3. Scanning arrays and generating intensity (CEL) file data.

The following file types are produced:

- Sample (ARR files)
- DAT Files
- CEL Files
- Audit
- JPG

See the instrument control user guide for more information.

Probe-level analysis of CEL file data

Copy number data is handled differently from genome-wide genotyping data in this step.

Note: You need to have the appropriate ChAS library files installed to perform these analyses for different array types. A 64-bit system is required to analyze CytoScan™ CEL files.

- For CytoScan™ arrays, this analysis is performed in ChAS and produces CYCHP or XNCHP files (depending on array type) and contain the data shown in Table 6. See "CN/LOH/Mosaicism analysis" on page 41.
- For CytoScan™ HTCMA Arrays, the analysis is performed in the RHAS which produces a RHCHP file for viewing in ChAS.
- Genome-Wide Human SNP Array 6.0 data: The probe level analysis on CEL file data is performed in GTC and produces the CNCHP file data types shown in Table 6. See the GTC User Guide for more information.
- For OncoScan™ data: The probe level analysis on CEL file data is performed in ChAS and produces the OSCHP file data types show in Table 6.

Table 6 OSCHP file data types.

	Analysis results[1]			
	CytoScan™ array[2]	**Genome-Wide Human SNP Array 6.0[3]**	**OncoScan™ arrays[4]**	**CytoScan™ XON[5] Array**
Graph data for the individual CN and SNP probes				
Copy Number State	Yes	Yes	Yes	No
Log2 Ratio	Yes	Yes	Yes	Yes
Weighted Log2 Ratio	Yes	No	Yes	Yes
LOH	Yes	Yes	Yes	Yes
Allele Difference	Yes	Yes	Yes	Yes
Genotype Calls	Yes	No	No	Yes
Smooth Signal	Yes	Yes	Yes	Yes
Variant Data	No	No	Yes (CNV Plus only)	No
B-allele Frequency	Yes	No	Yes	Yes
Segment data				
Gain and Loss segments based on runs of aberrant Copy Number State data	Yes	Yes	Yes	Yes
Mosaic Gain and Loss segments of non-integer Copy Number States between CN=1 and CN=3	Yes	No	No	No
LOH (Loss of Heterozygosity) based on runs of SNPs where heterozygote calls are absent	Yes	Yes	Yes	Yes
Exon Region Gain and Loss segments	No	No	No	Yes
Mosaic LOH (Loss of Heterozygosity) segments based on runs of SNPs where a percentage of heterozygote calls are absent in the sample.	Yes	No	No	No

[1] CYCHP for CytoScan™, CNCHP for Genome-Wide Human SNP 6.0 Array, XNCHP for CytoScan™ XON Array, and OSCHP for OncoScan™ array.
[2] For more details on CytoScan™ array data, see *Table 21 on page 163*.
[3] For more details on Genome-Wide SNP Array 6.0 data, see *Table 24 on page 166*.
[4] For more details on OncoScan™ FFPE Assay data, see *Table 25 on page 166*.
[5] For more details on CytoScan™ XON Array data, see *Table 23 on page 165*.

Note: Segment types drawn with flat ends (Gain and Loss) are the result of algorithms which can ascertain precise marker-to-marker breakpoints. Segment Types drawn with rounded ends (LOH, GainMosaic, LossMosaic, and MosaicLOH) are the output of algorithms which closely approximate breakpoints based on the data.

Load data into ChAS for display

You perform the same steps for the different types of analysis data (CYCHP, CNCHP, XNCHP, OSCHP and ZIP (ReproSeq™ Aneuploidy), but ChAS handles these types of data differently.

CytoScan™ array (CYCHP files)

When loading CYCHP files into ChAS for viewing, the software:

1. Selects the segments in the CYCHP file to display as segments.
2. Applies any smoothing or joining that would alter the length and other properties of segments.
3. Displays the segments and graph data:
   - Segment Data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity (LOH)
     - Mosaic Gain/Loss
     - Mosaic LOH
   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Weighted Log2 Ratio
     - Smooth Signal
     - Loss of Heterozygosity (LOH)
     - Allele Difference
     - B-allele Frequency
     - Genotype calls

CytoScan™ XON Array (XNCHP files)

When loading XNCHP files into ChAS for viewing the software:

1. Selects the segments in the XNCHP file to display as segments.
2. Displays the segments and graph data:
   - Segment Data
     - Loss of Heterozygosity
     - XON Region Gain/Loss
     - Whole Genome Gain/Loss Segments
   - Graph Data
     - Log2 Ratio
     - Weighted Log 2 Ratio
     - Smooth Signal
     - Loss of Heterozygosity (LOH)
     - Allele Difference
     - B-allele Frequency (BAF)
     - Genotype Calls

CytoScan™ HTCMA Array (RHCHP files)

When loading RHCHP files into ChAS for viewing, the software:

1. Selects the segments in the RHCHP file to display as segments.
2. Displays the segments and graph data:
   - Segment Data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity (LOH)
   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Smooth Signal
     - Loss of Heterozygosity (LOH)
     - Allele Difference
     - B-allele Frequency
     - Variant Data

Genome-wide SNP Array 6.0 (CNCHP files)

When loading CNCHP files into ChAS for viewing, the software:

1. Performs segment generation by analyzing the CN and LOH graph data in the CNCHP file.
2. Applies any smoothing or joining that would alter the length and other properties of Copy Number segments.

   In GTC software, these steps were performed in the Segment Reporting Tool.
3. Displays the segments and graph data:
   - Segment data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity (LOH)
   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Allele Difference
     - SmoothSignal
     - Loss of Heterozygosity (LOH)

OncoScan™ array (OSCHP files)

When loading OSCHP files into ChAS for viewing, the software:

1. Selects the segments in the OSCHP file to display as segments.
2. Displays the segments and graph data:
   - Segment data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity
   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Weighted Log2 Ratio
     - Allele Difference
     - B-allele frequency
     - Smooth Signal
     - Loss of Heterozygosity
     - Variants/Somatic Mutations (OncoScan™ CNV Plus only)

ReproSeq™ Aneuploidy (ZIP files)

When loading ZIP files from Ion Reporter™ Software into ChAS for viewing, the software displays the following segments and graph data:

- Segment data
  - Copy Number Gain/Loss
- Graph Data
  - Copy Number State (sequence tiles)

View data and features of interest using the ChAS display controls

ChAS provides the following options for viewing and studying your loaded analysis results data:

- Graphic Displays

  See “Display data in graphic views” on page 142.
- Tables

  See “Display data in table views” on page 305.

After the data is loaded, you can:

- Filter the segments by Segment Parameters to hide segments that do not meet your requirements for significance. See “Filter segments” on page 205.
- Select a region information file for use as a CytoRegion file and:
- Perform differential filtering for segments in CytoRegions and in the rest of the genome. See “Use CytoRegions” on page 251.
- Display only segments that appear in CytoRegions using Restricted Mode.
- Query segments from a loaded sample against segments previously uploaded to a ChAS Database. See "Query a segment from the segment table" on page 365.
  - See which samples had segments similar to the current sample.
  - View the Calls and Interpretations of previous segments to help in the analysis of the current sample.
- Select a region information file for use as an Overlap Map and use the Overlap filter to identify or conceal segments that appear in the Overlap Map regions. See “Use the overlap map and filter” on page 262.

- Add selected features of the genome to new or existing Region (AED) files, and edit annotation data on existing annotations. (To open a BED or AED file, click the icon or select **File** → **Open** on the menu bar.) See "Create and edit AED files" on page 269.
- View genotype data from Next Generation Sequencing data via VCF files. See "VCF files" on page 300.
- Prepare reports on your findings by exporting graphics and table data in PDF and other formats. See "Export results" on page 386.
- Save setups of ChAS for different tasks in user profiles and named settings. See "User profiles and named settings" on page 408.

Working with ChAS

Access functions in ChAS

Commands in ChAS can be accessed in the following ways:

- Main menus
- Toolbar
- Right-click menu options in:
  - Files List
  - Data Types List
  - Karyoview
  - Selected Chromosome View
  - Detail View
  - Table Headers

Change pane sizes

Do one of the following to change the size of the panes in the ChAS window, as shown in Figure 9 on page 40.

- Click and drag the dividers between panes.
- Click the arrows in the dividers (or) to hide or maximize an entire pane.

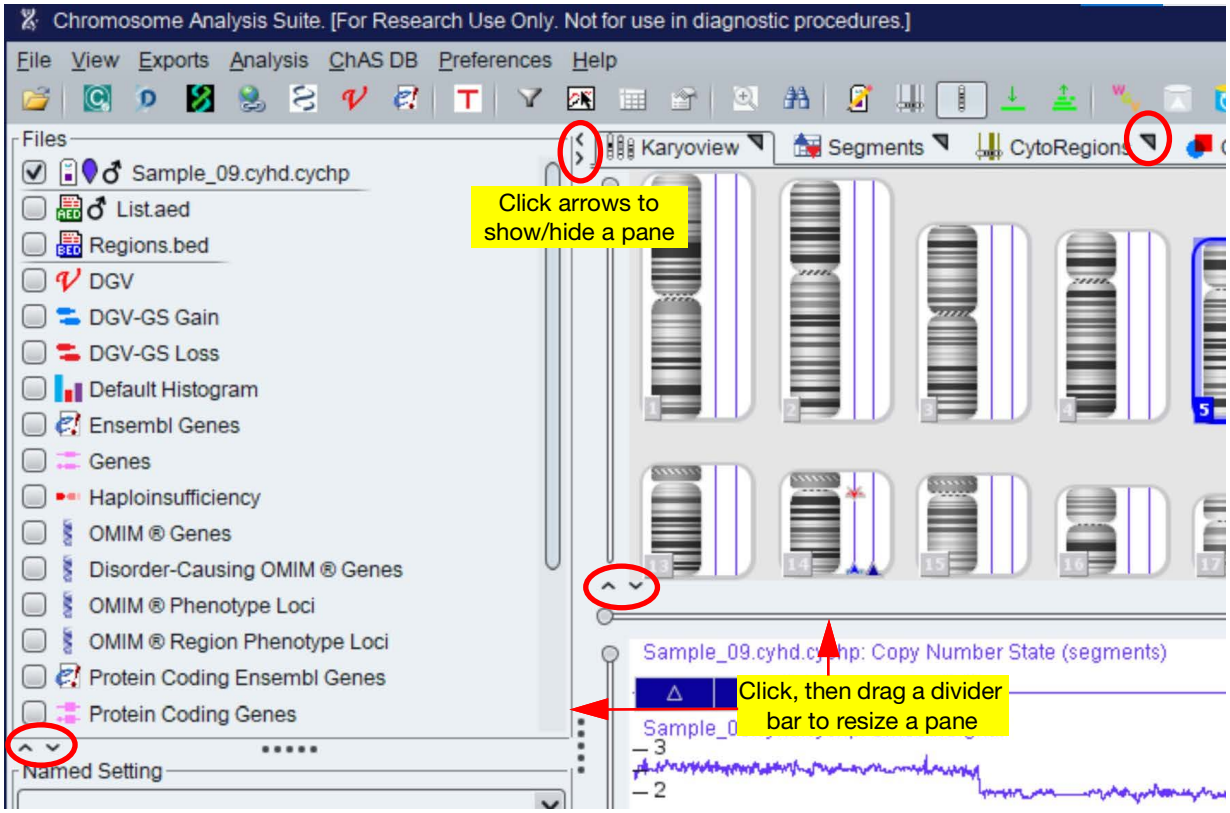


Figure 9 Resize or show/hide panes.

Open panes in separate windows

You can display a pane in a separate window by clicking the icon on the tab. To close the window and return the information to the tab panel, click the icon in the window.

4 CN/LOH/Mosaicism analysis

ChAS analyzes the intensity data (CEL file) from both CytoScan™ and OncoScan™ arrays. The software performs a single sample analysis which compares the data in a CEL file to a previously created reference file, using analysis parameters specified in the **.chasparam** file. The analysis generates a CYCHP/XNCHP/OSCHP data file that you load and view in ChAS. The analysis detects segments that exhibit are as follows:

- **Copy Number State Gain or Loss**: Regions of integer copy number gain or integer copy number loss.
- **Mosaic Gain and Loss (CytoScan™ HD, CytoScan™ 750k, CytoScan™ Optima, and CytoScan™ HD Accel only)**: Regions of non-integer copy number gain or loss (CN states between 1 and 3).
- **XON Region Gain and Loss (CytoScan™ XON only)**: Regions of gain or loss at the exon level.

  Note: The mosaicism segmentation analysis is currently only available for CytoScan™ array CEL files. However, xxCHP files for the other array types contain the SmoothSignal data type which displays non-integer copy number changes.

- **Loss of Heterozygosity (LOH)**: Regions where the preponderance of SNPs do not display heterozygosity.
- **Mosaic Loss of Heterozygosity (LOH)**: Regions in which a percentage of the sample has no heterozygous SNP calls.

For more details on loading and viewing CHP data, see "Load data" on page 110.

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

Note: Reference files are provided as part of the complete Library file packages. You can also create your own reference file using ChAS.

Load Genome-Wide Human SNP Array 6.0 CNCHP into ChAS to display and detect Copy Number and Loss of Heterozygosity segments. See "Load data" on page 110.

Load CytoScan™ HTCMA Array RHCHP into ChAS to display and detect Copy Number segments, Loss of Heterozygosity segments, variant data, and SMN data. See "Load data" on page 110.

IMPORTANT! It is recommended to perform analysis operations with all associated analysis files in a locally stored folder(s).

Single sample analysis

Single Sample Analysis compares the values in 1 or more user-selected CEL files with the values in a reference file that is created from a set of sample files. You can use the included default reference file or create your own (For more details, see "Create a reference file" on page 81)

ChAS analysis file compatibility

Table 7 lists the compatibility between ChAS Analysis file versions for the CytoScan Arrays.

Note: ChAS automatically prevents you from selecting an incompatible analysis file version for analysis or when viewing analysis results.

Table 7 Compatibility table.

CytoScan™ array analysis file set version	ChAS v4.6	ChAS v4.5	ChAS v4.4	ChAS v4.3
NA36 (hg38)	Yes	Yes	Yes	Yes
NA36 (hg19)	Yes	Yes	Yes	Yes
NA33(hg19)	Yes	Yes	Yes	Yes

Note: See the ChAS Release Notes for data equivalency information between the ChAS software and the Library file versions used to create CHP files.

Introduction to single sample analysis

Single sample analysis requires:

- ChAS analysis files for the array. See "Analysis file download" on page 24.
- A previously created reference model file.

  Note: You can use the included reference model file or create one using your own CEL file data and the Reference File creation function. The Reference Model file in the CytoScan™ array set includes 380 microarrays which were run as part of a larger set of microarrays by 9 operators processing ~48 unique samples in 2 rounds each, with randomization of the placement of sample DNAs across the PCR plates and randomization of the reagents and instruments used. The source DNA includes:
  - 284 HapMap samples including at least 1 replicate of each of 270 HapMap samples: 90 from each of the Yoruban, Asian, and Caucasian ethnic groups, from cell-line derived DNAs from the Coriell Institute of Medical Research.
  - 96 DNA samples from blood of phenotypically healthy male and female individuals obtained from BioServe Biotechnologies.

- CEL file data

- During the analysis, ChAS generates CYCHP files with:
- Graph Data
  - Copy Number State
  - Log2 Ratio
  - Weighted Log2 Ratio
  - LOH
  - Allele Difference
  - Smooth Signal
  - Genotype Calls
  - B-allele Frequency
- Segment Data
  - Copy Number Gain/Loss
  - Mosaicism Gain/Loss
  - Loss of Heterozygosity (LOH)
  - Mosaic Loss of Heterozygosity (LOH)

The CYCHP files can be loaded into ChAS for viewing and study.

Figure 10 on page 44 shows an overview of single sample analysis for the CytoScan Array.

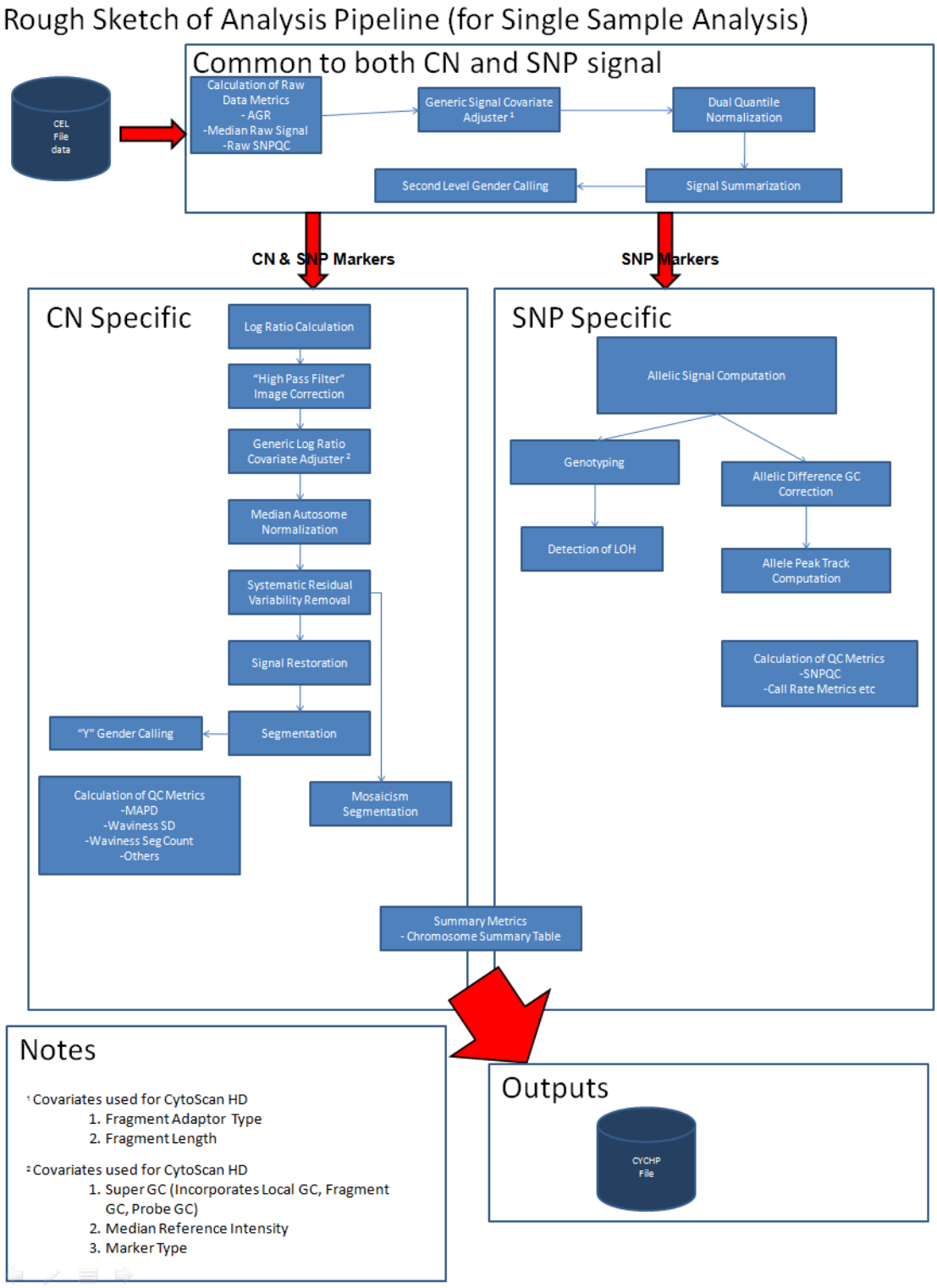


Figure 10 CytoScan™ Single Sample Analysis workflow example.

Copy number segments on the X and Y chromosomes

The expected copy number state on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes, and the fact that they share extensive homology with each other only in the Pseudo Autosomal Regions (PARs) that they each have at either end. PAR1 is at the top of the p-arm and PAR2 at the bottom of the q-arm.

Markers occurring in the PAR regions are mapped exclusively to the X Chromosome. Therefore, in normal males the PAR regions of the X are expected to be CN=2 (probes on the X and Y contribute to the signal), while the rest of the Chr X is expected CN=1 for normal males. As a result, we treat the two X PARs in males as independent units (CN=2 expected) from the rest of the X chromosome (CN=1 in males) when generating Copy Number Segments.

Aberrant segments that cross PAR/non-PAR boundaries may be normalized into 1 segment if they have equivalent type (Gain or Loss) and CN State. During this normalization process, ChAS does not combine an aberrant (Gain or Loss) segment with a normal segment across PAR/non-PAR boundaries, even if they have the same CN State. If smoothing is subsequently applied, aberrant segments with different copy number state may be combined. If joining is subsequently applied, aberrant segments separated by a non-aberrant segment may be combined.

Because only Y-specific probes are mapped to the Y chromosome, the expected state of the entire Y chromosome is 1 for males and is 0 for females.

Mosaic copy number segments on the X chromosome

The expected copy number state on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes and the fact that they share extensive homology with each other only in the Pseudo Autosomal Regions (PARs) that they each have at either end. PAR1 is at the top of the p-arm and PAR2 at the bottom of the q-arm.

Markers occurring in the PAR regions are mapped exclusively to the X Chromosome. Therefore, in normal males the PAR regions of the X are expected to be CN=2 (probes on the X and Y contribute to the signal), while the rest of the Chr X is expected CN=1 for normal males.

Mosaic Segments whose boundaries start and end entirely in one of the PAR regions use CN=2 as normal to determine the type (GainMosaic or LossMosaic) of Mosaic segment to draw.

Because the Mosaicism algorithm can generate segments which cross the PAR boundaries, Mosaic Segments that touch the non-PAR region of the X chromosome use the gender call of the sample to determine the Type of Mosaic segment to draw.

Because only Y-specific probes are mapped to the Y chromosome, the expected state of the entire Y chromosome is 1 for males and is 0 for females.

LOH segments on X and Y chromosomes

CytoScan arrays

For normal XY male samples, the X chromosome has single-copy based LOH calls (CN = 1). Male samples with more than one X chromosome (for example, XXY) may have LOH calls on the X chromosome, depending on the constitution of the X chromosomes' origins.

The following tables briefly describe how the array-specific algorithms call LOH segments for the X or Y chromosome.

Table 8 Expected LOH calls on the X and Y chromosomes for the CytoScan arrays.

LOH segments	X chromosome	Y chromosome
Normal male sample (XY)	LOH calls that are single copy-based LOH call (CN = 1).	No LOH calls are made for the Y chromosome. Genotype calling is not performed on the Y chromosome.
Male sample with multiple X chromosomes (for example, XXY)	LOH calls are possible, depending on the constitution of the X chromosomes' origins.	
Normal female sample (XX)	LOH calls are possible, depending on the constitution of the X chromosomes' origins.	
Female sample with a single X chromosome (X0)	LOH calls on X regions which have only a single copy. Heterozygous SNP genotypes are possible, but are due to the low inherent Heterozygote call error rate noise, not the true presence of 2 alleles.	

Table 9 Expected LOH calls on the X and Y chromosomes for the Genome-Wide Human SNP Array 6.0.

LOH segments	X chromosome	Y chromosome
Normal male sample (XY)	LOH calls on the non-PAR region of the X chromosome resulting from "forced" homozygote-only calls due to the presence of the Y chromosome. Heterozygous calls are ignored on the X chromosome in males.	LOH calls that are due to single copy genotyping calls (CN = 1).
Male sample with multiple X chromosomes (for example, XXY)	LOH calls are possible, depending on the constitution of the X chromosomes' origins. SNP genotypes are not constrained to homozygous calls. Heterozygous calls are ignored on the X chromosome in males.	
Normal female sample (XX)	LOH calls are possible, depending on the constitution of the X chromosomes' origins.	LOH analysis is not performed on the Y chromosome since it is assumed that there not substantial Y chromosomal Female sample with a single X material.
Female sample with a single X chromosome (X0)	LOH calls on X regions with only a single copy. Heterozygous SNP genotypes are possible, but are due to the low inherent Heterozygote call error rate noise, not the true presence of 2 alleles.	

Table 10 Expected LOH calls on the X and Y chromosomes for the OncoScan arrays.

LOH segments	X chromosome	Y chromosome
Normal male sample (XY)	LOH calls that are single copy-based LOH call (CN = 1).	No LOH calls are made for the Y chromosome.
Male sample with multiple X chromosomes (for example, XXY)	LOH calls are possible where there is either loss or low heterozygosity.	
Normal female sample (XX)	LOH calls are possible depending on the constitution of the X origins or in regions of either loss or low heterozygosity.	
Female sample with a single X chromosome (X0)	LOH regions on X which have only a single copy. Is called LOH where there is single copy X.	

Perform a single sample analysis

The following procedure is for setting up a manual analysis. To setup an automatic analysis see "Automatic CEL file analysis" on page 469.

You only need to perform the following steps once, as the data and selections you input (throughout this section) are retained for your convenience with future single sample analysis runs.

Set up and run a single sample analysis

Note: If you want to setup and run an OncoScan™ analysis, see "Set up and run an OncoScan™ single sample analysis" on page 63. If your samples are cancer samples and you suspect aberrations for at least 50% of the genome, then running a Normal Diploid Analysis is recommended. For more information, see "Set up and run a normal diploid analysis" on page 61.

1. From the **Analysis** menu, select **Perform Analysis Setup.** (Figure 11)

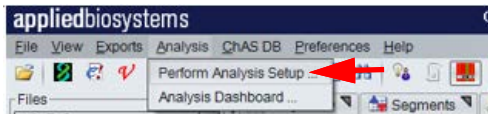


Figure 11 Analysis dropdown list.

The **Analysis setup** tab opens. (Figure 12)

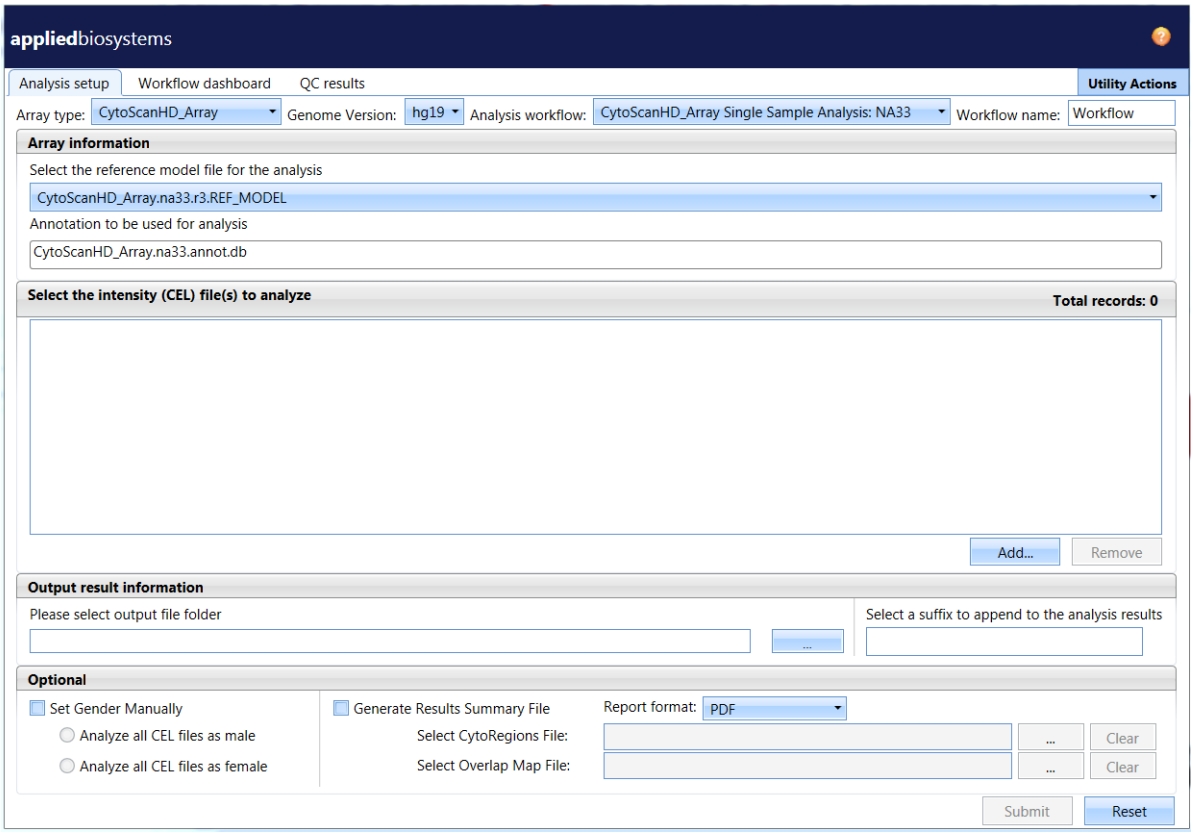


Figure 12 Analysis Workflow.

2. From the **Select array type** dropdown list, click to select CytoScan™ array type. (Example: CytoScanHD_Array)

Note: After you select the array type, analysis workflow, and reference model file, then the annotation file are auto selected for you based on your earlier selections. The Select array type dropdown list includes only the array types for which library (analysis) files have been downloaded from the NetAffx™ Analysis Center or copied from the library package provided with the installation.

3. Choose a Genome Build. (Example: **hg38**)
4. From the **Select analysis workflow** dropdown list, click to select an analysis workflow. (Example: CytoScanHD_Array Single Sample Analysis: NA33 or higher)
5. By default, the Set workflow name is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.
6. From the **Select the reference model file for the analysis** dropdown list, click to select a reference model file for the analysis. (Example: **CytoScanHD_Array.na33.r2.REF_MODEL** ***or higher***).

Note: For Single Sample Analysis, the **Annotation to be used for analysis** field is auto-populated and set with the annotation filename used when the reference model file was generated.

7. At the **Select the intensity (CEL) file(s) to analyze** pane, click **Add.**

Note: The Workflow Analysis window retains the dropdown selections used in your last submitted analysis. However, it does not display a previously set workflow name, CEL files to analyze list, or any suffix used to append your last analysis results. These 3 fields must be completed again.

The following window appears: (Figure 13)

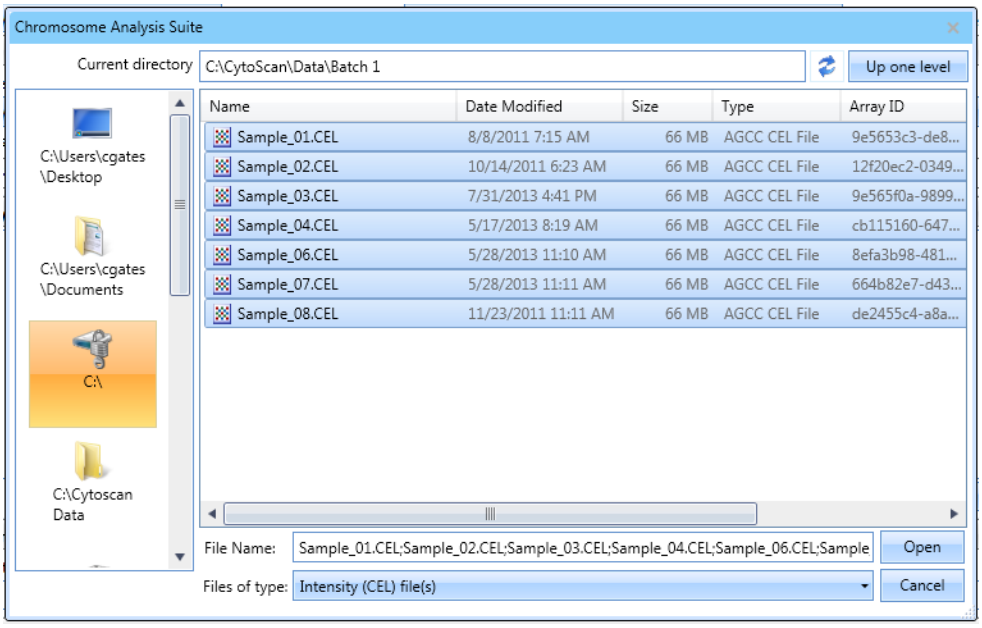


Figure 13 CEL file folder.

8. If your CEL files are located somewhere other than your input path location, navigate to the desired folder. Click, Ctrl+click, Shift+click, or Ctrl+A to select multiple CEL files.
9. Click **Open**.

The **Select the intensity (CEL) file(s) to analyze** pane is now populated with your CEL files, as shown in Figure 14.

Note: You can load several CEL files at a time for a Single Sample Analysis.

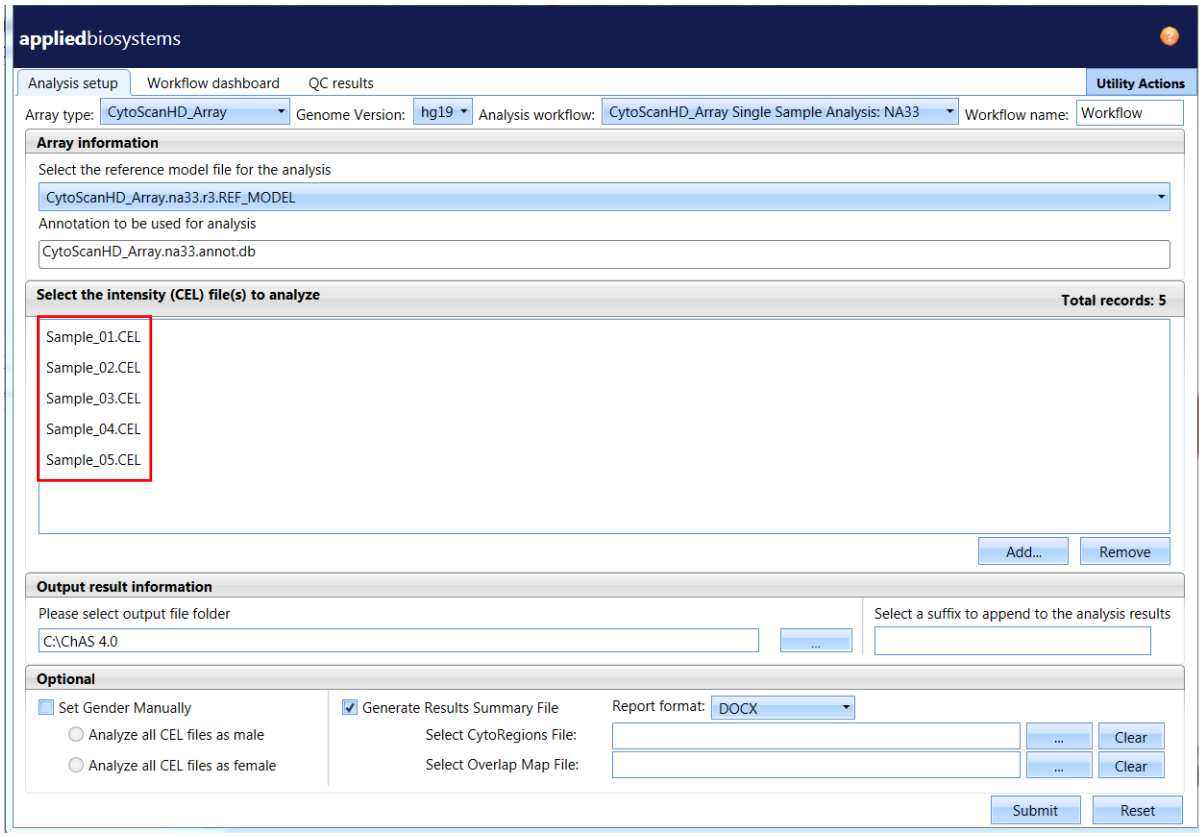


Figure 14 Select the intensity (CEL) file(s) to analyze pane.

To remove a CEL file from this list, click to highlight it, then click **Remove**.

10. At the **Output result information** pane, confirm the path shown for your output file folder. To change the current path/folder, click **…** button to select a different output path/folder.

Note: To better organize your output results, you can add sub-folders to your assigned output result path/folder.

Add sub-folders to your assigned result path/folder

1. Click the ••• button to return to your assigned output path and/or folder.
2. Click **Create New Folder**.
3. Enter a sub-folder name.
4. Click **OK**. Repeat the above steps to add more sub-folders.

   The newly created sub-folders now appear in the output result information window. (Figure 15)

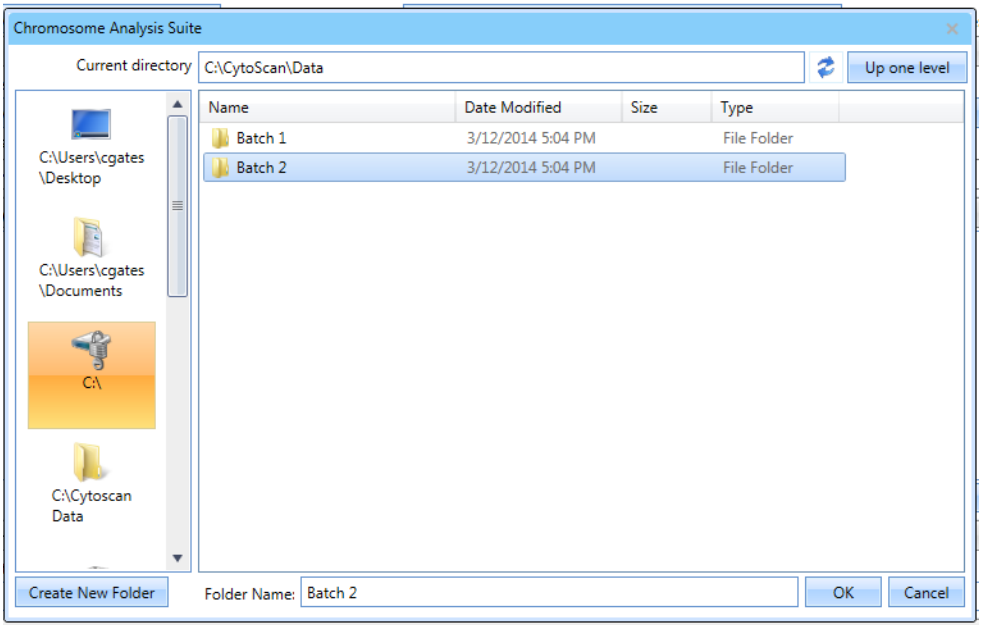


Figure 15 Output result information window with sub-folders example.

5. If you are using a previously analyzed CEL file(s) to verify new CHP data (against CHP data generated from previous versions of ChAS and Library files), you may want to use a suffix to append the new resulting CHP file(s). To do this, click inside the **Select a suffix to append to the analysis results** field to enter an appending file suffix. (Figure 16)

IMPORTANT! If you are saving the same .CYCHP file into the same output file folder that contains your originally run CYCHP file, a "1" is automatically added into the filename (in addition to any suffix you may add) to differentiate the 2 runs of identical CEL file names. Example: na33(1).cyhd.cychp

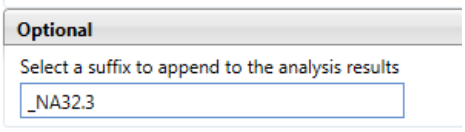


Figure 16 Add a suffix.

6. Optional: If you have a CEL file(s) in which the Y chromosome is partially/fully deleted and therefore determined to be female by the gender calling algorithm, go to the Analysis Setup's Optional pane (Figure 17), select the **Set Gender Manually** checkbox, then click to select the appropriate option.

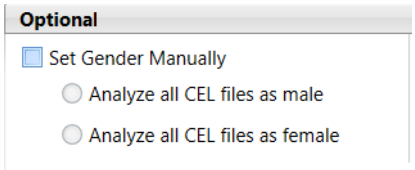


Figure 17 Set Gender option.

7. Optional: If you want to have an automatic export of the Karyoview, Segments Table, and Detail View for Copy Number and LOH Segments in the CHP file, select the checkbox **Generate a Results Summary File**, then and select the output format of either PDF or DOCX. (Figure 18)

Note: You can assign a CytoRegion and Overlap Map region file that highlights these regions in the export. The export is placed in the same folder as the CYCHP file. This automatic export feature is only available for CytoScan™ arrays.

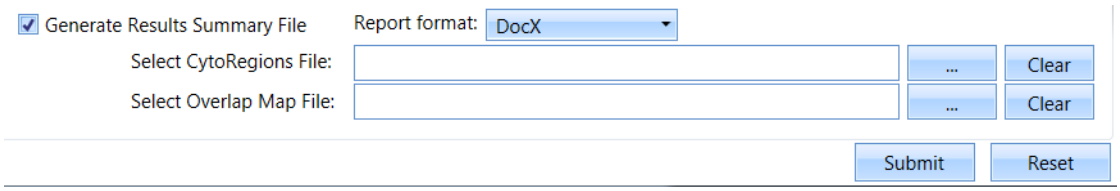


Figure 18 Results Summary File.

8. Click **Submit**.

   If the following warning message appears (Figure 19), acknowledge it, then click **OK**.

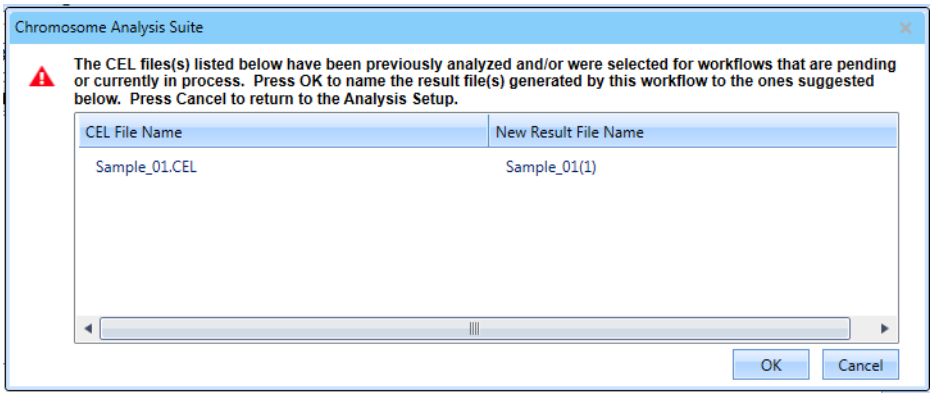


Figure 19 CEL warning message.

The Workflow dashboard window appears and your annotation files begin to load. (Figure 20).

The Analysis Workflow dashboard tracks ongoing analysis tasks for ChAS. It also delivers the results of the analyses and can restart the Browser (if it was shut down to free up memory for the analysis).

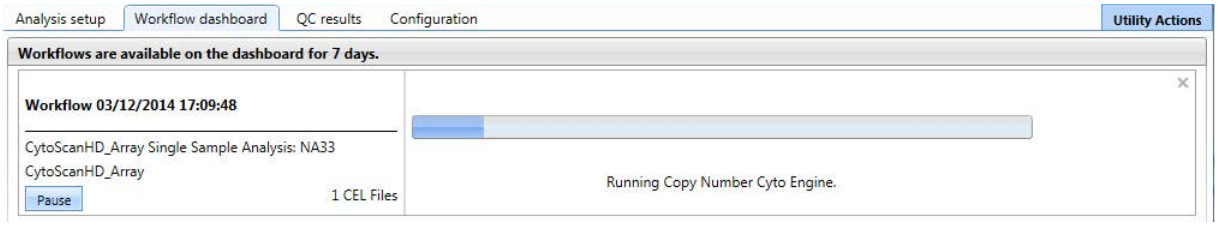


Figure 20 CEL files loading inside the Workflow dashboard.

After loading is complete, a Workflow completed successfully message appears. (Figure 21)

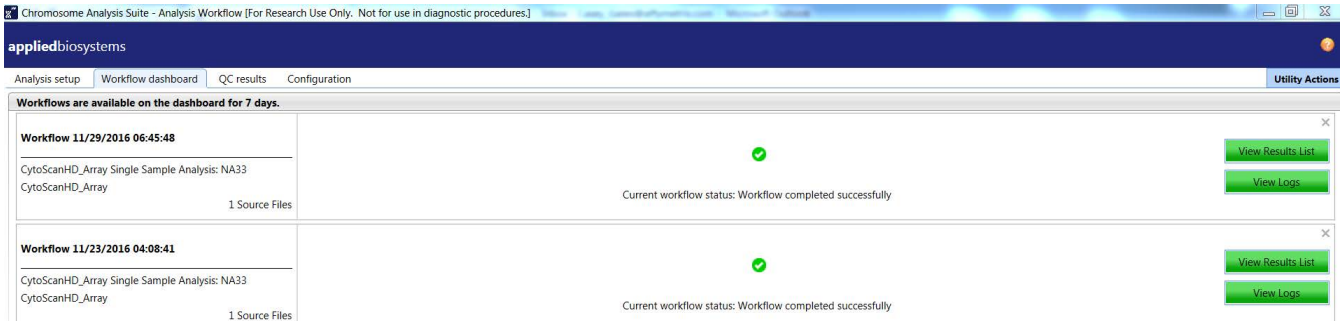


Figure 21 Workflow Dashboard example with multiple Single Samples loaded.

Note: The **View Logs** button accesses the algorithm pipeline logs which can be useful if you have a workflow that fails to complete.

9. Click to choose the analysis you want to view, then click **View Results List**.

   The QC Results tab window appears showing the Basic View QC settings. A Detail View QC setting, which provides more columns of data, is also available in the QC Settings dropdown list. (Figure 22)

Note: QC parameters can also be viewed in the ChAS Browser see setting QC parameters in ChAS Browser.

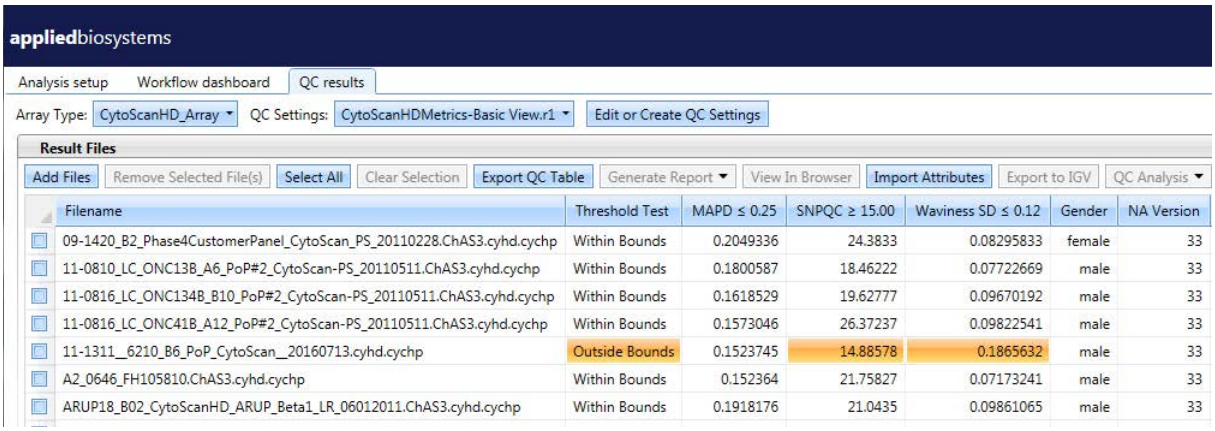


Figure 22 QC results window tab.

10. Select each sample's checkbox or click the **Select All** button to select all samples.

Create your own custom QC setting

1. Click the **Edit or Create QC Settings** button.
   The **New QC Setting** window appears. (Figure 23)

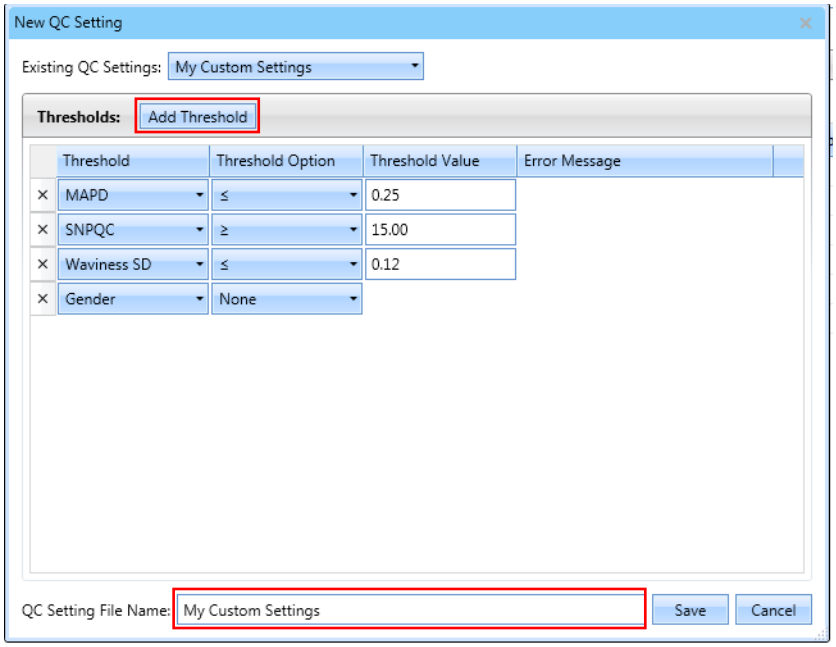


Figure 23 New QC Setting window.

2. Click **Add Threshold** (Figure 23) to create a new row.
3. Select the threshold you want to view in your custom QC Setting.

Note: The threshold metric you select is not required to have a threshold value.

4. Name your custom QC settings. (Example: My Custom Settings) (Figure 23), then click **Save**.
   Your custom QC Setting is now available from the QC Settings dropdown list, as shown in Figure 24.

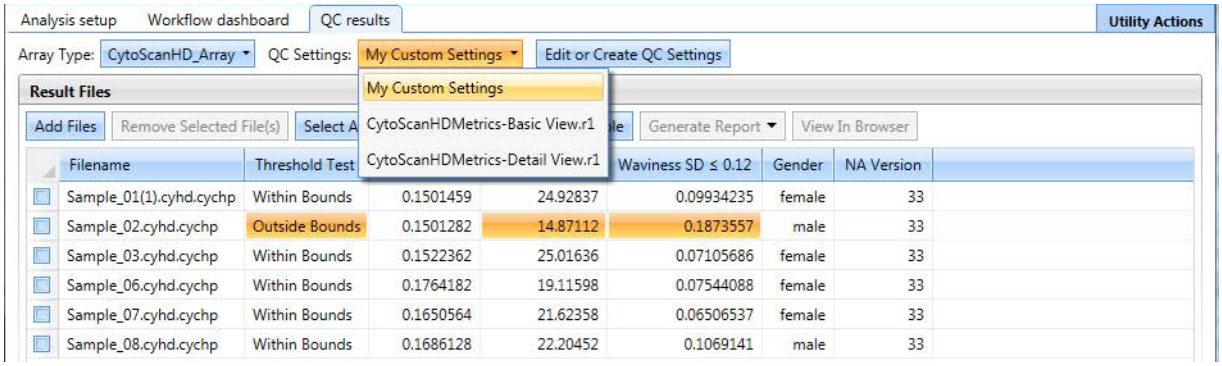


Figure 24 New QC Settings menu item.

View results in the browser

At the QC Results window, click the **View in Browser** button or the **View in MSV** button. For more MSV information, see the *Reproductive Health Research Analysis Software User Guide* (Pub. No. MAN0027973).

If the following warning message appears (Figure 25), acknowledge it, then click **OK**.

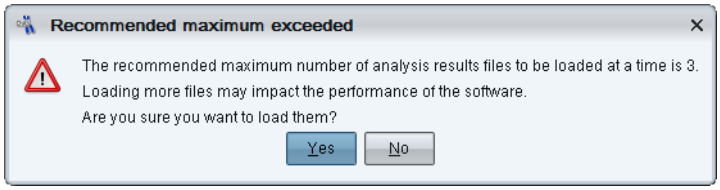


Figure 25 Recommended maximum exceeded message.

If the following warning message appears (Figure 26), acknowledge it, then click **OK**.

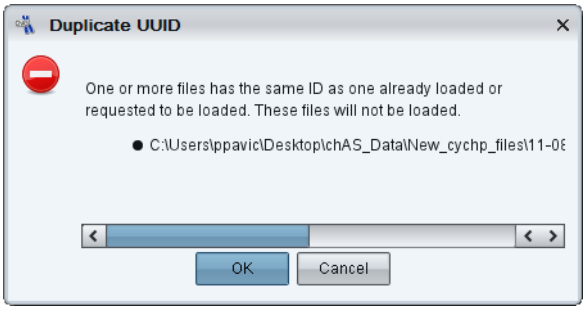


Figure 26 Duplicated files message.

If the following warning message appears (Figure 27), click **Yes** to acknowledge it.

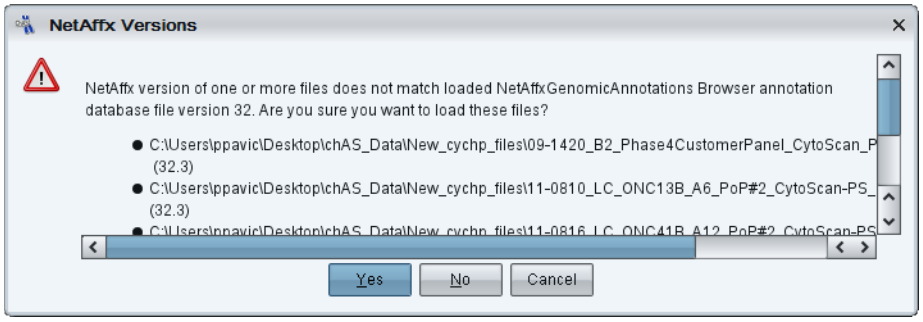


Figure 27 NetAffx™ versions message.

A progress bar appears.

Note: The ChAS Browser allows for loading of xxCHP files analyzed from different versions of ChAS. However, xxCHP files analyzed from different genome versions (hg18, hg19, hg38) cannot be loaded at the same time.

After a few moments, the ChAS Browser featuring your selected samples appears. (Figure 28)

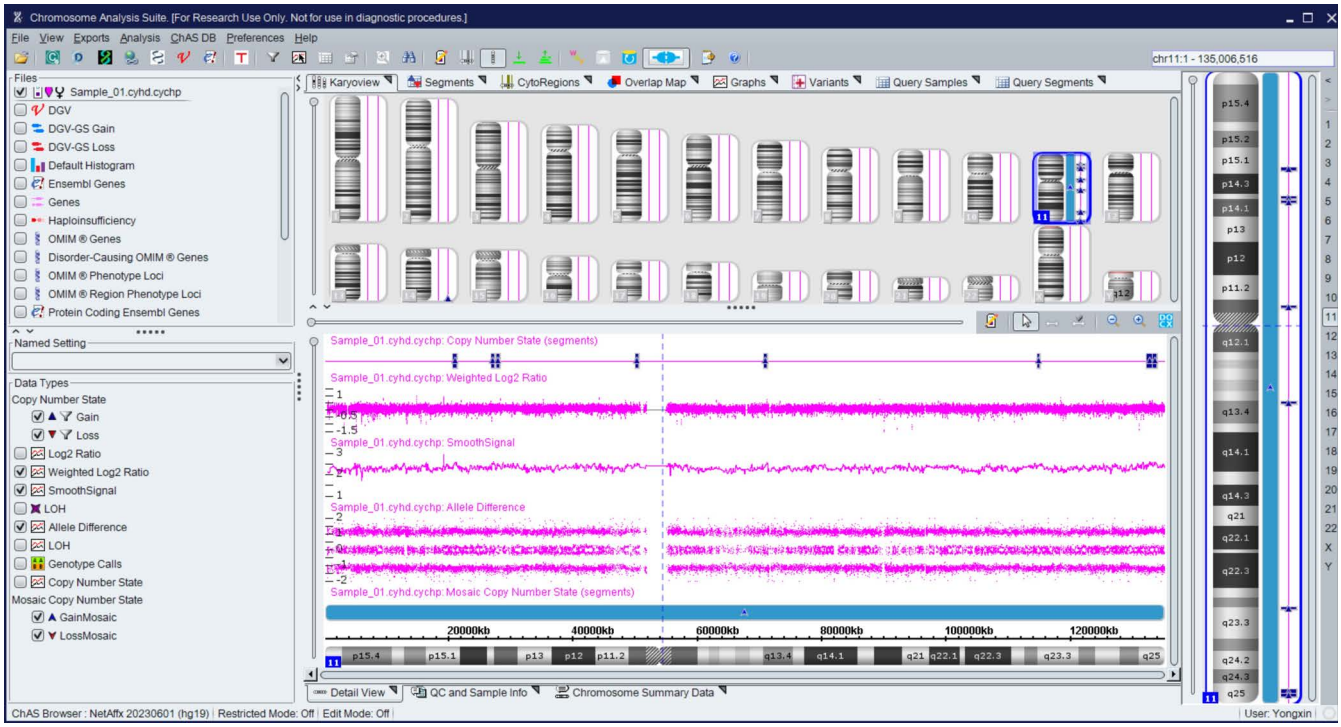


Figure 28 ChAS Browser.

Recenter CytoScan™ HD, 750K, HD Accel, and Optima, arrays

Due to the complexity and low diploid count in a small fraction of cancer samples, there may be a need to manually assign the diploid region of the sample or recenter it.

In Figure 29, Chromosome 1 is called as a mosaic copy number loss, the log 2 ratio data is shifted downward, the smooth signal averages 1.75 copies, but the Allele Difference (AD) and B- Allele Frequency (BAF) Graphs are displaying 3 tracks.

Note: Since it is unlikely to have 3 tracks in AD/BAF data in a region of loss (unless the loss is CN=0), this sample needs to be re-centered.

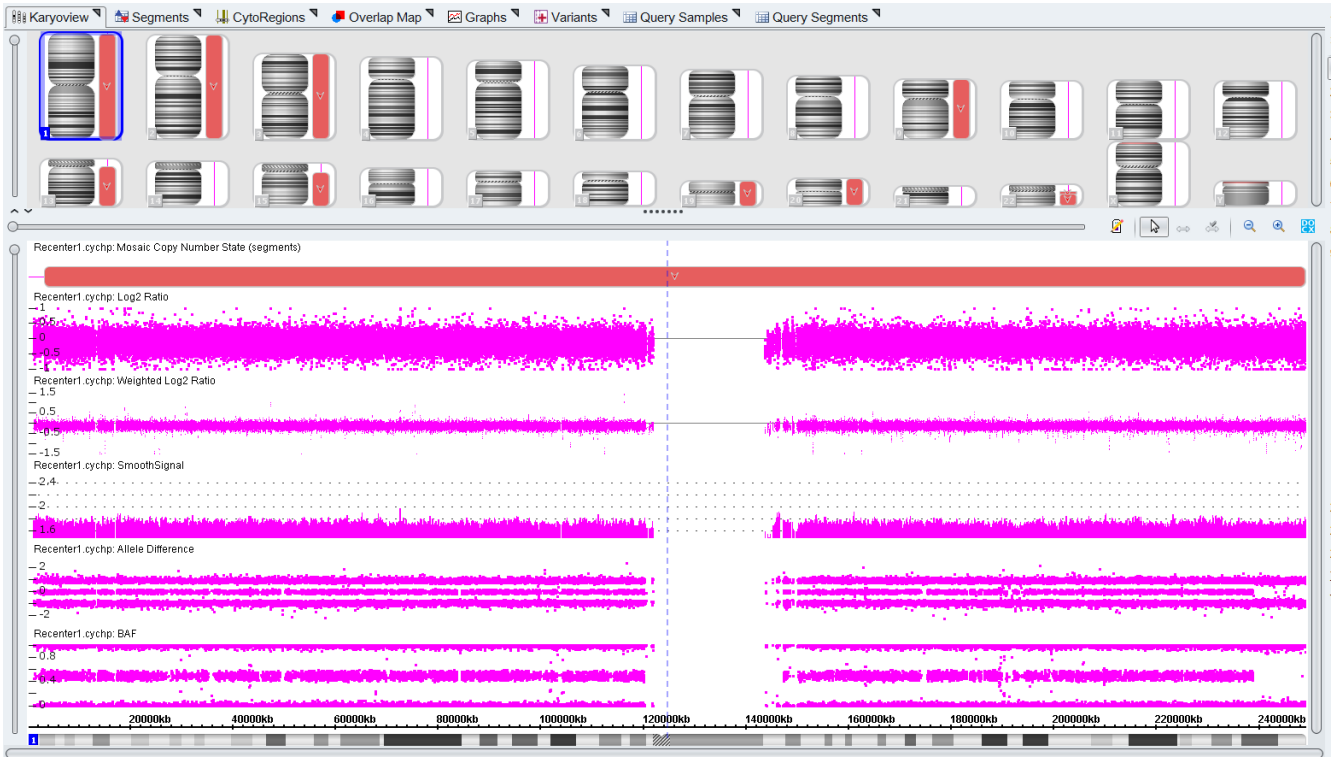


Figure 29 Need for re-centering example.

If the region that is true diploid is a whole chromosome, use **Method 1**. If the region that is true diploid is part of a chromosome, use **Method 2**.

Method 1: Determine the median Log 2 ratio for the region in the sample that is truly diploid

1. Open the ChAS Browser.
2. Click the **Chromosome Summary Data** tab, then click the dropdown list to select **MedianSignal**. (Figure 30)

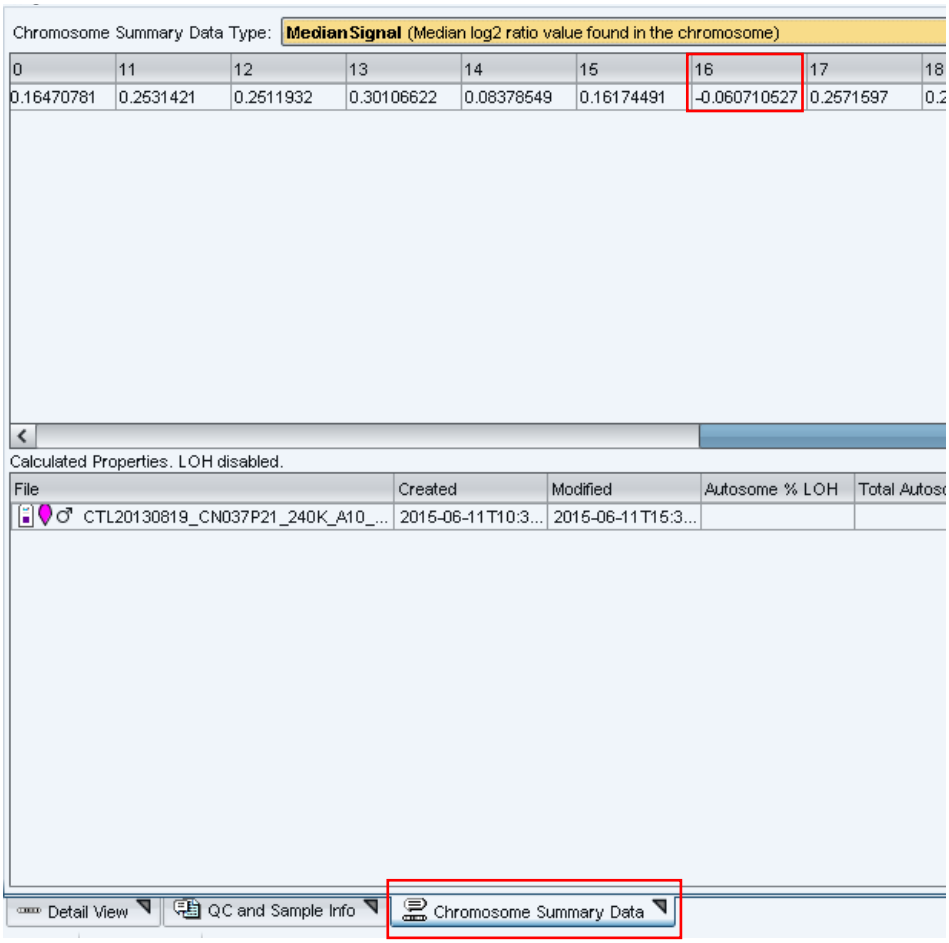


Figure 30 Chromosome Summary tab - median log2 ratio value for Chromosome 16 example.

Method 2: Determine the median Log 2 ratio for the region in the sample that is truly diploid

1. Open the ChAS Browser.
2. In the Detail View (Figure 29), zoom into the region determined to be diploid.
3. Go to the **Graphs** tab, then click  to include only the selected view.
4. Highlight the **Log2 Ratio** column, then right-click to select **Sum, mean and median**. (Figure 31)

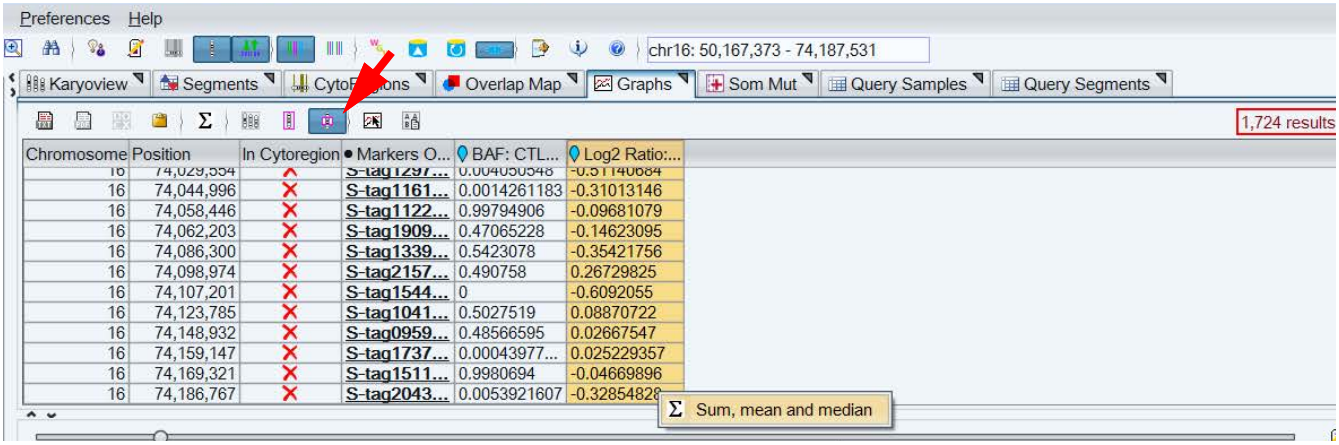


Figure 31 Graphs tab - Log 2 Ratio column.

A sum, mean, and median window appears. (Figure 32)

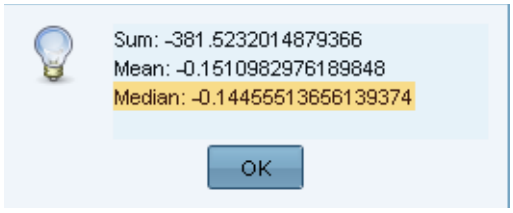


Figure 32 Sum, mean, and median message.

5. Acknowledge the message, then click **OK**.
6. In the Analysis Workflow, set up the CEL file in the Single Sample Workflow, as described in "Perform a single sample analysis" on page 48.
7. Select the **Use Manual Recentering** checkbox to enable the parameter fields, then enter the value of the median Log2 (determined by the browser) into the **Adjust this log 2 to 0** text field. Optional: Enter a suffix.

Note: A suffix is recommended to differentiate the re-centered CYCHP file from the original.

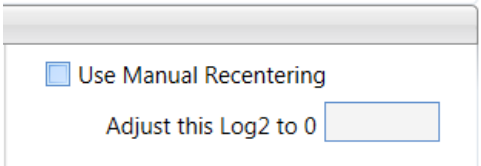


Figure 33 Use Manual Recentering checkbox.

8. Click **Submit**.

Note: By entering a median Log2 Ratio value (for the region you have determined to be diploid, The Recentering Algorithm has re-centered the log2 ratio data (for the region determined to be diploid) around 0 and there is no longer a mosaic loss segment called in this region, as shown in the Chromosome 1 example. (Figure 34)

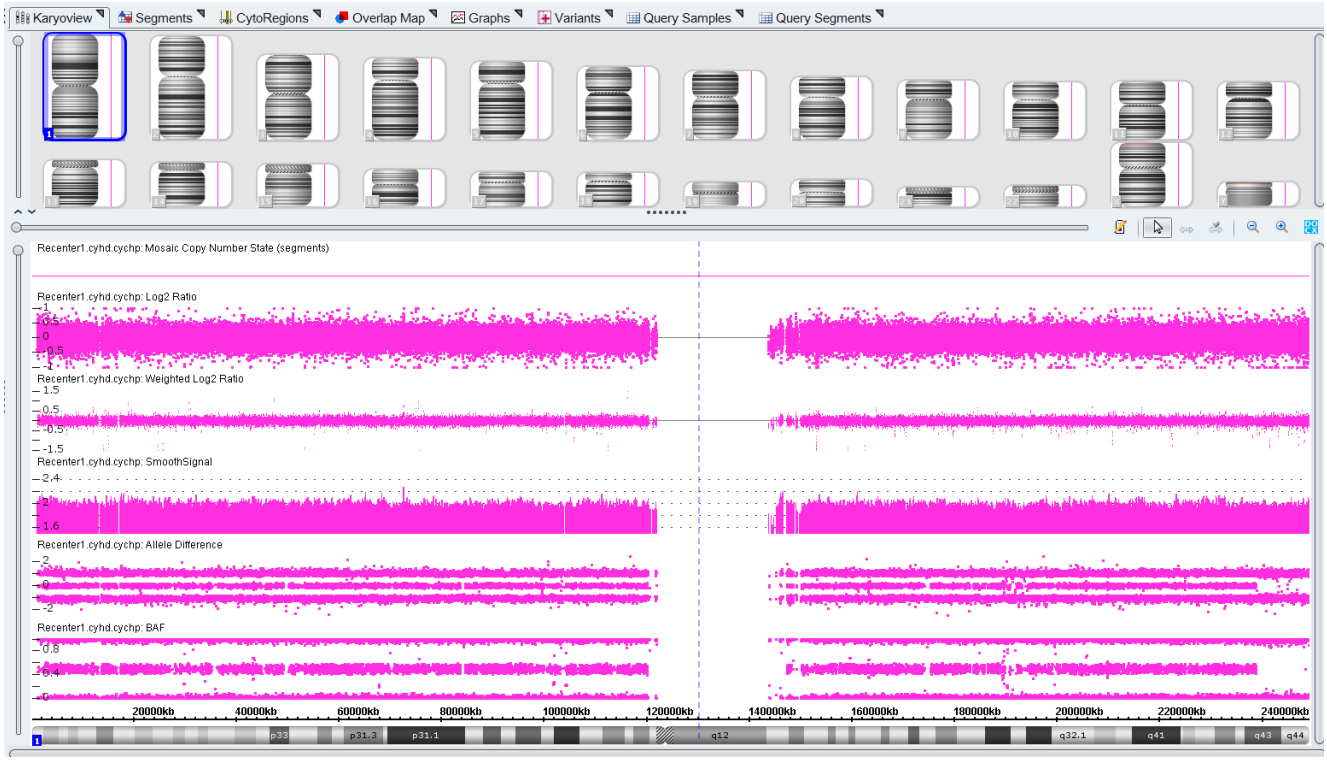


Figure 34 Re-centered Chromosome 1 example.

No gender single sample analysis

The No Gender Single Sample Analysis (Figure 35) is the same analysis as described previously for Single Sample Analysis with the exception that no gender information is displayed in ChAS. The gender is not reported and no segment or probe level data from X or Y chromosomes are displayed.

The metric, **Sex Chromosomes Aberrated** can be added to the QC table and reports either a Yes or No.

- **Yes**: Indicates that the sample does have segments meeting the following default thresholds: 50 Markers/ 200 kb for copy number and 50 Markers/10,000 kb for LOH segments.
- **No**: Indicates no copy number or LOH segments meet the previously defined thresholds.

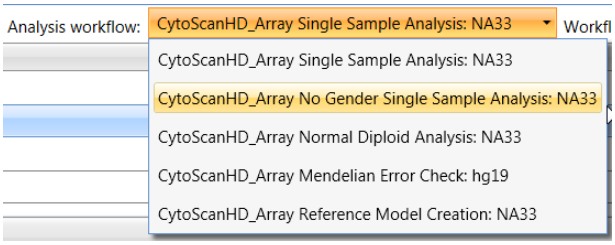


Figure 35 No gender workflow.

Set up and run a normal diploid analysis

The Normal Diploid Analysis for CytoScan™ microarrays is recommended for cancer samples in which >50% of the genome is likely to be rearranged. This analysis automatically determines the normal diploid regions and normalize the rest of the sample based on those regions resulting in properly centered data.

Note: Normal diploid analysis is *not* supported for CytoScan™ HD Accel Arrays.

A Normal Diploid Analysis has the identical setup steps as "Set up and run a single sample analysis" on page 48. The only difference is you must select **Normal Diploid Analysis** from the **Select analysis workflow** dropdown list, as shown in Figure 36.

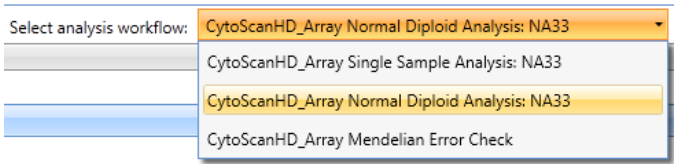
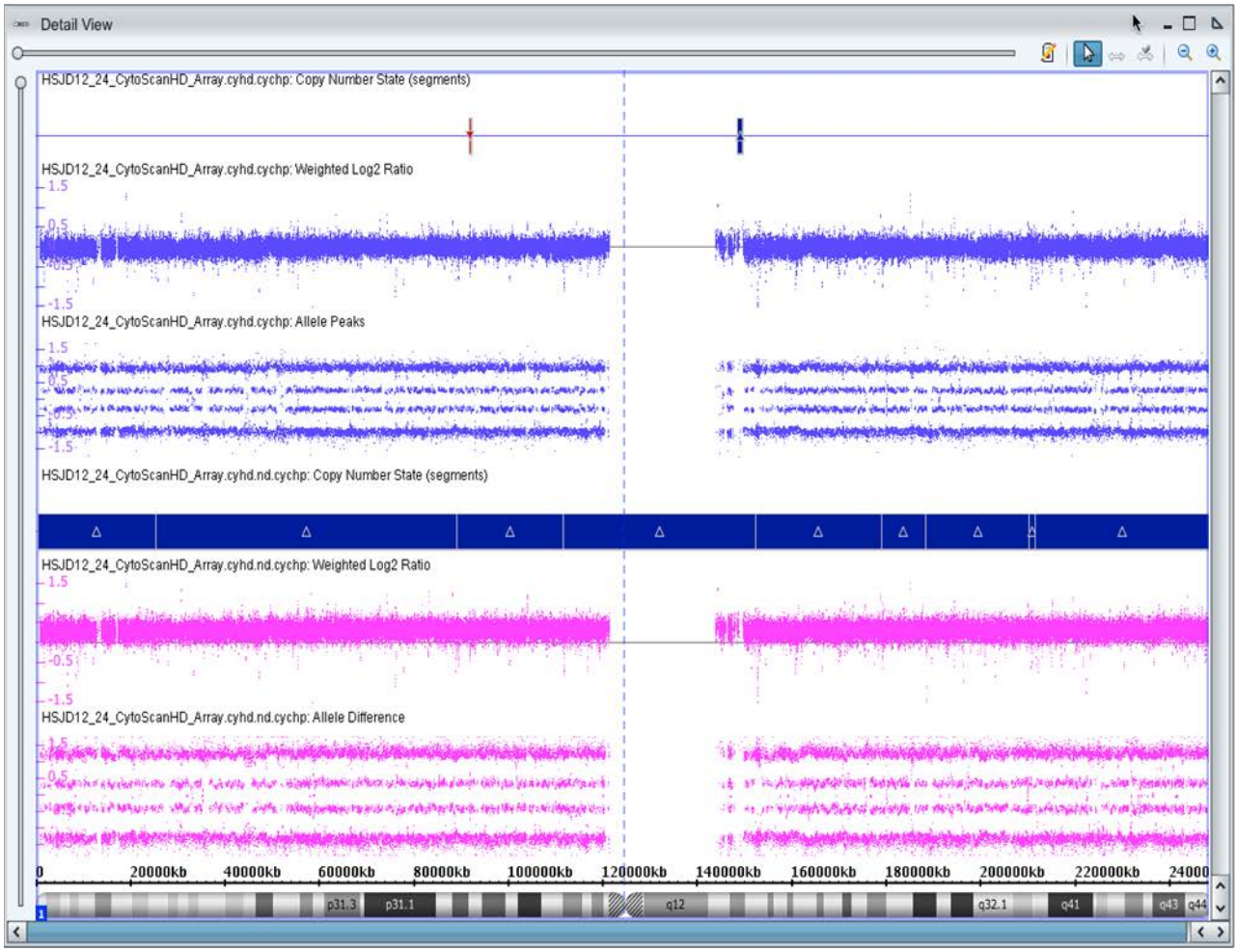


Figure 36 Normal Diploid Analysis.



The data shown above is from a cancer sample in which >50% of the genome was non-diploid. The top graph (purple) shows the sample run through the traditional single sample analysis. There are no Copy Number Segments called, the weighted log2 is centered around 0, but there are 4 allele difference tracks indicating more than 2 copies of this chromosome. In the bottom graph (pink), this same sample is run through the Normal Diploid normalization algorithm. The Copy Number Gain segment is called, the weighted log2 is shifted above the 0 line which is in agreement with the 4 allele difference tracks.

Figure 37 Example: Sample with and without a Normal Diploid Analysis.

Recommended QC metrics for Normal Diploid Analysis

- ndSNPQC
- MAPD
- ndwavinessSD
- SNPQC
- wavinessSD

View the recommended QC metrics (listed above)

Note: For samples run through the Normal Diploid Analysis, the following QC metrics are recommended:

- MAPD < 0.25
- SNPQC or ndSNPQC ≥ 15
- wavinessSD or ndwavinessSD < 0.12

1. From the Analysis Workflow, click the **QC Results** window tab.
2. Click the **Settings** dropdown list, then select **NDN View.r1**, as shown in Figure 38.

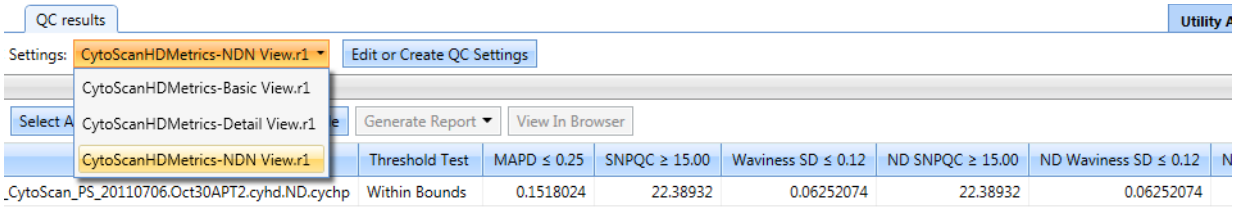


Figure 38 NDN View.r1 from the Setting dropdown list.

Set up and run an OncoScan™ single sample analysis

1. From the **Analysis** menu, select **Perform Analysis Setup.** (Figure 39)

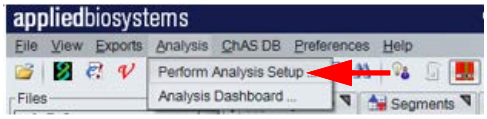


Figure 39 Analysis dropdown list.

The **Analysis setup** tab opens. (Figure 40)

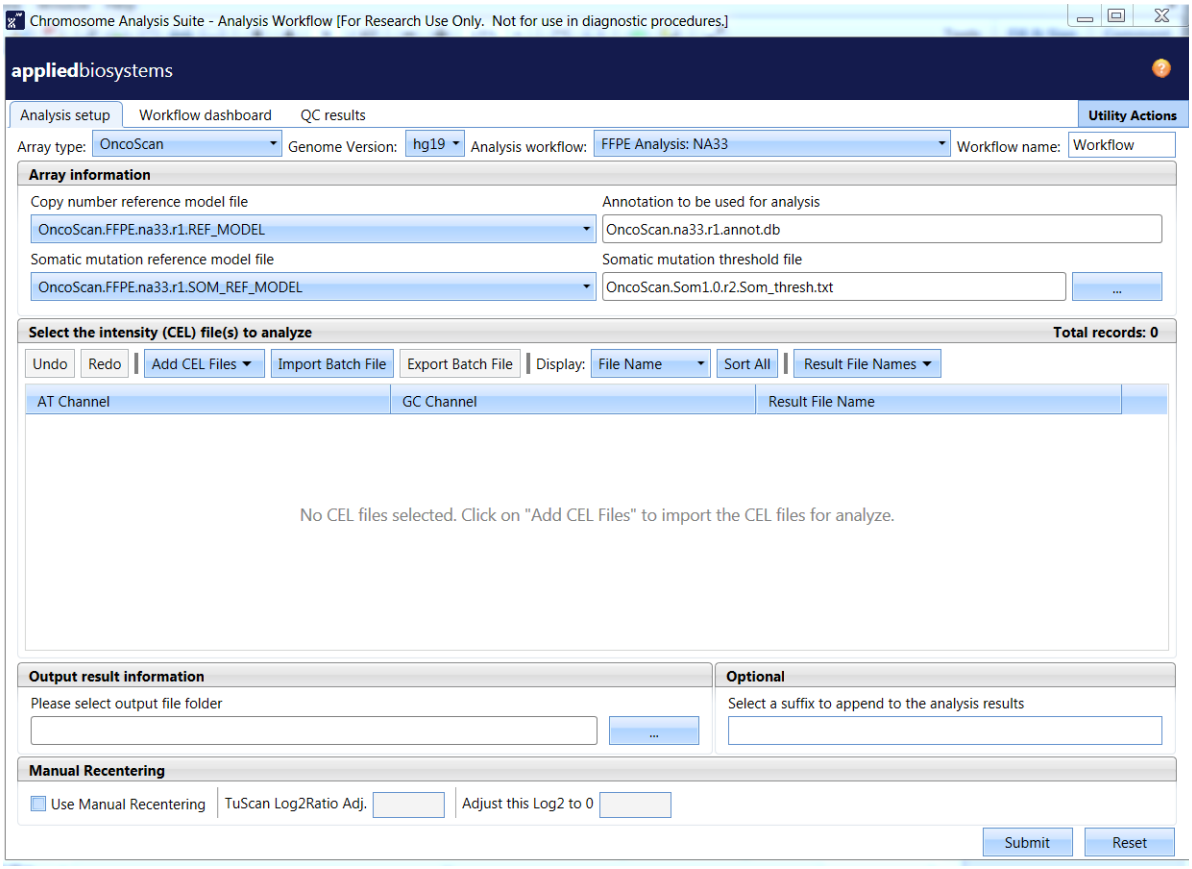


Figure 40 Analysis workflow.

2. From the **Select array type** dropdown list, click to select **OncoScan**.

Note: After you select the array type, analysis workflow, and reference model file, then the annotation file is auto selected for you based on your earlier selections.

IMPORTANT! The Select array type dropdown list includes only the array types for which library (analysis) files have been downloaded from the NetAffx™ Analysis Center or copied from the library package provided with the installation.

3. Select the appropriate Genome Build.
4. From the **Select analysis workflow** dropdown list, click to select an appropriate analysis workflow.

IMPORTANT! For FFPE samples use the FFPE Analysis NAXX workflow. For Control DNA use the Control DNA Analysis.

5. By default, the **Set workflow name** is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.
6. Click the **Copy number reference model file** from the dropdown list, then click to select an appropriate file.
7. Click the **Somatic mutation reference model file** from the dropdown list, then click to select an appropriate file.

8. At the **Select the intensity (CEL) file(s) to analyze** pane, click the **Add CEL Files** dropdown, then click to select **AT Channel.**

   A File Explorer window appears.

9. Highlight the CEL file(s) using Ctrl+click or Shift+click, then click **Open**.

10. At the **Select the intensity (CEL) file(s) to analyze** pane, click the **Add CEL Files** dropdown, then click to select **GC Channel.**

    A File Explorer window appears.

11. Highlight the CEL file(s) using Ctrl+click or Shift+click, then click **Open**.

    The **Select the intensity (CEL) file(s) to analyze** pane is now populated with AT and GC Channel CEL files.

    IMPORTANT! After loading the CEL files, check that the AT lines up with the matching GC CEL file.

12. Click the **Result File Names** dropdown list to enable ChAS to automatically generate Output Names.

    Note: Output file names are only auto-generated if the 2 CEL files have the same root name. It is recommended to use an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "_AS_05A.CEL" is an AT Channel file, while "_AS_05C.CEL" is a GC Channel file.

    You can also clear this (populated) column by clicking **Clear Column**.

13. Optional: To choose a different output folder from the saved output path that is displayed, click the Output result information's **Browse** button.

    A File Explorer window appears.

14. Navigate to an output folder location, then click **OK**.

    Note: To better organize your output results, you can add sub-folders to your assigned output result path/folder.

Add sub-folders to your assigned Output Results folder

1. Click the ••• button to return to your assigned output path and/or folder.
2. Click **Create New Folder**.
3. Enter a sub-folder name.
4. Click **OK**. Repeat the above steps to add more sub-folders.

   The newly created sub-folders now appear in the output result information window.

5. If you are using a previously analyzed CEL file(s) to verify new CHP data (against CHP data generated from previous versions of ChAS and Library files), you may want to use a suffix to append the new resulting CHP file(s). To do this, click inside the **Select a suffix to append to the analysis results** field to enter an appending file suffix. (Figure 41)

   IMPORTANT! If you are saving the same OSCHP file into the same output file folder that contains your originally run OSCHP file, a "1" is automatically added into the filename (in addition to any suffix you may add) to differentiate the 2 runs of identical CEL file names. Example: na33(1).oschp

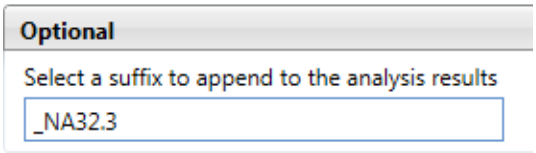


Figure 41 Add a suffix.

6. Optional: If you have a CEL file(s) in which the Y chromosome is partially/fully deleted and therefore determined to be female by the gender calling algorithm, go to the Analysis Setup's Optional pane (Figure 42), select the **Set Gender Manually** checkbox, then click to select the appropriate option.

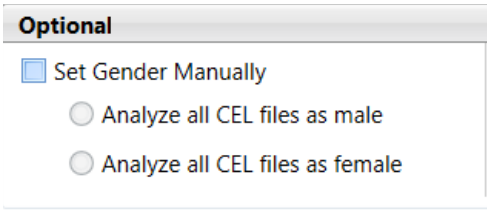


Figure 42 Set Gender option.

7. Click **Submit**.

   If the following warning message appears (Figure 43), acknowledge it, then click **OK**.

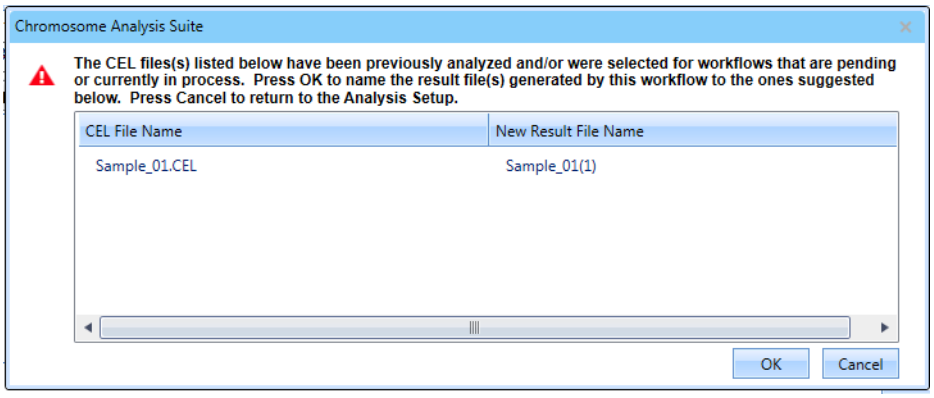


Figure 43 CEL warning message.

The **Workflow dashboard** window appears and your annotation files begin to load. (Figure 44).

The Analysis Workflow Dashboard tracks ongoing analysis tasks for ChAS. It also delivers the results of the analyses and can restart the Browser (if it was shut down to free up memory for the analysis).

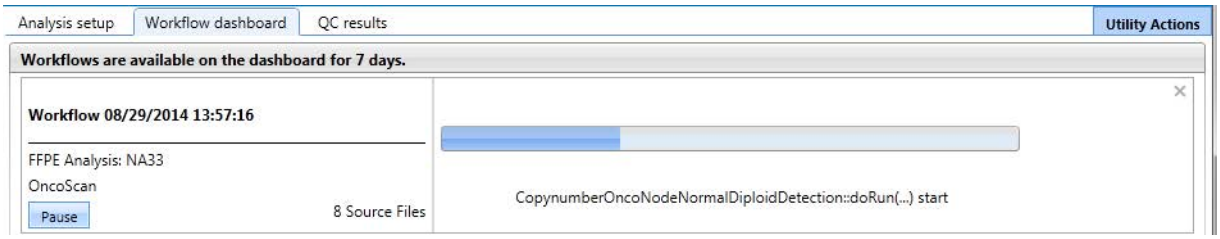


Figure 44 CEL files loading inside the Workflow dashboard.

After loading is complete, a Workflow completed successfully message appears. (Figure 45)

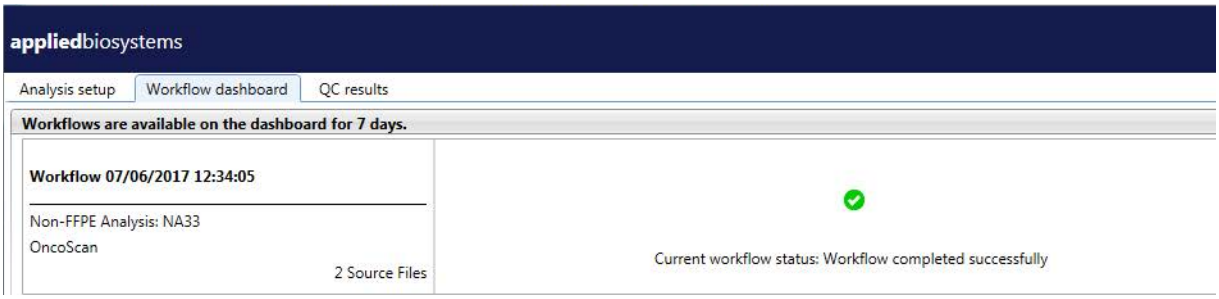


Figure 45 Workflow dashboard with Single Samples loaded.

Note: The **View Logs** button accesses the algorithm pipeline logs which may be useful if you have a workflow that fails to complete.

8. Click to choose the analysis you want to view, then click **View Results List**.

   The **QC results** tab window appears showing the Basic View QC settings. (Figure 46) A Detail View QC setting (which provides more columns of data) is also available in the QC settings dropdown list.

Note: QC parameters can also be viewed in the ChAS Browser see "Set QC parameters in the ChAS Browser" on page 124.

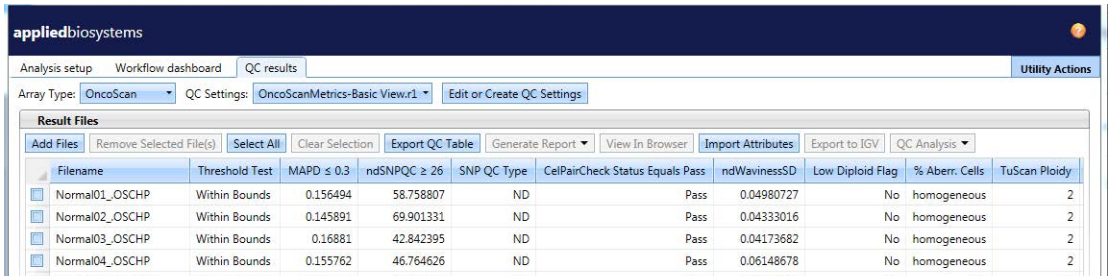


Figure 46 QC Results window tab.

9. Select each sample's checkbox or click the **Sample File** checkbox to select *all* samples.

Create your own custom QC settings

See "Create your own custom QC setting" on page 54

View results in the browser

1. At the QC Results window, click **View In Browser**.

   If the following warning message appears (Figure 47), click **Yes** to acknowledge it.

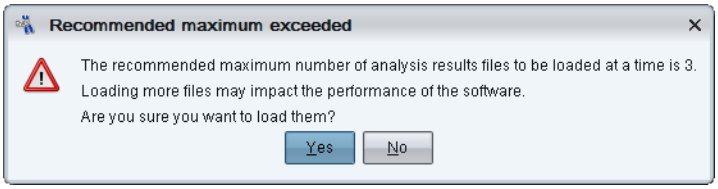


Figure 47 Recommended maximum exceeded message.

If the following warning message appears (Figure 48), acknowledge it, then click **OK**.

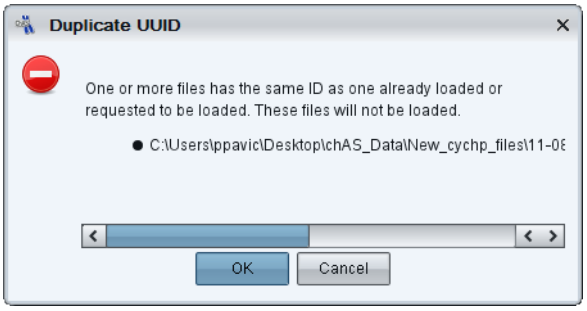


Figure 48 Duplicated files message.

If the following warning message appears (Figure 49), click **Yes** to acknowledge it.

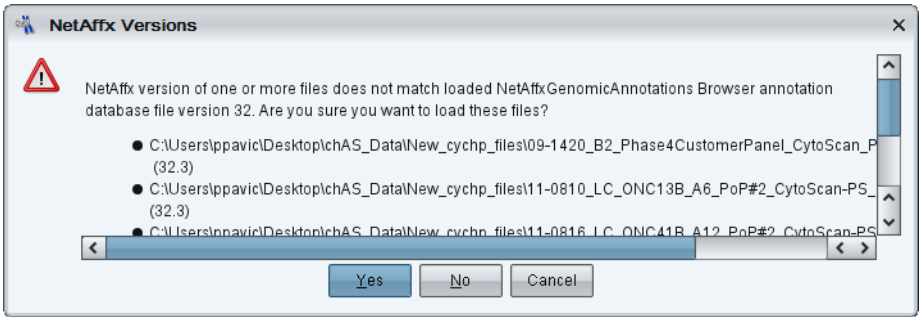


Figure 49 NetAffx™ versions message.

A progress bar appears.

Note: The ChAS Browser allows loading files analyzed using different NetAffx™ version at the same time (as long as the versions are all from all the same reference and genome builds). If NetAffx™ versions are from different builds of the genome (for example Hg18 and Hg19), The ChAS Browser does not load the files.

After a few moments, the ChAS Browser featuring your selected samples appears.

Recenter OncoScan™ CNV and OncoScan™ CNV Plus Arrays

Due to the complexity and low diploid count in a small fraction of cancer samples, there may be a need to manually assign the diploid region of the sample or "recenter" it.

In Figure 50, Chromosome 16q is called as a loss, the log 2 ratio data is shifted downward, but the Allele Difference Graph is displaying 3 tracks representing AA, AB, BB calls. Having an Allele Difference graph with 3 tracks means this region must have at least 2 copies. Since you cannot have 3 Allele Difference tracks in a region of loss, this sample needs to be recentered. For more information, see "Manual re-centering algorithm (OncoScan™ data)" on page 462.

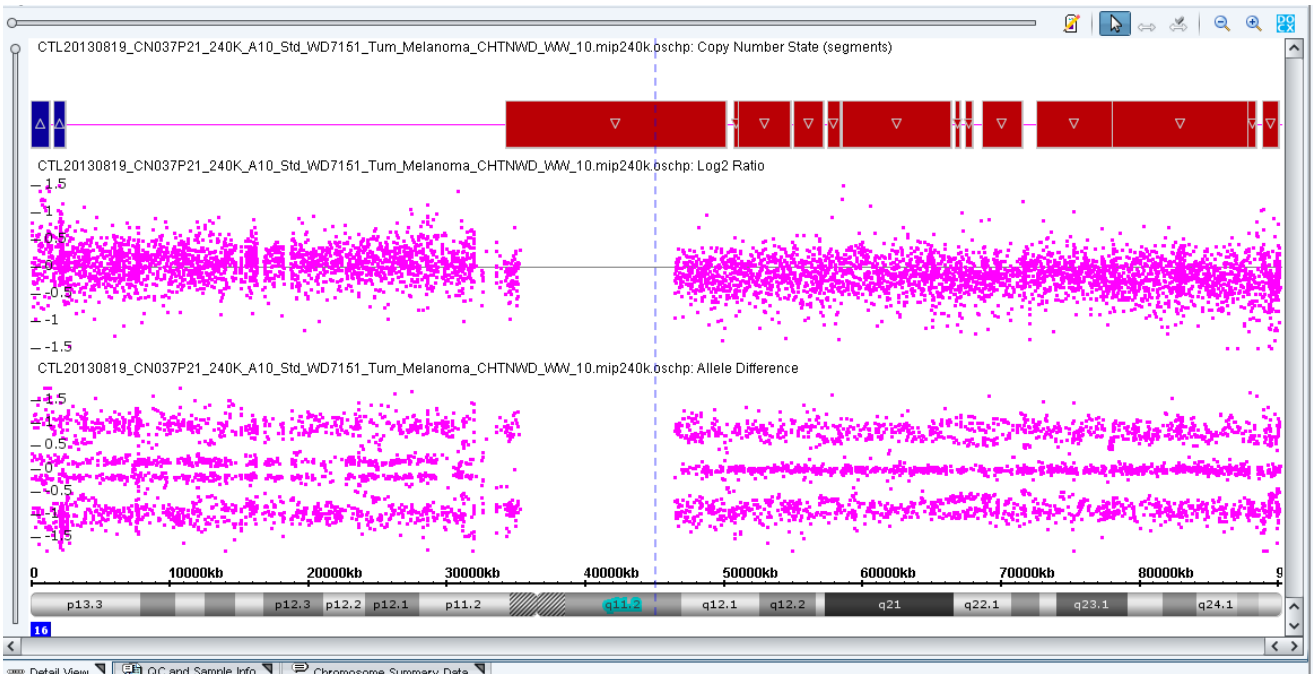


Figure 50 Example of 3 Allele Difference tracks in a Region of Loss.

If the region that is true diploid is a whole chromosome, use **Method 1**. If the region that is true diploid is part of a chromosome, use **Method 2**.

Method 1: Determine the median Log 2 ratio for the region in the sample that is truly diploid

1. Open the ChAS Browser.
2. Click the **Chromosome Summary Data** tab, then click the dropdown list and select **MedianSignal**. (Figure 51)

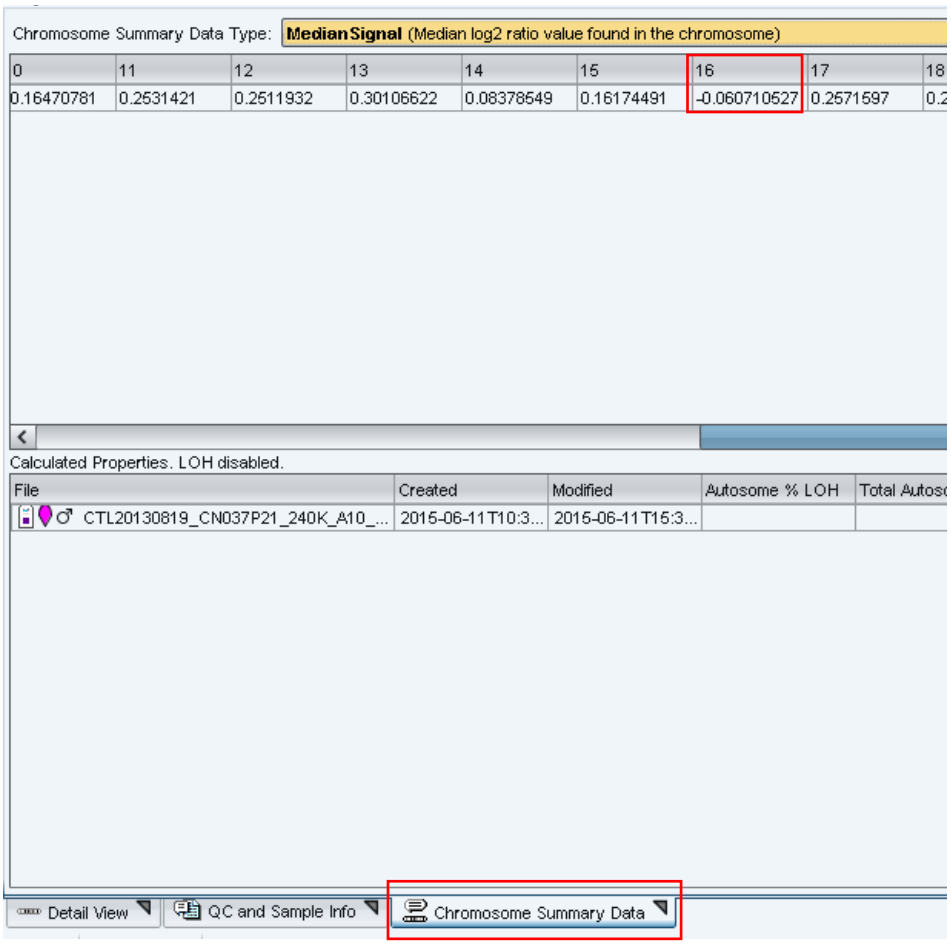


Figure 51 Chromosome Summary tab - median log2 ratio value for Chromosome 16 example.

Method 2: Determine the median Log 2 ratio for the region in the sample that is truly diploid

1. Open the ChAS Browser.
2. In the Detail View (Figure 50), zoom into the region determined to be diploid.
3. Go to the **Graphs** tab, then click  to include only the selected view.
4. Highlight the **Log2 Ratio** column, then right-click to select **Sum, mean and median**. (Figure 52)

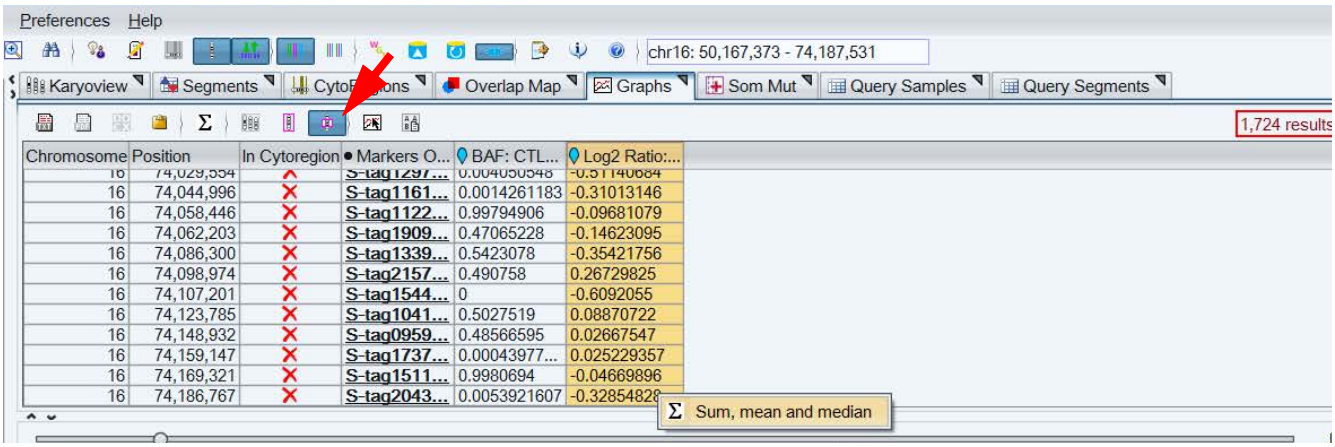


Figure 52 Graphs tab - Log 2 Ratio column.

A Sum, mean, and median window appears. (Figure 53)

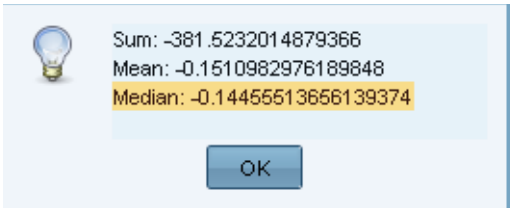


Figure 53 Sum, mean, and median message.

5. Acknowledge the message, then click **OK**.
6. Open the **ChAS Analysis Workflow**, then click the **QC Results** tab. (Figure 54)

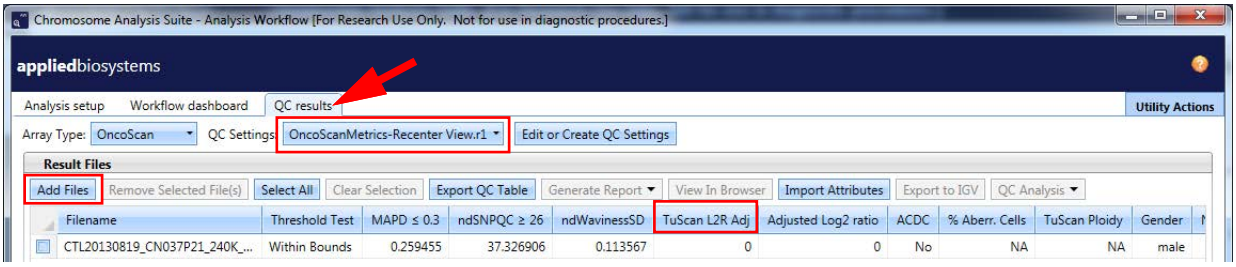


Figure 54 ChAS Analysis Workflow - QC Results window tab.

7. Load the OSCHP file into the **QC Results** tab by clicking the **Add Files** button. (Figure 54)

8. From the **QC Settings** dropdown list, select **Recenter View**, then make a note of the **TuScan L2R Adj** value. (Figure 54)
9. Click the **Analysis setup** tab. (Figure 55)

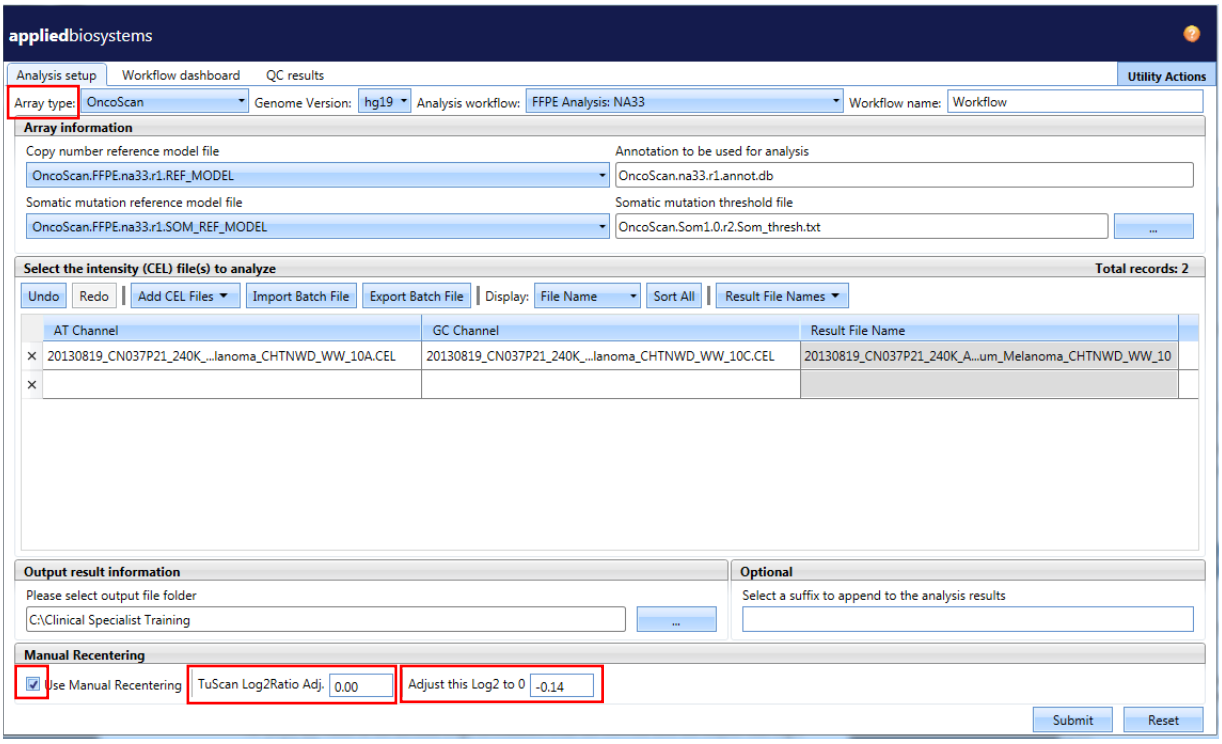


Figure 55 ChAS Analysis Workflow - Analysis setup window tab.

10. Select the FFPE or non-FFPE analysis based on the sample type.
11. Load in the 2 CEL files into the appropriate channel.
12. Select the **Manual Recenter** checkbox to enable the parameter fields. (Figure 55)
13. Enter the **TuScan Log2 Adjustment** value you noted earlier.
14. Enter the value of the **median Log2** determined from the browser into the **Adjust this log 2 to 0** field.
15. Enter a suffix if desired.

Note: An RC is automatically appended onto any OSCHP file that goes through Manual Recentering for an RC.OSCHP extension.

Figure 56 shows the original OSCHP file (pink data) and the manually recentered RC.OSCHP (green data).

By inputting both the TuScan Log2 Ratio value (derived from the algorithm) and the median Log2 Ratio value (for the region you have determined to be diploid, Chromosome 16q for our example), the Recentering Algorithm has recentered the log2 ratio data (for the region determined to be diploid) around 0 and there is no longer a loss segment called in this region.

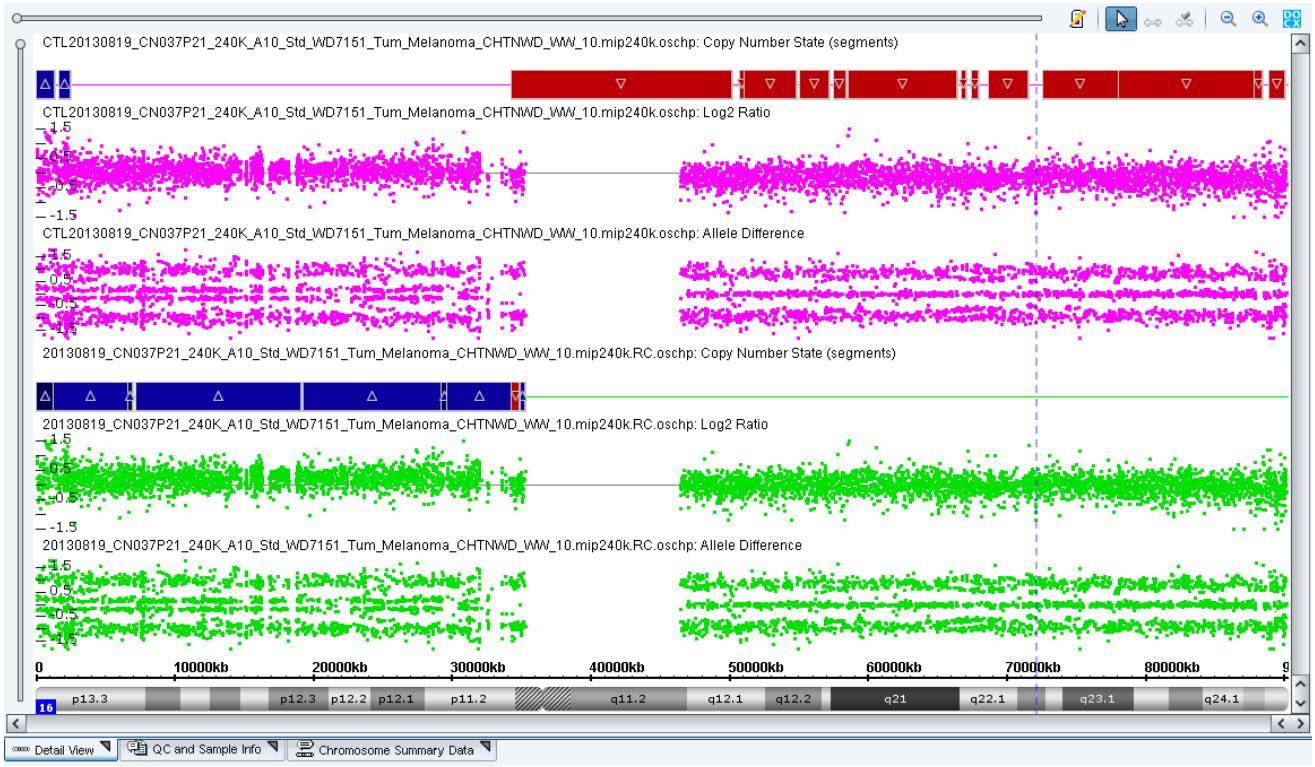


Figure 56 Example original OSCHP file (pink data) and the manually re-centered RC.OSCHP (green data).

Set up and run an OncoScan™ matched normal analysis

As long as your library file folder contains the necessary analysis files for the array, your configuration paths are established (Figure 57) your **Array information** fields auto-populate. (Figure 58)

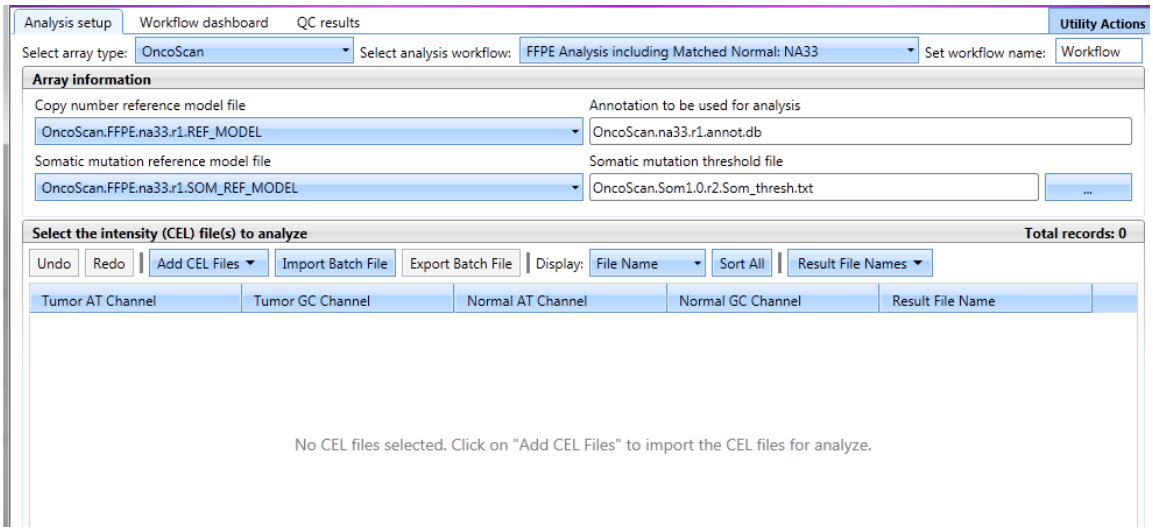


Figure 57 Matched Normal Analysis Setup window/tab - Overview.

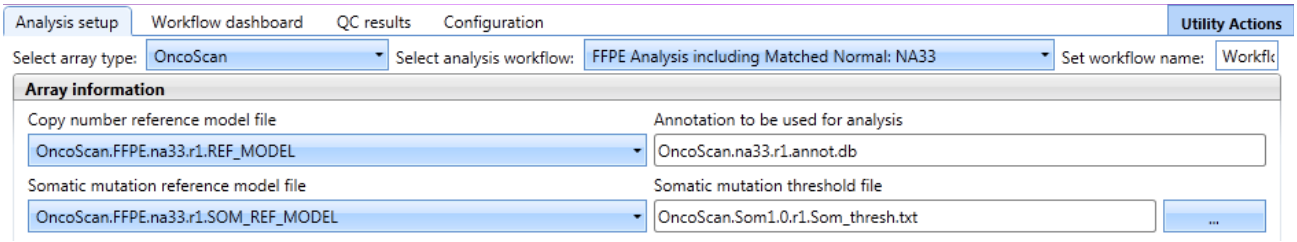


Figure 58 Matched Normal Analysis Configuration.

1. From the **Select array type** dropdown list, select **OncoScan**.

Note: The Select array type dropdown list includes only the array types from the library (analysis) files that have been downloaded from the NetAffx™ Analysis Center or copied from the library package provided in the OncoScan™ installation package.

IMPORTANT! After adding new library files to the library file folder, always close and re-launch OncoScan™ Console to ensure the newly added files are recognized by the software.

---

2. From the **Select analysis workflow** dropdown list, click to select **FFPE Analysis including Matched Normal NAXX**.

   Other available Analysis Workflow options are:

   - **Control DNA Analysis NAXX**: Use this workflow for the Control DNA in the OncoScan Kit.
   - **Non-FFPE Analysis NAXX**: Use this workflow with cell line DNA.
   - **FFPE Analysis NAXX**: Use this workflow for a standard analysis.

3. Enter a Workflow name (optional). By default, the Set workflow name is **Workflow**. Click **Workflow** (upper right) to enter a different workflow name.

   Note: Customizing a Workflow name can be a useful tool in keeping track of analysis workflows as all the related output files (outside of the OSCHP file) are pre-fixed with this workflow name.

   The Annotation file is automatically selected for you and is based on your selected reference model file. (Example: **OncoScan.na33.v1.annot.db**)

   Note: The Annotation to be used for analysis field is auto-populated based on your Ref Model file selection. The analysis is not be permitted to run if the appropriate annotation file is not available in your Library folder.

4. Select a Somatic mutation reference model file. By default, it is set to **OncoScan.na33.v1.SOM_REF_MODEL**. If you created your own reference model file, click the dropdown list to select your .SOM_REF_MODEL.
5. Confirm that the displayed Somatic mutation threshold file to be used is correct. If you need to change it, click the **Browse** button, navigate to the appropriate threshold TXT file, then click **OK**.

   IMPORTANT! If the Reference Model File and Somatic mutation Reference Model File were created independently of each other, a warning message appears after you click Submit (to start the Workflow Analysis process). Click OK to acknowledge the message.

Add CEL files to analyze

You can manually add CEL files or import them as a tab-delimited text file.

Manually add CEL files to analyze

1. At the **Select the intensity (CEL) file(s) to analyze** pane, click the **Add CEL files** dropdown list.
2. Click **Tumor AT Channel**.

   The CEL file window appears. (Figure 59)

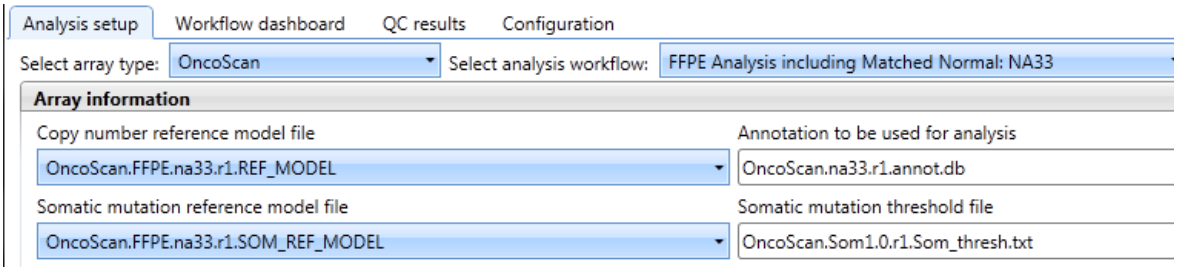


Figure 59 CEL file folder example.

3. Click any header to sort your files or click the **Files of type** dropdown to filter your CEL files by AT Channel, as shown in Figure 60.

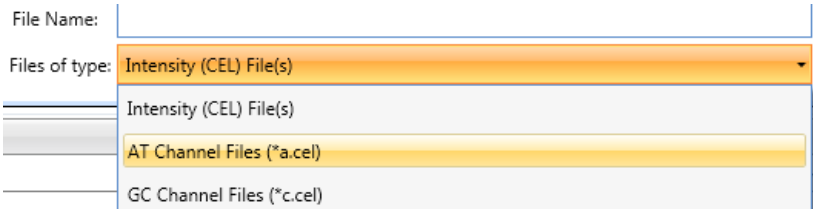


Figure 60 Files of type dropdown list.

4. Click, Ctrl+click, or Shift+click to select multiple Tumor AT Channel files.

IMPORTANT! It is recommended to use an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "_AS_05A.CEL" is an AT Channel file, while "_AS_05C.CEL" is a GC Channel file. See Figure 59.

5. Click **Open**.

   The Tumor AT Channel fields are now populated. (Figure 61)

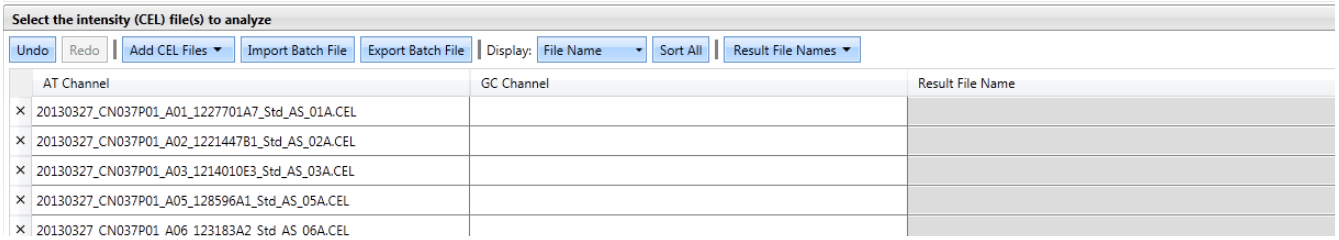


Figure 61 Tumor AT Channel file list.

6. Click the **Add CEL files** dropdown list.
7. Click **Tumor GC Channel**. The CEL file window appears. (Figure 59 on page 75)
8. Click, Ctrl+click, or Shift+click to select multiple Tumor GC Channel files.
9. Click **Open**.

   The Tumor GC Channel fields are now populated. (Figure 62)

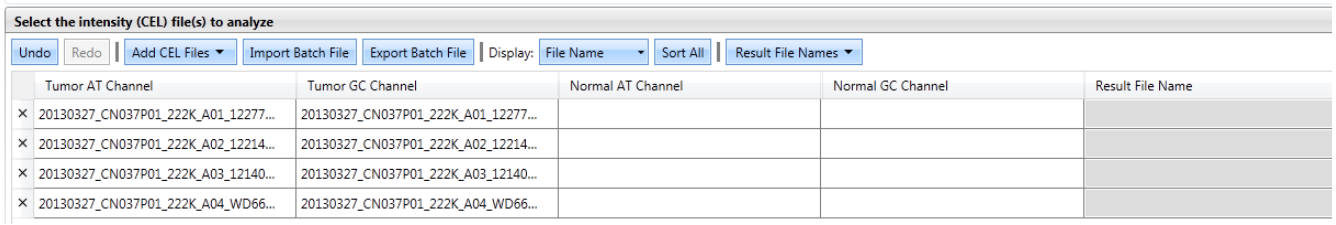


Figure 62 Tumor GC Channel file list.

10. Click the **Add CEL files** dropdown list.
11. Click **Normal AT Channel**. The CEL file window appears. (Figure 59)
12. Click, Ctrl+click, or Shift+click to select multiple Normal AT Channel files.
13. Click **Open**.

    The Normal AT Channel fields are now populated. (Figure 63)

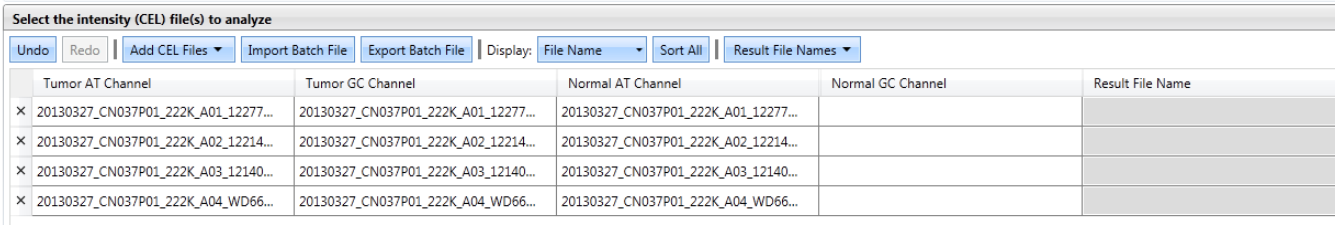


Figure 63 Normal AT Channel file list.

14. Click the **Add CEL files** dropdown list.
15. Click **Normal GC Channel**. The CEL file window appears. (Figure 59)
16. Click, Ctrl+click, or Shift+click to select multiple Normal GC Channel files.
17. Click **Open**.

    The Normal GC Channel fields are now populated. (Figure 64)

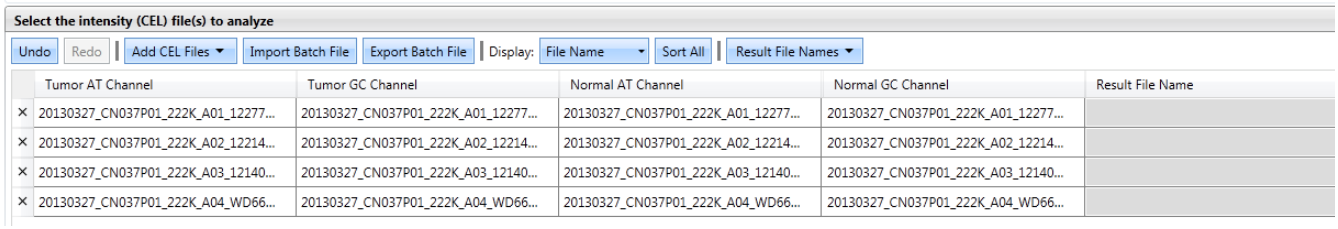


Figure 64 Normal GC Channel file list.

CEL file display options (optional)

The **File Name** dropdown list (Figure 65) is dynamically populated and based on what attributes are populated in the ARR file.

To use this display option, you must:

1. Provide the appropriate attributes at the time of sample registration in AGCC.
2. The ARR files must reside in the same folder as the CEL files.

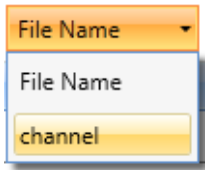


Figure 65 Example: File Name Display Choices.

To see "channel" as an option in the dropdown list, you must use a template or the OncoScan™ template provided in the AGCC library files that contains a "channel" attribute. The resulting ARR file must also reside in the same folder as the CEL files you are analyzing.

You can display one of the attributes from the ARR file in the table. For example, "Channel" can be chosen (Figure 65) to confirm that the assignment of a CEL file to its appropriate channel.

Select a File Name display attribute

1. Click the **File Name** dropdown list, then click to select the attribute you want displayed along with your CEL file names.

   The 2 examples (Figure 66 and Figure 67) show how the table appears with the display set to Filename, then to Channel.

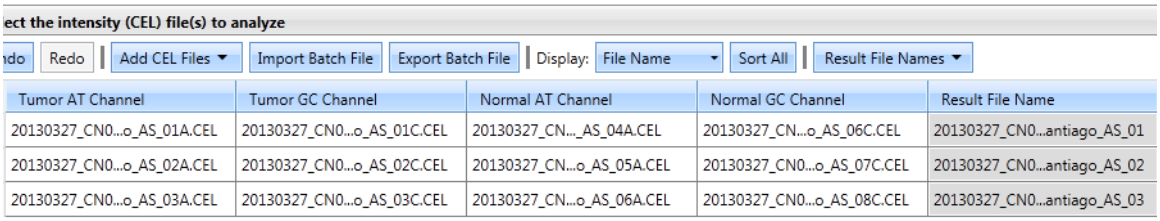


Figure 66 Table with Filename displayed.

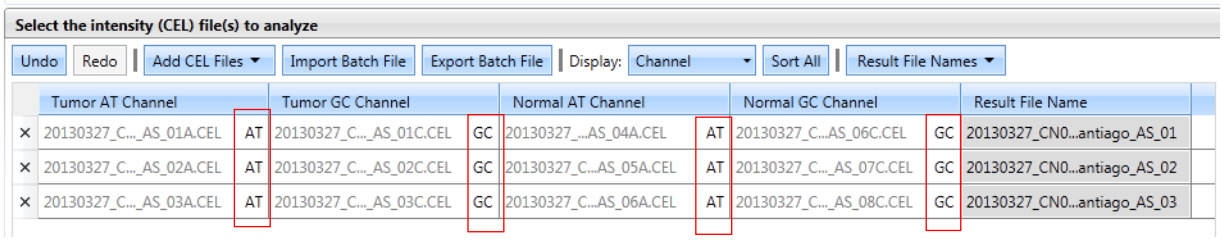


Figure 67 Table with Channel displayed.

Import CEL files using batch import

The batch file must be saved as a text (tab-delimited) format and include the full directory path for your CEL files, as shown in Figure 68.

Note: The resulting OSCHP files are saved to your output path location, therefore it is not necessary to include a path under RESULT. Simply enter the desired results filename in this column.

The format for this tab-delimited file is 5 columns (A, B, C, D, and E) with the headers:

- ATCHANNELCEL
- GCCHANNELCEL
- ATChannelMatchedNormalCel
- GCChannelMatchedNormalCel
- RESULT

You must provide the full path to the CEL files for each Channel column.
(Example: **C:\Desktop\OncoScan\Data\Sample1.cel**)

	A	B	C	D	E
1	ATCHANNELCEL	GCCHANNELCEL	ATChannelMatchedNormalCel	GCChannelMatchedNormalCel	RESULT
2	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	20130327_CN037P01_222K_A0
3	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	20130327_CN037P01_222K_A0
4	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	L:\OncoScan\AnalysisWorkflow\Onco	20130327_CN037P01_222K_A0

Figure 68 List from an Excel™ spreadsheet.

1. Click **Import Batch File**

   A File window appears.

2. Navigate to your text (tab delimited) file location, then click the file you want to import.

   IMPORTANT! The Excel™ spreadsheet must be closed before you import.

3. Click **Open**.

   The Tumor AT, Tumor GC, Normal AT, Normal GC and Result File Name fields are now populated. (Figure 69)

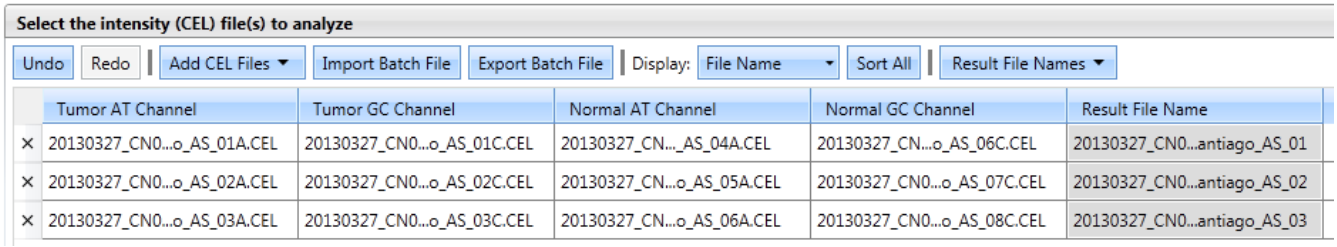


Figure 69 Tab-delimited text file imported into OncoScan™ Console.

Reference files

This section explains how to create a reference file which is required to perform single sample analysis in ChAS. The software analyzes a sample file by comparing it to a reference file. You can use the reference file provided with ChAS, or you can create a reference file using your own sample data.

See Figure 70 for an overview of the analyses involved in creating a reference file for the CytoScan™ arrays.

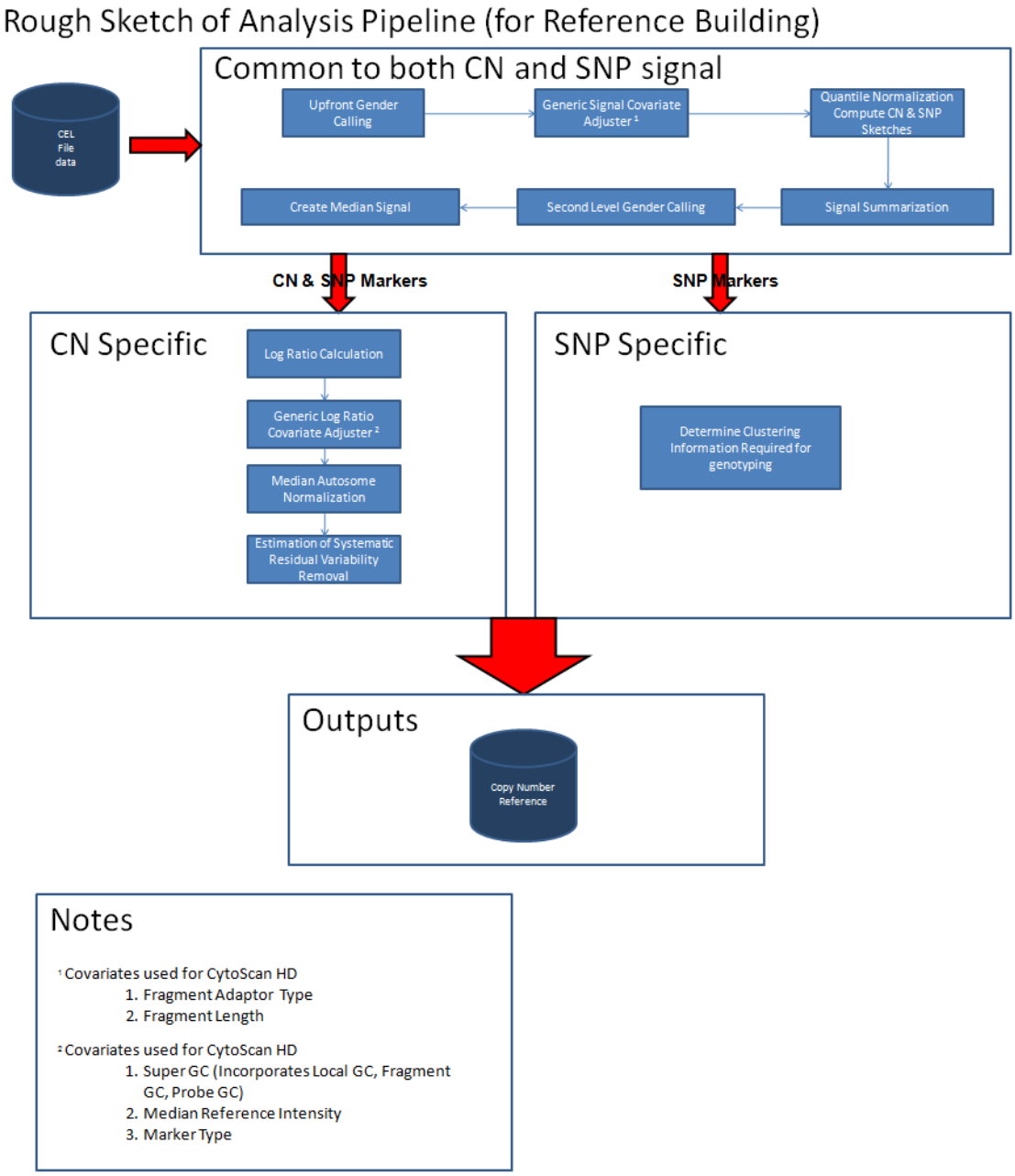


Figure 70 Overview of creating a reference file.

Create a reference file

IMPORTANT! When creating a CytoScan reference file, it is recommended that you use a minimum of 44 CEL files. These CEL files must include at least 20 male and 20 female samples.

1. From the Analysis menu, select **Perform Analysis Setup.** (Figure 71)

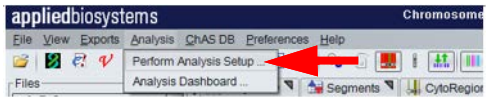


Figure 71 Analysis dropdown list.

The Analysis Workflow window tab opens. (Figure 72)

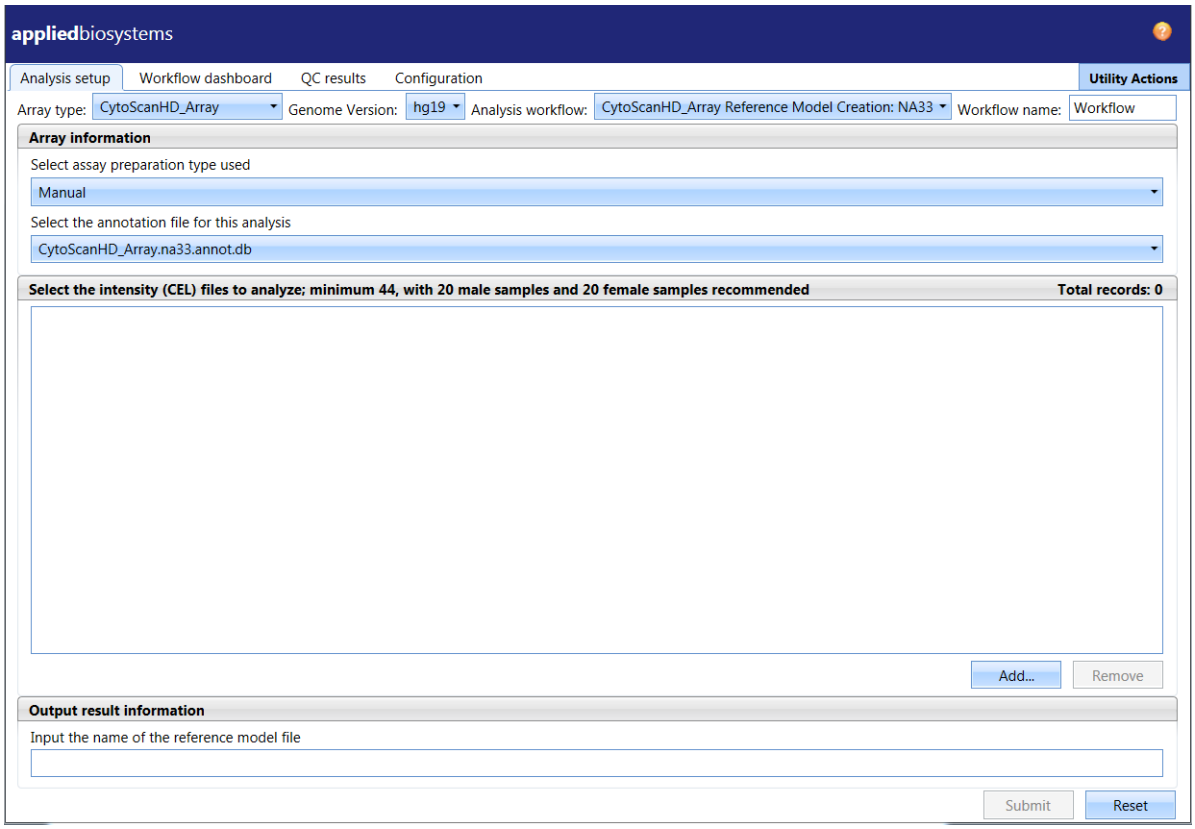


Figure 72 Analysis Workflow.

2. From the **Select array type** dropdown list, click to select an array type (Example: CytoScanHD_Array.

Note: The Select array type dropdown list includes only the array types for which library (analysis) files have been downloaded from the NetAffx™ Analysis Center or copied from the library package provided with the installation.

3. Select a Genome Build. (Example: hg19)
4. From the **Select analysis workflow** dropdown list, click to select an analysis workflow. (Example: CytoScanHD_Array Reference Model Creation:NA33)
5. By default, the Set workflow name is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.

6. Click the **Assay Preparation type used** dropdown list, then select one of the following:
   - **Manual** (CEL files whose assays were performed by hand)
   - **Automation** (CEL files whose assays were assisted by a robot)
   - **Automation, Manual** (a mix of CEL files whose assays were performed either by hand or with the assistance of a robot.

   Note: The Annotation file for analysis is auto-selected after the array type, analysis workflow and assay preparation type used fields are selected.

7. From the **Select the annotation file for this analysis** dropdown list, verify the selection of the annotation file. (Example, CytoScanHD_Array.na.33.annot.db)
8. At the **Select the intensity (CEL) file(s) to analyze** pane, click **Add.**

   IMPORTANT! The same annotation file you used to create a Reference Model File MUST also be used with future Single Sample Analyses runs that utilize your created Reference Model File.

   The following window appears: (Figure 73)

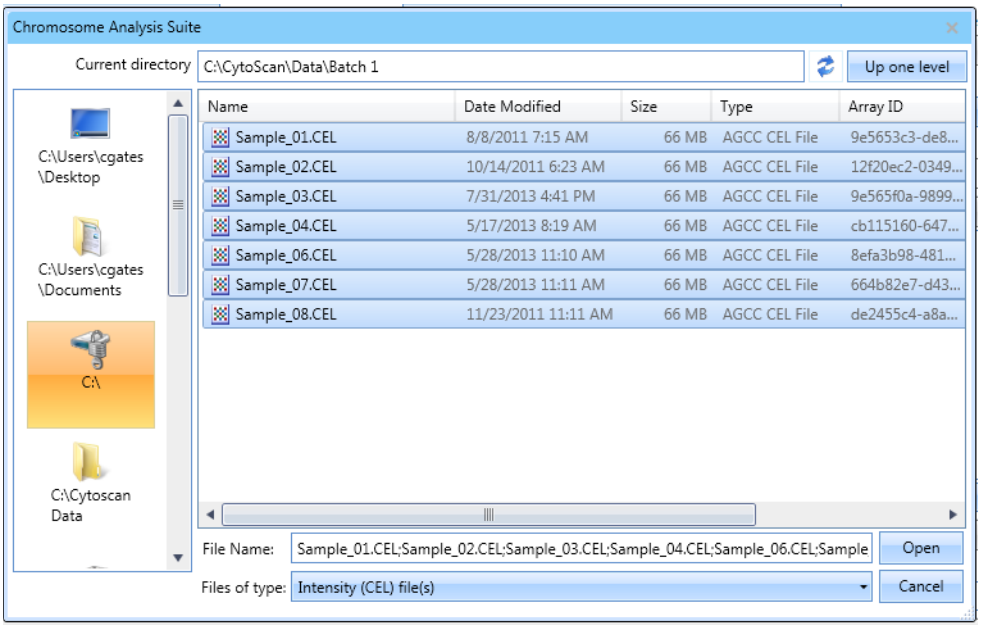


Figure 73 >CEL file folder.

9. Click, Ctrl+click, or Shift+click to select multiple CEL files, then click **Open**.

   The **Select the intensity (CEL) file(s) to analyze** pane is now populated with your CEL files. (Figure 74)

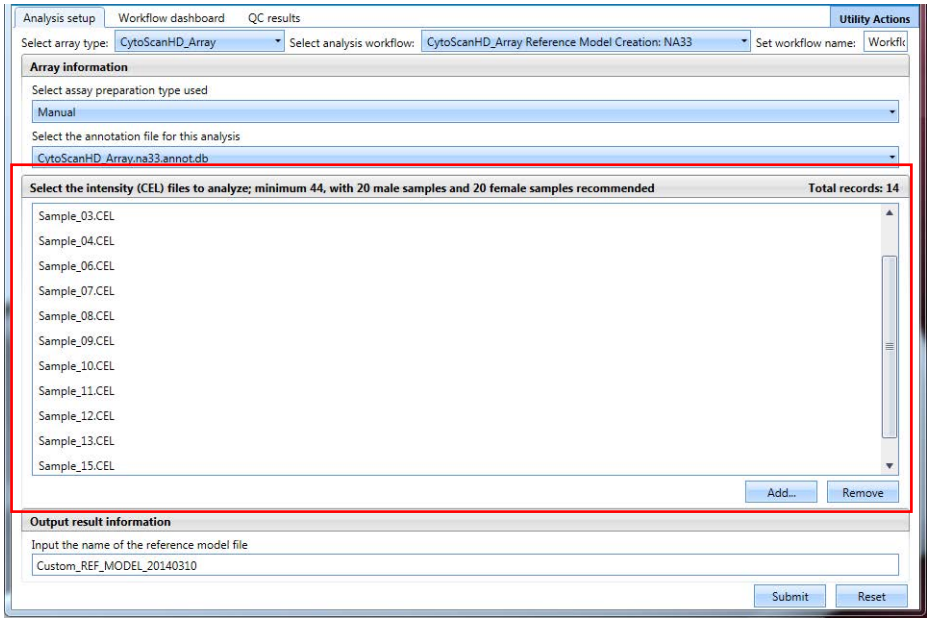


Figure 74 Select the intensity (CEL) file(s) to analyze pane.

To remove a CEL file from this list, click to highlight it, then click **Remove**.

10. At the **Output result information** pane, enter a name for your reference model file. (Figure 75)

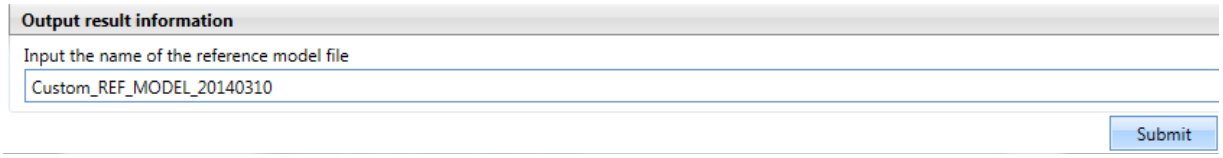


Figure 75 Name your Reference Model File name.

11. Click **Submit**.

5 Analysis workflow exports and QC tools

Display and export data from the analysis workflow

Add files to the QC results table

1. Click the **QC results** tab. (Figure 76)

   The QC results window tab appears.

2. Click **Add Files**.

   A Chromosome Analysis Suite window appears.

3. Navigate to your folder's location, then select the xxCHP files you want to add.

4. Click **Open**.

   Your selected files appear in the Export QC Table window tab, as shown in Figure 76.

Export QC table information

1. Select the adjacent checkbox next to the file(s) you want to export or click the **Select All** icon (atop the checkboxes) to auto-check all the displayed files.

2. Click the **Export QC Table** tab.

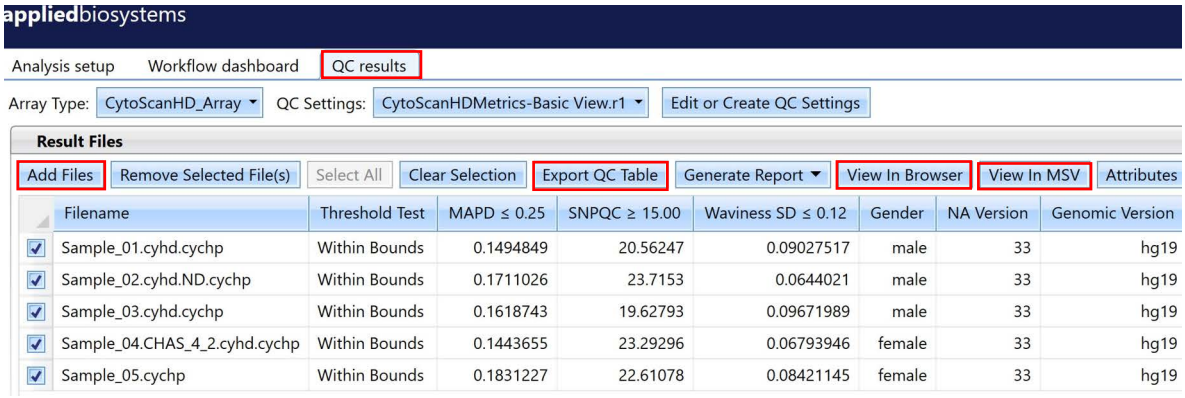


Figure 76 Workflow Dashboard loaded.

   A File Explorer window appears.

3. Navigate to the export location you want, then enter a name for your QC file.

4. Click **Save**.

View analysis files in the ChAS Browser

1. Check the adjacent box next to the file(s) you want to open in the ChAS Browser.
2. Click the **View in Browser** button to open the files in the ChAS Browser or click the **View in MSV** button to open the files in the Multi-Sample Viewer. For more information on the MSV see, the *Reproductive Health Research Analysis Software User Guide* (Pub. No. MAN0027973).

Export probe level data

Export CytoScan™ probe level data

1. In the **QC Results** tab, select the checkbox adjacent to the Results File(s) you want to generate a report for or click the **Select All** icon (atop the checkboxes), as shown in Figure 77.
2. To export probe-level data, click the **Generate Report** dropdown list. (Figure 77)

   The following export reporting options appear: (Figure 77)

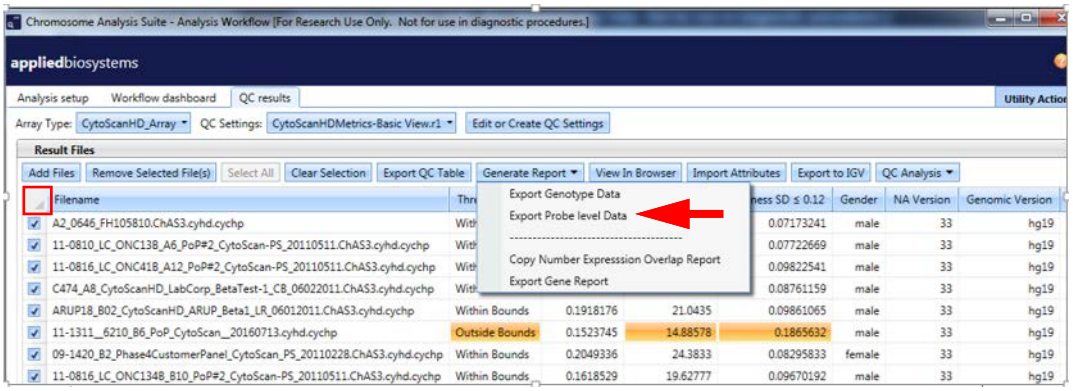


Figure 77 Generate Report dropdown list.

3. Click to select **Export Probe Level Data**. (Figure 77)

   Your previously assigned Output folder file window appears.

   Note: The default root filename is Result. Click inside the File Name field to enter a different root filename.

4. Enter a File Name for your text (tab-delimited) reporting file, then click **Save**, or navigate to different save location.

Export OncoScan™ probe level data

1. In the **QC Results** tab, select each checkbox next to the Results File(s) you want to generate a report for. Click the **Select All** icon (atop of the checkboxes) to auto-check all the displayed files.
2. To export probe-level data, click the **Generate Report** dropdown list.

The following export reporting options appear: (Figure 78)

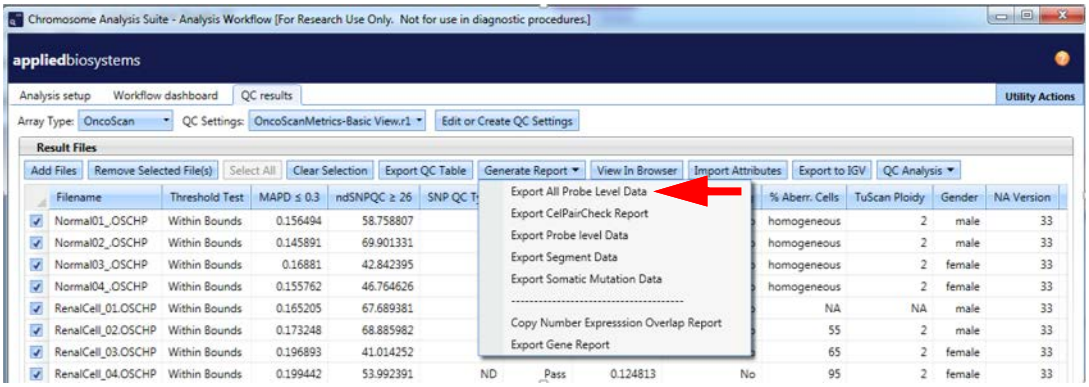


Figure 78 Generate Report dropdown list.

3. If you want to export all 4 available reports at one time, click to select **Export All Probe Level Data**. Otherwise, click to select the specific report(s) you want export.

   The appropriate (previously assigned) folder file window appears.

   Note: The default root filename is Result. Click inside the File Name field to enter a different root filename.

4. Enter a File Name for your reporting file, then click **Save** or navigate to a different save location.

Export a gene report (CytoScan™ or OncoScan™ arrays)

This report summarizes the copy number segments that overlap user defined regions of interest (for example, Genes) as defined in the selected BED file.

1. From the **QC Results** tab, click the **Generate Report** dropdown list and select **Export Gene Report**. (Figure 79)

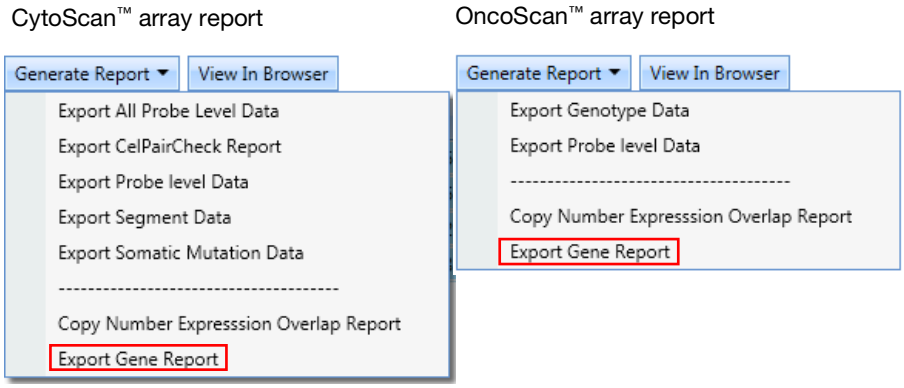


Figure 79 Generate Report dropdown list.

The following window appears. (Figure 80)

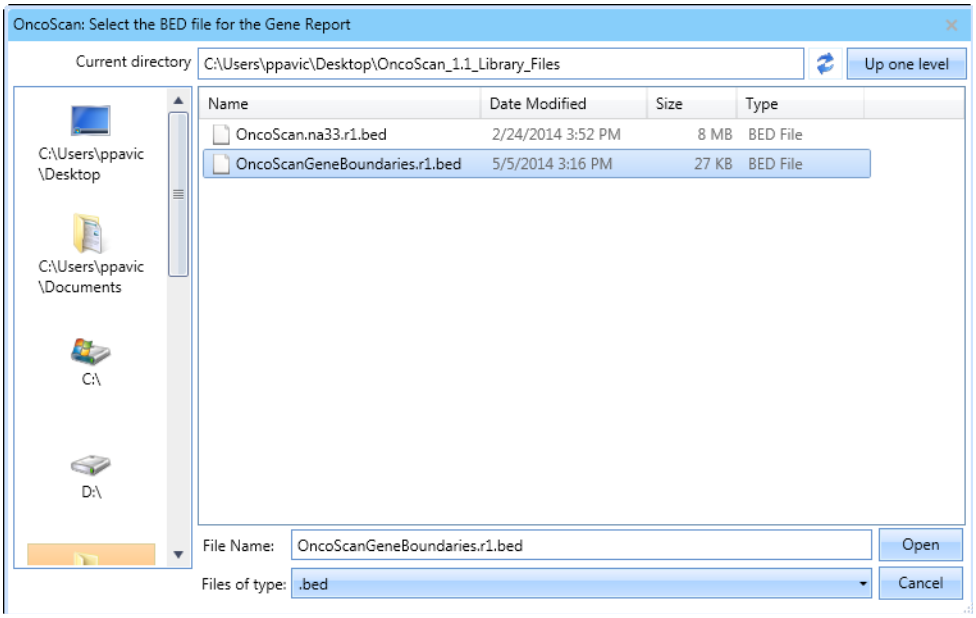


Figure 80 Select the BED file for the Gene Report window.

2. Click to select the appropriate BED file, then click **Open**.

Note: Any BED file can be used to generate the Gene Report on any regions of interest contained within the BED file.

Your previously assigned Output folder file window appears. (Figure 81)

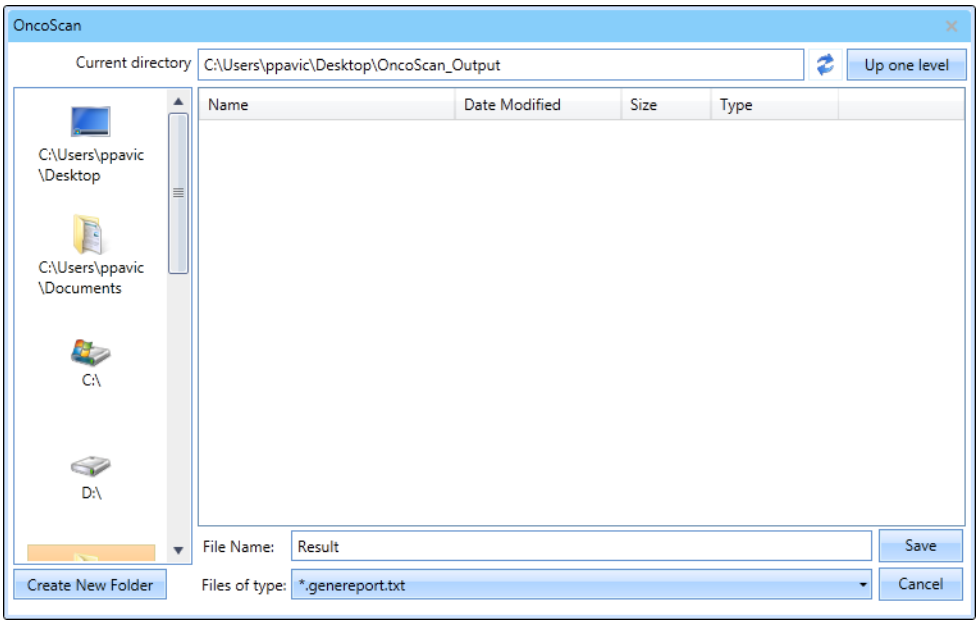


Figure 81 OncoScan Output folder window.

3. The default root filename is Result. Click inside the File Name field to enter a different root filename, then click **Save**.

A progress bar appears while your report generates, followed by a message window. (Figure 82)

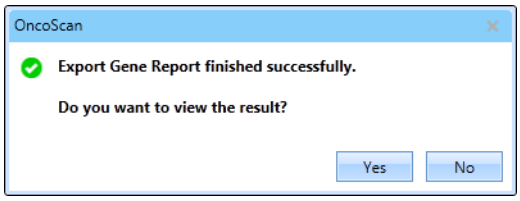


Figure 82 Gene Report finished successfully.

4. Click **Yes**.

   The Results Output folder window appears.

5. Locate the Gene Report text file, then open it in an Excel™ spreadsheet.

   The following window appears. (Figure 83)

Filename	Chromosome	Start Position	End Position	Genes	Threshold Test	% Aberr. Cells	TuScan Ploidy	Low Diploid Flag	Median Log2 Ratio	Median BAF	State	LOH
Normal02	1	59236462	59259785	JUN	Within Bounds	homogeneous	2	No	0.007	0.484	2	-
Normal02	1	156020966	156050295	RAB25	Within Bounds	homogeneous	2	No	0.011	0.485	2	-
Normal02	2	16070682	16097129	MYCN	Within Bounds	homogeneous	2	No	0.013	0.486	2	-
Normal02	2	61098751	61160178	REL	Within Bounds	homogeneous	2	No	0.013	0.486	2	-
Normal02	2	99051320	99208284	INPP4A	Within Bounds	homogeneous	2	No	0.011	0.485	2	-
Normal02	3	10173318	10205354	VHL	Within Bounds	homogeneous	2	No	0.009	0.486	2	-
Normal02	3	69778585	70027488	MITF	Within Bounds	homogeneous	2	No	0.009	0.486	2	-
Normal02	7	55076724	55234644	EGFR	Within Bounds	homogeneous	2	No	0.009	0.484	2	-
Normal02	7	116302458	116448440	MET	Within Bounds	homogeneous	2	No	0.009	0.484	2	-
Normal02	8	128738314	128763680	MYC	Within Bounds	homogeneous	2	No	-0.177	NaN	1.5	-
Normal02	9	21957750	21984826	CDKN2A	Within Bounds	homogeneous	2	No	0.006	0.485	2	-
Normal02	9	21992901	22019312	CDKN2B	Within Bounds	homogeneous	2	No	0.006	0.485	2	-
Normal02	10	89613194	89738532	PTEN	Within Bounds	homogeneous	2	No	-0.002	0.486	2	-
Normal02	11	69445872	69479242	CCND1	Within Bounds	homogeneous	2	No	0.002,-0.432	0.485,NaN	2,1	-
Normal02	12	58131509	58156230	CDK4	Within Bounds	homogeneous	2	No	-0.003	0.485	2	-
Normal02	12	69191970	69249212	MDM2	Within Bounds	homogeneous	2	No	-0.003	0.485	2	-
Normal02	13	32879616	32983809	BRCA2	Within Bounds	homogeneous	2	No	0.016	0.485	2	-
Normal02	13	48867882	49066026	RB1	Within Bounds	homogeneous	2	No	0.016	0.485	2	-
Normal02	17	7561719	7588811	TP53	Within Bounds	homogeneous	2	No	-0.016	0.486	2	-
Normal02	17	37834392	37894915	ERBB2	Within Bounds	homogeneous	2	No	0.021	0.488	2	-
Normal02	17	41186311	41286132	BRCA1	Within Bounds	homogeneous	2	No	-0.097	0.488	1.5	-
Normal02	17	48702217	48755288	ABCC3	Within Bounds	homogeneous	2	No	-0.027	0.485	2	-
Normal02	19	1195797	1238434	STK11	Within Bounds	homogeneous	2	No	0.407	0.484	2.5	-
Normal02	19	40726223	40801302	AKT2	Within Bounds	homogeneous	2	No	-0.046	0.486	2	-
Normal02	X	66753873	66960461	AR	Within Bounds	homogeneous	2	No	-0.851	NaN	1	LOH
Normal02	1	3559128	3662765	TP73	Within Bounds	homogeneous	2	No	0.328,-0.038	0.487,0.485	2.5,2	-
Normal02	1	3763844	3811993	DFFB	Within Bounds	homogeneous	2	No	-0.038	0.485	2	-
Normal02	1	9701789	9799172	PIK3CD	Within Bounds	homogeneous	2	No	-0.038	0.485	2	-
Normal02	1	11156587	11332608	MTOR	Within Bounds	homogeneous	2	No	-0.038	0.485	2	-

Result.genereport

Ready 100%

Figure 83 Gene Report.

Table 11 Gene Report table columns.

Column name	Description
Filename	Name of the xxCHP file containing the data.
Chromosome	Chromosome on which the probeset is located.
Start Position	Start position of gene or region as defined in the bed file.
End Position	End position of gene or region as defined in the bed file.
Genes	This column is populated from the name column of the bed file. In most cases, it contains gene names.
Threshold Test	Displays Outside Bounds if any of the QC metrics fail to meet a threshold test. For more information on thresholds, see "Create your own custom QC setting" on page 54.
% Aberr.Cells (OSCHP only)	If % AC = 100%, we return “homogeneous” because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.
TuScan Ploidy (OSCHP bnly)	TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred. TuScan Ploidy is assigned the median CN state of all markers, provided that %AC could be determined and integer copy numbers are returned. If %AC cannot be determined, NA (Not Available) is reported for both ploidy and %AC.
Low Diploid Flag (OSCHP only)	An essential part of the algorithm is the identification of “normal diploid” markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that “normal diploid markers” result in a log2 ratio of 0 (for example, copy number 2). The algorithm might later determine that the “normal diploid” markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy reports 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of “normal diploid” markers and no “normal diploid calibration occurs. This event triggers “low diploid flag” = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.
Median Log2 Ratio (OSCHP only)	Log2 Ratio is the log2 ratio of the normalized intensity of the sample over the normalized intensity of a reference with further correction for sample specific variation. The Median Log2 Ratio is computed for each segment.
Median BAF (OSCHP only)	B-allele frequency (BAF) is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. Median BAF is reported for each segment and is the median BAF of the markers identified as heterozygous, after mirroring any marker BAFs above 0.5 to the equivalent value below 0.5. If the number of heterozygous markers in the segment is below 10 or the percent of homozygous markers is above 85% no value is reported,
State	This is a comma separated list of the copy number state of the segments that overlap the gene or region.
LOH	Flag to indicate whether the gene or region is in a Loss of Heterozygosity region (0=No, 1=Yes).

Export an XON region report (CytoScan™ XON only)

Note: This export provides XON Region gains/loses for a defined list of genes.

1. From the QC Results tab, select the checkbox for the sample file(s) for which you would like to generate an XON Region report.
2. Click the Generate Report dropdown list and select Export XON Region Report.
3. In the browse window, click to select the appropriate aed/bed file, then click **Open**.
4. Click the **Browse** button to assign an output path.

   Note: The default root filename is **Result**.

5. Optional: Click inside the Filename field to enter a difference root filename, then click **Save**.
6. Click the appropriate option (for each XNCHP file) to either export all data into a single text file or into individual text files.
7. Locate the XON Region Report text file, then open it in an Excel™ spreadsheet.

Table 12 XON Region Report columns.

Column name	Description
Filename	Name of the XNCHP file containing the data
Gene	This column is populated from the name column of the bed file. In most cases, it contains gene names.
Chromosome	Chromosome on which the probeset is located.
Gene Start Position	Start position of the gene or region as defined in the bed file.
Gene End Position	End position of gene or region as defined in the bed file.
XON Region Start Position	Start position of the Exon Region segment as defined in the XNCHP file.
XON Region Stop Position	End position of the Exon Region segment as defined in the XNCHP file.
XON Region Type	Gain or Loss
Size (bp)	Size of the Exon Region Segment.
Marker Count	Number of markers in the Exon Region Segment.

Export a copy number expression overlap report

This report summarizes the copy number segments with the fold change from expression data that overlap user defined regions of interest (for example, Genes) as defined in the selected AED or BED file.

1. In the **QC Results** tab, click the **Generate Report** dropdown list, then select **Copy Number Expression Overlap Report**. (Figure 84)

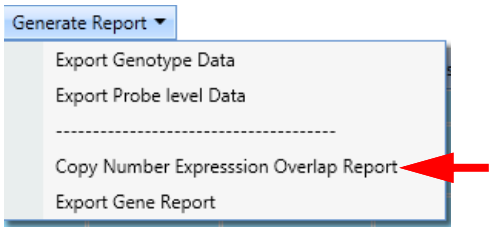


Figure 84 Generate Report dropdown list.

The following window appears: (Figure 85)

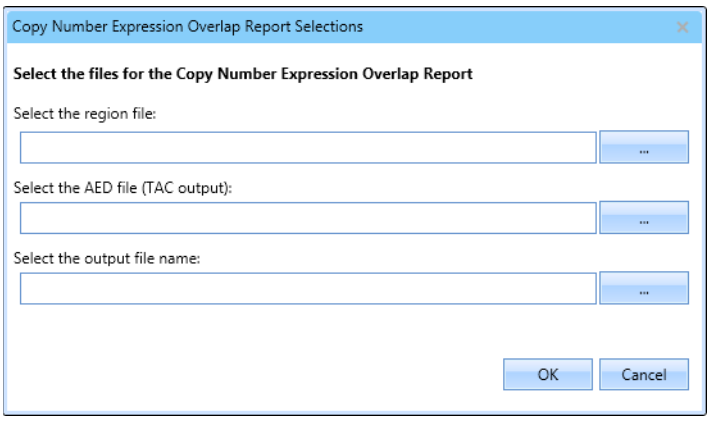


Figure 85 Select files for the Overlap Report window.

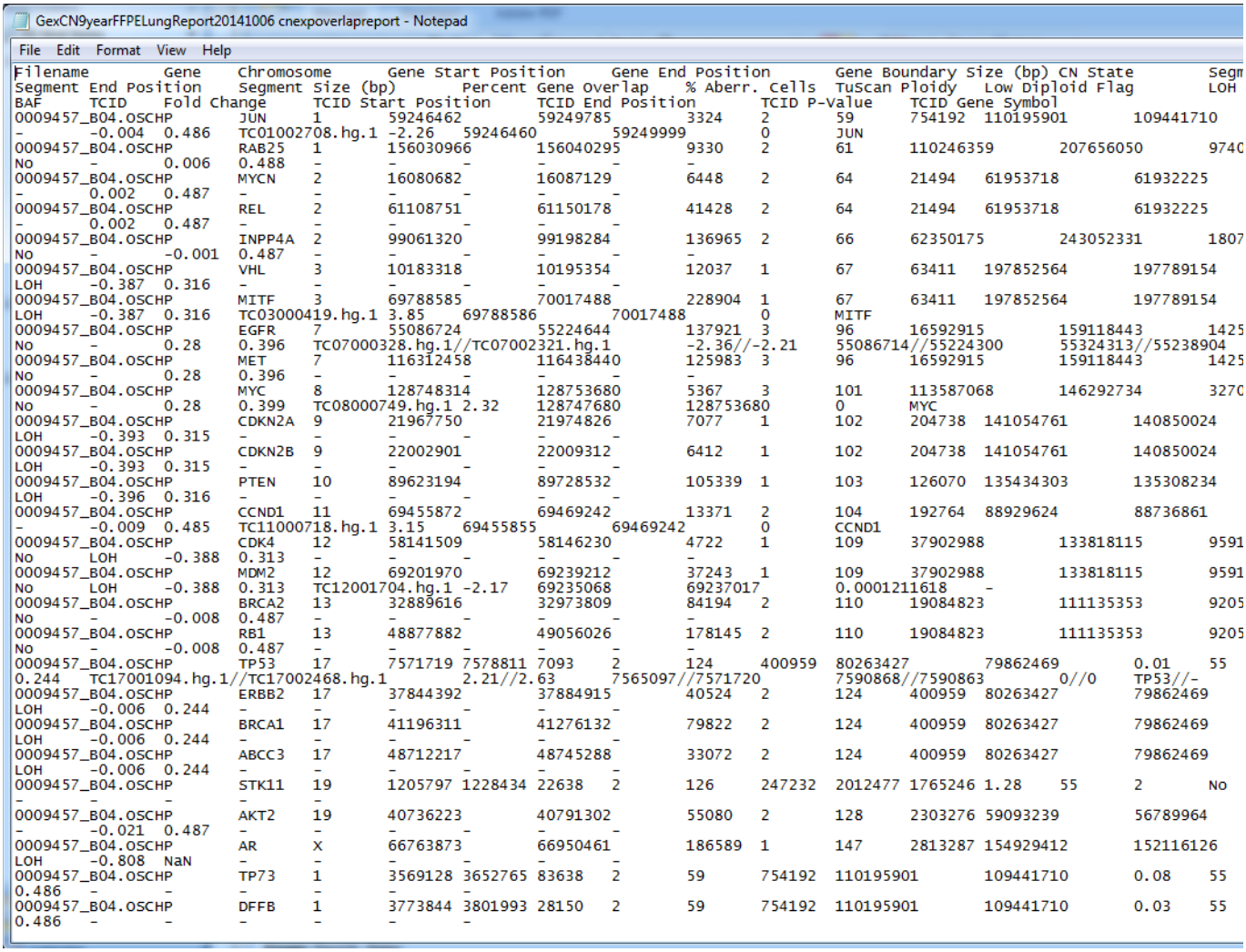
2. Click the Select the region file **Browse** button to navigate to and select the appropriate file.

Note: For the Regions file, you can use the default bed files provided in the in the library files for use with the Copy Number Expression Overlap report. You may also create your own AED/BED file containing your custom regions of interest.

3. Click the Select the AED file (TAC output) **Browse** button to navigate to and select the appropriate file. See the *Transcriptome Analysis Console (TAC) 4.0 User Manual* for analyzing and exporting expression data as an AED file.
4. Click the Select output file name **Browse** button to navigate to an existing report location, or click inside the text field to enter a different root filename (other than the default Results filename), then click **OK**.

   A progress bar appears while your report generates.
5. Locate the **cnexoverlapreport.txt** file, then double-click to open. It is recommended to open the tab-delimited file an Excel™ spreadsheet for easier viewing.

The following window appears. (Figure 86)



GexCN9yearFFPELungReport20141006 cnexpoverlapreport - Notepad

File Edit Format View Help

Filename	Gene	Chromosome	Gene Start Position	Gene End Position	Gene Boundary Size (bp)	CN State	Segm
Segment End Position	Segment Size (bp)	Percent Gene Overlap	% Aberr. Cells	TuScan Ploidy	Low Diploid Flag		LOH
BAF	TCID	Fold Change	TCID Start Position	TCID End Position	TCID P-Value	TCID Gene Symbol	
0009457_B04.OSCHP	JUN	1	59246462	59249785	3324	2	59
754192	110195901	109441710					
-	-0.004	0.486	TC01002708.hg.1	-2.26	59246460	59249999	0
JUN							
0009457_B04.OSCHP	RAB25	1	156030966	156040295	9330	2	61
110246359	207656050	9740					
No	-	0.006	0.488	-	-	-	-
0009457_B04.OSCHP	MYCN	2	16080682	16087129	6448	2	64
21494	61953718	61932225					
-	0.002	0.487	-	-	-	-	-
0009457_B04.OSCHP	REL	2	61108751	61150178	41428	2	64
21494	61953718	61932225					
-	0.002	0.487	-	-	-	-	-
0009457_B04.OSCHP	INPP4A	2	99061320	99198284	136965	2	66
62350175	243052331	1807					
No	-	-0.001	0.487	-	-	-	-
0009457_B04.OSCHP	VHL	3	10183318	10195354	12037	1	67
63411	197852564	197789154					
LOH	-0.387	0.316	-	-	-	-	-
0009457_B04.OSCHP	MITF	3	69788585	70017488	228904	1	67
63411	197852564	197789154					
LOH	-0.387	0.316	TC03000419.hg.1	3.85	69788586	70017488	0
MITF							
0009457_B04.OSCHP	EGFR	7	55086724	55224644	137921	3	96
16592915	159118443	1425					
No	-	0.28	0.396	TC07000328.hg.1//TC07002321.hg.1		-2.36//-2.21	55086714//55224300
55324313//55238904							
0009457_B04.OSCHP	MET	7	116312458	116438440	125983	3	96
16592915	159118443	1425					
No	-	0.28	0.396	-	-	-	-
0009457_B04.OSCHP	MYC	8	128748314	128753680	5367	3	101
113587068	146292734	3270					
No	-	0.28	0.399	TC08000749.hg.1	2.32	128747680	128753680
0	MYC						
0009457_B04.OSCHP	CDKN2A	9	21967750	21974826	7077	1	102
204738	141054761	140850024					
LOH	-0.393	0.315	-	-	-	-	-
0009457_B04.OSCHP	CDKN2B	9	22002901	22009312	6412	1	102
204738	141054761	140850024					
LOH	-0.393	0.315	-	-	-	-	-
0009457_B04.OSCHP	PTEN	10	89623194	89728532	105339	1	103
126070	135434303	135308234					
LOH	-0.396	0.316	-	-	-	-	-
0009457_B04.OSCHP	CCND1	11	69455872	69469242	13371	2	104
192764	88929624	88736861					
-	-0.009	0.485	TC11000718.hg.1	3.15	69455855	69469242	0
CCND1							
0009457_B04.OSCHP	CDK4	12	58141509	58146230	4722	1	109
37902988	133818115	9591					
No	LOH	-0.388	0.313	-	-	-	-
0009457_B04.OSCHP	MDM2	12	69201970	69239212	37243	1	109
37902988	133818115	9591					
No	LOH	-0.388	0.313	TC12001704.hg.1	-2.17	69235068	69237017
0.0001211618	-						
0009457_B04.OSCHP	BRCA2	13	32889616	32973809	84194	2	110
19084823	111135353	9205					
No	-	-0.008	0.487	-	-	-	-
0009457_B04.OSCHP	RB1	13	48877882	49056026	178145	2	110
19084823	111135353	9205					
No	-	-0.008	0.487	-	-	-	-
0009457_B04.OSCHP	TP53	17	7571719	7578811	7093	2	124
400959	80263427	79862469	0.01	55			
0.244	TC17001094.hg.1//TC17002468.hg.1		2.21//2.63	7565097//7571720		7590868//7590863	0//0
TP53//-							
0009457_B04.OSCHP	ERBB2	17	37844392	37884915	40524	2	124
400959	80263427	79862469					
LOH	-0.006	0.244	-	-	-	-	-
0009457_B04.OSCHP	BRCA1	17	41196311	41276132	79822	2	124
400959	80263427	79862469					
LOH	-0.006	0.244	-	-	-	-	-
0009457_B04.OSCHP	ABCC3	17	48712217	48745288	33072	2	124
400959	80263427	79862469					
LOH	-0.006	0.244	-	-	-	-	-
0009457_B04.OSCHP	STK11	19	1205797	1228434	22638	2	126
247232	2012477	1765246	1.28	55	2	No	
-	-	-	-				
0009457_B04.OSCHP	AKT2	19	40736223	40791302	55080	2	128
2303276	59093239	56789964					
-	-0.021	0.487	-	-	-	-	-
0009457_B04.OSCHP	AR	X	66763873	66950461	186589	1	147
2813287	154929412	152116126					
LOH	-0.808	NaN	-	-	-	-	-
0009457_B04.OSCHP	TP73	1	3569128	3652765	83638	2	59
754192	110195901	109441710	0.08	55			
0.486	-	-	-	-	-		
0009457_B04.OSCHP	DFFB	1	3773844	3801993	28150	2	59
754192	110195901	109441710	0.03	55			
0.486	-	-	-	-	-		

Figure 86 Expression Gene Report.

Table 13 Expression Gene Report columns.

Column name	Description
Filename	Name of the OSCHP file containing the data.
Gene	This column is populated from the name column of the bed file. In most cases, it contains gene names.
Chromosome	Chromosome on which the probeset is located.
Gene Start Position	Start position of gene or region as defined in the bed file.
Gene End Position	End position of gene or region as defined in the bed file.
CN State	This is a comma separated list of the copy number state of the segments that overlap the gene or region.

Table 13 Expression Gene Report columns. (Continued)

Column name	Description
Segment ID	The unique identifier for the copy number segment.
Segment Start Position	Start position of the overlapping copy number segment in the xxCHP file.
Segment End Position	End position of the overlapping copy number segment in the xxCHP file.
Segment Size (bp)	The Segment Stop Position minus The Segment Start Position.
Percent Gene Overlap	Gene Boundary Size/Segment Size.
% Aberr.Cells (OncoScan only)	If % AC = 100%, we return “homogeneous” because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.
TuScan Ploidy (OncoScan only)	TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred. Algorithmically it is the CN state of the markers identified by the algorithm as normal diploid before %AC and ploidy are determined. When a high ploidy is determined the "normal diploid" is deemed to correspond to a higher CN and the log2 ratio gets adjusted appropriately. If ploidy cannot be determined NA (Not Available) is reported.
Low Diploid Flag (OncoScan only)	An essential part of the algorithm is the identification of “normal diploid” markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that “normal diploid markers” result in a log2 ratio of 0 (for example, copy number 2). The algorithm might later determine that the “normal diploid” markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy reports 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of “normal diploid” markers and no “normal diploid calibration occurs. This event triggers “low diploid flag” = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.
Median Log2 Ratio (OncoScan only)	Log2 Ratio is the log2 ratio of the normalized intensity of the sample over the normalized intensity of a reference with further correction for sample specific variation. The Median Log2 Ratio is computed for each segment.
Median BAF (OncoScan only)	B-allele frequency (BAF) is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. Median BAF is reported for each segment and is the median BAF of the markers identified as heterozygous, after mirroring any marker BAFs above 0.5 to the equivalent value below 0.5. If the number of heterozygous markers in the segment is below 10 or the percent of homozygous markers is above 85% no value is reported,
LOH	Flag to indicate whether the gene or region is in a Loss of Heterozygosity region (0=No, 1=Yes).
Fold Change	The level of fold change as determined from the TAC software.
TCID	The Transcript Cluster ID overlapping the Gene or Region defined in the bed file.
TCID Start Position	Start Position: Start position of the overlapping TCID(s).
TCID End Position	End Position of the overlapping TCID(s).
TCID P-Value	ANOVA p-value (Condition1 vs Condition2)
TCID GeneSymbol	Gene Symbol: the Gene Symbol assigned to the TCIS(s) based on the TAC analysis.

Export genotype data using the analysis workflow

Note: For exporting genotypes from an entire sample or multiple samples at one time, exporting genotypes from the Analysis Workflow is generally faster than from the ChAS Browser.

1. In the **QC Results** tab, click the **Generate Report** dropdown list, then select **Export Genotyping Data**. (Figure 87)

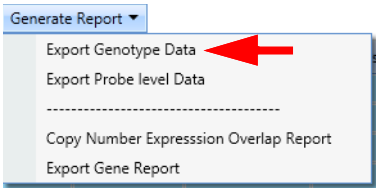


Figure 87 Generate Report dropdown list.

The following window appears: (Figure 88)

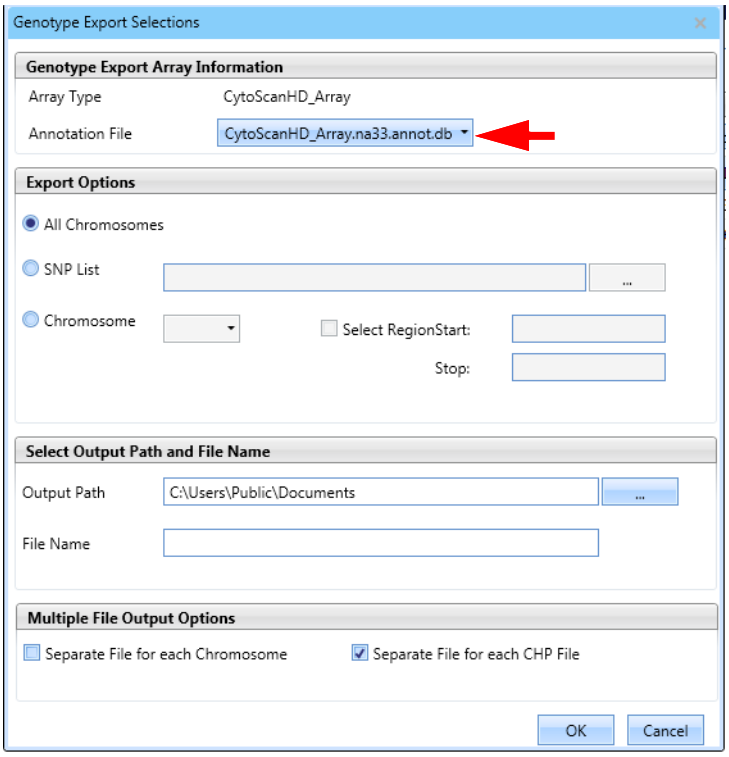


Figure 88 Genotype Export Selections window.

Export options

Note: The default export is All Chromosomes.

Export a SNP List

1. Click the Annotation File dropdown list (Figure 88), then click to select which array annotation file you want to use for exporting SNP information (along with the genotypes).
2. Click the **SNP List** option, then click the SNP List **Browse** button.

   The Select SNP List window appears. An example SNP List can be seen in Figure 380 on page 324.
3. Navigate to, then click to select the SNP List you want to export.
4. Click **Open**.

   Your SNP List is now set for exporting.

Export a specific chromosome

1. Click the **Chromosome** option.
2. Click the **Chromosome** dropdown list, then click to select the specific number or chromosome type you want to export.
3. Optional: Select the **Select Region** checkbox, then enter a **Start** and **Stop** value in the provided text fields.

Select an output path and filename

1. Click the Output Path **Browse** button.

   The **Select Output Path for the Genotype Export Report** appears.
2. Navigate to an existing report location, or click inside the text field to enter a different root filename, then click **OK.**
3. Click inside the File Name text field to enter a report name.

Multiple file output options

1. Select the **Separate File for each Chromosome** and/or **Separate File for each CHP File** checkbox.
2. Click **OK**.

   A P**lease Wait...Exporting Genotype Data** progress bar appears.
3. After a few moments, the **Export Genotype Data finished successfully** window appears. Click **Yes** to view the report, click **No** to view the report later.

   Clicking **Yes** opens your previously assigned Output folder window. Locate the newly exported file, then double-click to open it. It is recommended to open the tab-delimited text file with an Excel™ spreadsheet for easier viewing.

Save and import attributes

Sample attributes can be added to the Results table for use in the PCA and Export IGV functions. A tab-delimited text file containing sample attributes can also be added to the Results table, however, column A must contain the name of the xxCHP file. Subsequent columns can contain other sample attributes. Alternatively, sample attributes listed in an ARR file generated in AGCC are also automatically displayed in the Results table (as long as the ARR and xxCHP files are located in the same directory).

1. In the ChAS Analysis Workflow, select the **QC Results** tab.
2. Click **Add Files** to navigate to and load your CHP files.

   The **Attributes** button is enabled.
3. Click the **Attributes** dropdown list, select **Import**, then navigate to the corresponding TXT file.
4. Click **Open**.

   The attributes now appear in the table.
5. Optional: To save an individual text file of sample attributes for each loaded chp file, click the **Attributes** dropdown, then select **Save**.

Export to Integrative Genomics Viewer (IGV)

The ChAS Analysis Workflow enables you to export a variety of graphs for viewing in IGV. To access this viewer, go to: http://software.broadinstitute.org/software/igv/

1. In the ChAS Analysis Workflow, click the **QC Results** tab.
2. Load results files by clicking **Add Files**, then navigate to and highlight the CHP files.
3. Click **Open**.
4. Select files by either clicking the **Select All**, or checking each filename's adjacent checkbox.
5. Click **Export to IGV**.

   The IGV Exporter window appears. (Figure 89)
6. Click the **Browse** button to assign an output folder.
7. From the IGV Exporter window, select the checkbox(es) adjacent to the data you want to export, as shown in Figure 89.
8. Click **OK**.

Note: To include sample attribute information in the IGV Export, click **Attribute → Save** prior to running the IGV Export.

Note: XON Region segments are exported as CN segments when working with CytoScan™ XON Arrays.

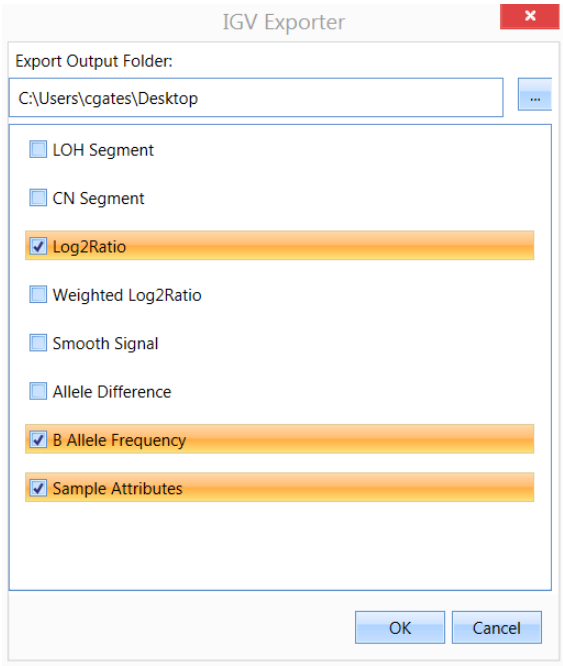


Figure 89 IGV Exporter window.

Note: The export process may take several minutes to complete, as it is dependent on your sample(s) and data type(s).

If the export was successful, an IGV Export Complete message appears. (Figure 90) Acknowledge the message, then click **OK**.

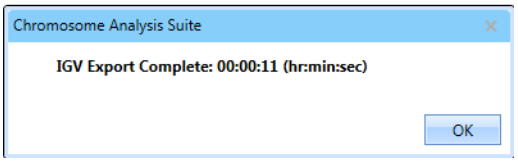


Figure 90 IGV Export Complete message.

Your data is now ready to be imported into IGV's browser.

For more information on importing and viewing your data in IGV, go to:
http://software.broadinstitute.org/software/igv/UserGuide

Principle component analysis

ChAS Analysis Workflow enables you to perform Principle Component Analysis (PCA) on signal (CHP) data. PCA identifies a new set of variables (PCA1, PCA2, and PCA3) that account for a majority of the variance in the original data set. The first principal component (PCA1) captures as much variability in the data as possible. PCA2 captures as much of the remaining variability (not accounted for by PCA1) as possible. PCA3 captures as much of the remaining variability (not accounted for by PCA2) as possible.

Generate a PCA plot

1. In the ChAS Analysis Workflow, select the QC Results tab. Load results files by clicking Add Files, and navigating to xxCHP files, then click **Open**.

   When files are loaded, the Import Attributes, Export to IGV, and QC Analysis buttons are enabled.
2. Select files, by either clicking **Select All** button or selecting files individually by ticking box to left of file.
3. Click the **QC Analysis** button and in the resulting dropdown list, select PCA.

   The 3-dimensional PCA graph(s) appears. The graph axes represent the top 3 variables (PCA1, PCA2, and PCA3) that account for the majority of the variability among the samples.

Sample display options

By default, the table is set to a vertical view. Click  to change the view to a horizontal view.

1. The file can be identified by clicking an icon in the plot.

   The corresponding file in the table is highlighted, as shown in Figure 91.

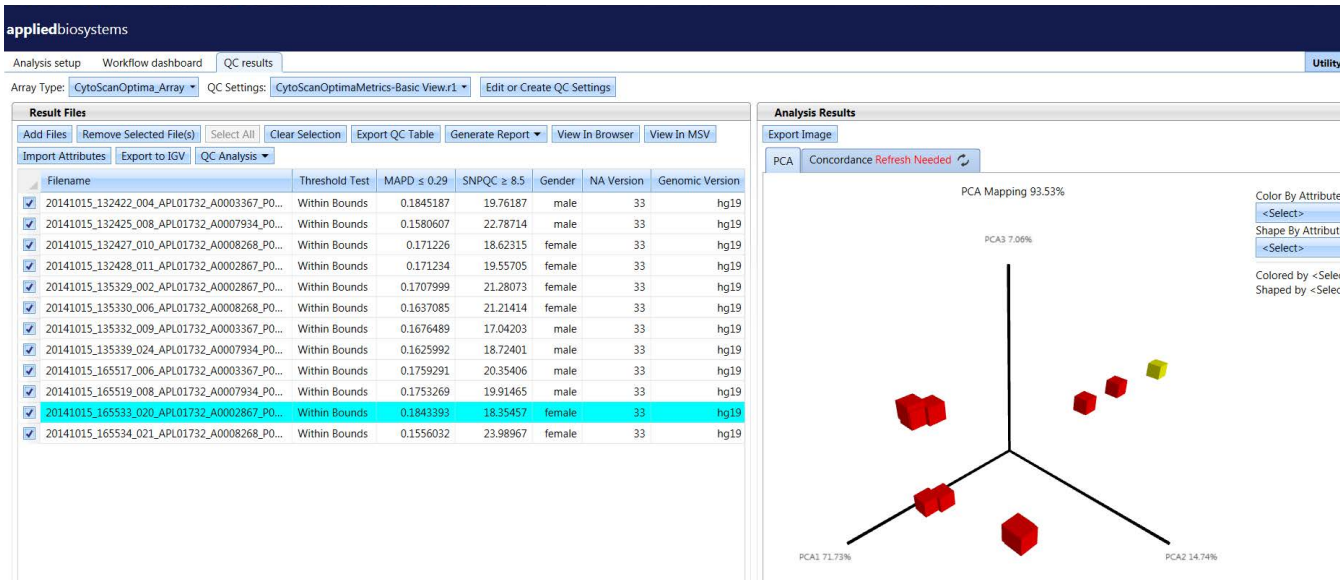


Figure 91 PCA display.

2. Icons can be lassoed by clicking and drawing circle around sample(s) of interest, as shown in Figure 92. This action also highlights the corresponding files in the table.

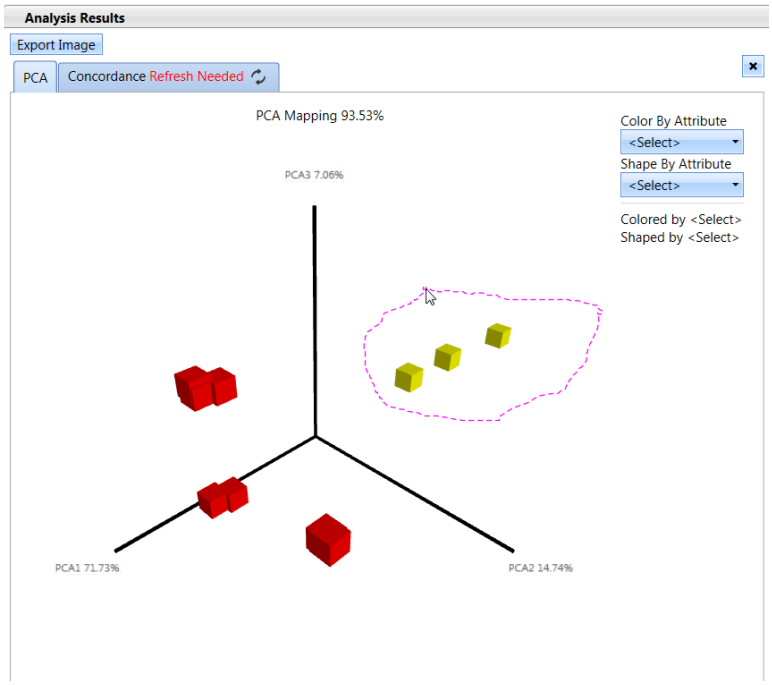


Figure 92 PCA display.

3. Points on the plot can also be hidden by right-clicking, then selecting the appropriate option.

Note: The selected samples are only hidden from the plot.

4. Use the dropdown lists (Figure 93) to select attributes for display by **Color By Attribute** and by **Shape By Attribute**.

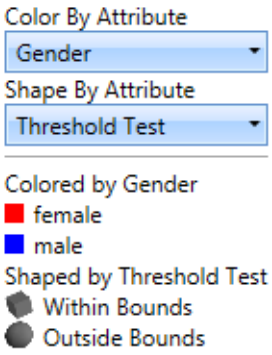


Figure 93 Sample Display.

Additional PCA graph display options

- To rotate the graph, right-click, then drag the graph to change its view perspective.
- Click **Export Image** to save the displayed plot as a PNG graphics file.

Concordance checks

The ChAS Analysis Workflow enables you to perform pairwise comparison concordance checks on genotype calls for all selected samples.

The concordance between all pairwise comparisons for the samples in the results table are reported.

A reference sample can be selected. After selected, concordances are displayed.

Compare to reference allows you to compare every sample to a single reference file.

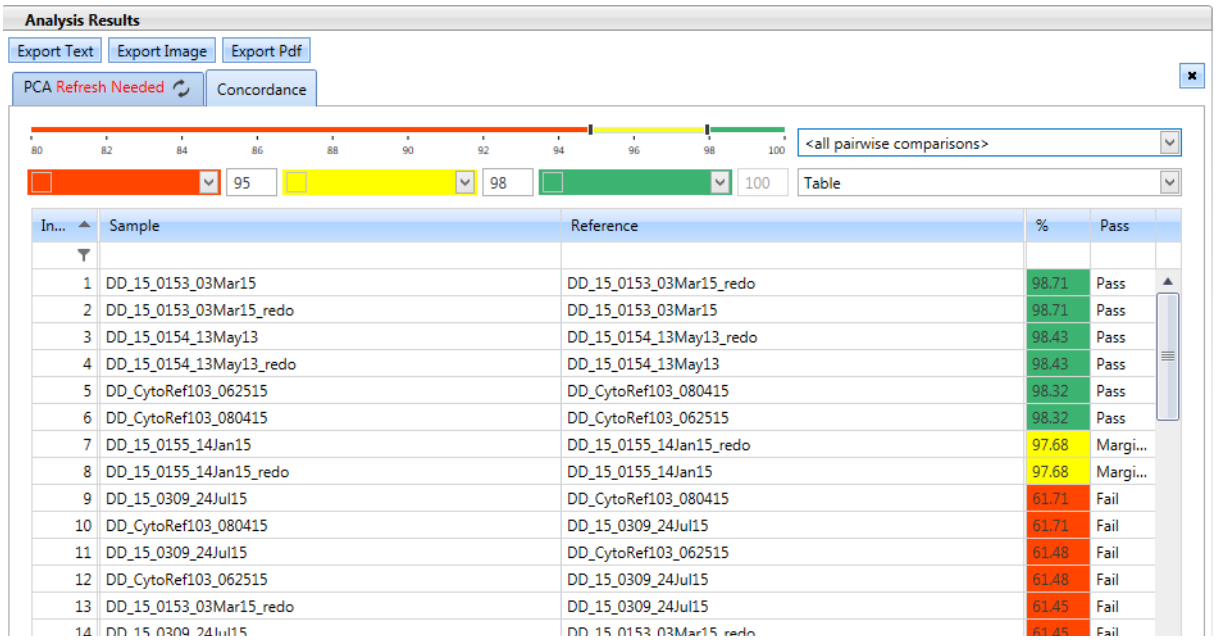
Perform a concordance check

1. In the ChAS Analysis Workflow, select the **QC Results** tab.
2. Load results files by clicking **Add Files**, then navigate to xxCHP files.

   After the files are loaded Import Attributes, Export to IGV, and QC Analysis buttons are enabled.
3. Select files, by either clicking **Select All** button or by selecting each file's (left) checkbox.
4. Click the **QC Analysis** button.

   A dropdown list appears.
5. Click **Concordance**.

   The concordance table with sample, reference and percent concordance is generated, as shown in Figure 94.

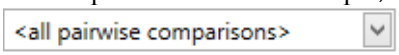


In...	Sample	Reference	%	Pass
1	DD_15_0153_03Mar15	DD_15_0153_03Mar15_redo	98.71	Pass
2	DD_15_0153_03Mar15_redo	DD_15_0153_03Mar15	98.71	Pass
3	DD_15_0154_13May13	DD_15_0154_13May13_redo	98.43	Pass
4	DD_15_0154_13May13_redo	DD_15_0154_13May13	98.43	Pass
5	DD_CytoRef103_062515	DD_CytoRef103_080415	98.32	Pass
6	DD_CytoRef103_080415	DD_CytoRef103_062515	98.32	Pass
7	DD_15_0155_14Jan15	DD_15_0155_14Jan15_redo	97.68	Margi...
8	DD_15_0155_14Jan15_redo	DD_15_0155_14Jan15	97.68	Margi...
9	DD_15_0309_24Jul15	DD_CytoRef103_080415	61.71	Fail
10	DD_CytoRef103_080415	DD_15_0309_24Jul15	61.71	Fail
11	DD_15_0309_24Jul15	DD_CytoRef103_062515	61.48	Fail
12	DD_CytoRef103_062515	DD_15_0309_24Jul15	61.48	Fail
13	DD_15_0153_03Mar15_redo	DD_15_0309_24Jul15	61.45	Fail
14	DD_15_0309_24Jul15	DD_15_0153_03Mar15_redo	61.45	Fail

Figure 94 Concordance table example.

Concordance table filter and display options

- To compare to reference sample, select the sample from drop down-menu.



- The default values are set at >98%, 95–98%, and below 95%.
- The 3 categories of QC are passing, failing, and marginal.
- Each QC category is represented by its own unique color, as shown in Figure 95.

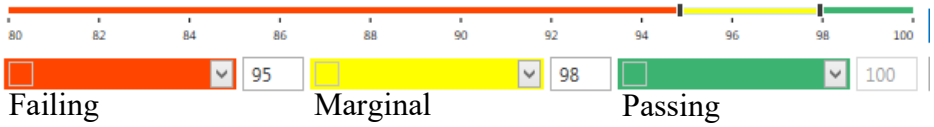


Figure 95 Displayed concordance QC example.

- Colors in the table can be changed by clicking the color dropdown (Figure 96), then choosing a different color from the pallet.

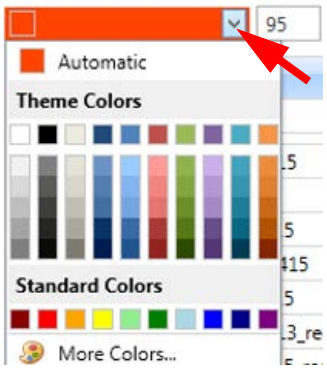


Figure 96 Concordance color options.

- The default values can be changed by either moving the hash marks on the line bar, or typing in a number in the designated category (Figure 96).
- The data can also be viewed in matrix format by selecting **Matrix** in the dropdown list, as shown in Figure 97.

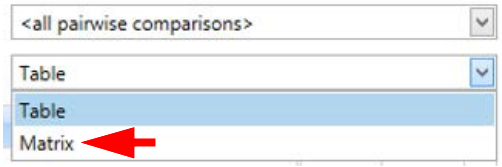


Figure 97 Table and Matrix options.

Filter

1. At the table, place your mouse cursor over a Sample or Reference cell.
   A filter icon appears. (Figure 98)

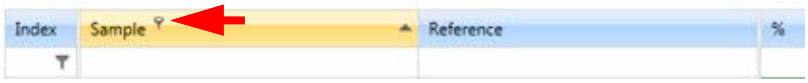


Figure 98 Filter icon.

2. Right-click the Filter icon.
   A dropdown list appears. (Figure 99)

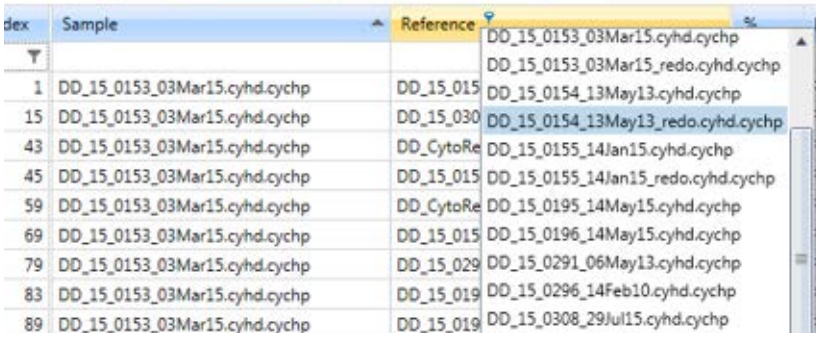


Figure 99 Dropdown list.

3. Click to select the samples you want to filter from the dropdown list.

Change the view and/or order of sample and reference columns

1. Click the Filter icon.
   A menu appears. (Figure 100)

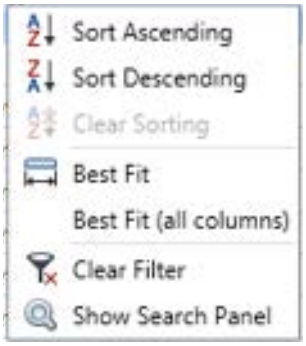


Figure 100 Filter icon menu.

2. Use the menu selections to customize your sample and reference columns.

Export the currently displayed table

1. Click the appropriate, Export Text | Export Image | Export Pdf button, then export the file as you normally would.

Mendelian error checking

Note: The Mendelian Error Checking feature is for the CytoScan™ family of arrays only.

Run an error checking analysis

1. From the Analysis menu, select **Perform Analysis Setup.**

   The Analysis Workflow window opens. (Figure 101)

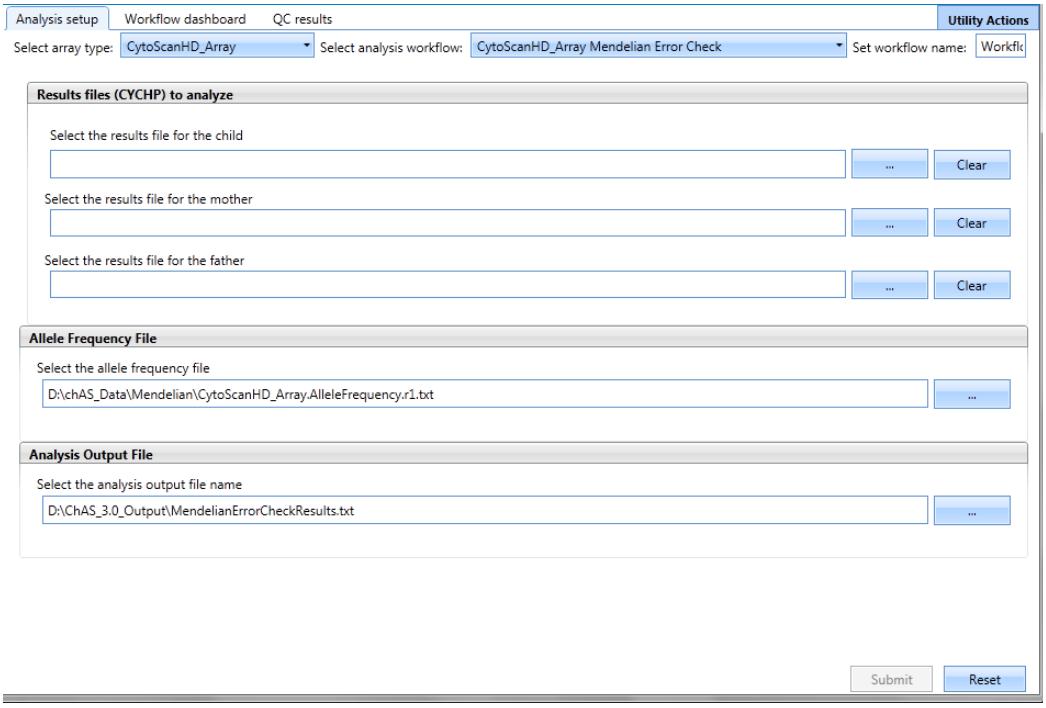


Figure 101 Analysis Workflow.

2. From the **Select array type** dropdown list, click to select an array type (Example: **CytoScanHD_Array**.

   Note: The **Select array type** dropdown list includes only the array types for which library (analysis) files have been downloaded from the NetAffx™ Analysis Center or copied from the library package provided with the installation.

3. From the **Select analysis workflow** drop down, click to select the appropriate Mendelian Error Check Workflow. (Example: **CytoScanHD_Array Mendelian Error Check**)
4. By default, the Set workflow name is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.
5. Click each of the 3 Results files (CYCHP or XNCHP) to analyze **Browse** buttons to navigate to and select the appropriate Results File for **Child**, **Mother**, and **Father**.

   You can also run a Mendelian Error Check using 2 Results files: **Child** and **Mother** or **Child** and **Father**.
6. Click the Allele Frequency File **Browse** button to navigate to and select the appropriate Allele text file.

   Note: An Allele Frequency file is provided in the library file package or you can create your own custom Allele Frequency File for use in this analysis.

7. Click the Analysis Output File **Browse** button to navigate to and enter a name (tab-delimited text filename) for your Mendelian Error Check output result. By default, the Output name is based on the CYCHP/XNCHP filename used for the Child.
8. Click **Submit**.

   An error appears if a male is assigned as mother or a female is assigned as father or if the gender in the actual cychp file is wrong.

   The Workflow Dashboard window appears. (Figure 102).

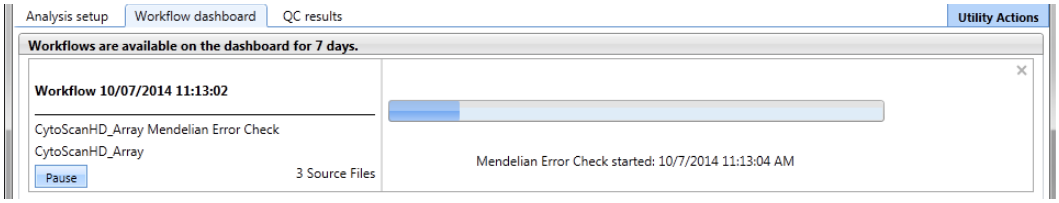


Figure 102 .Files loading inside the Workflow Dashboard.

After loading is complete, a Workflow completed successfully message appears. (Figure 103)

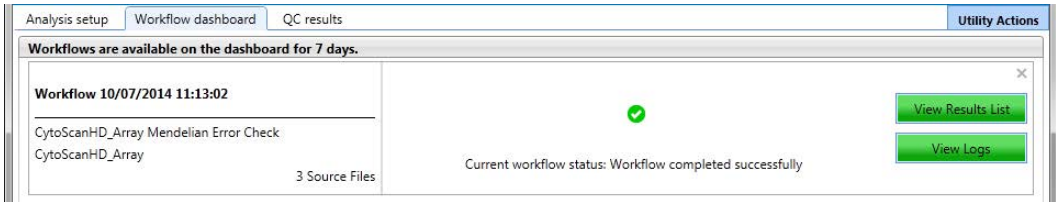


Figure 103 Workflow Dashboard loaded.

9. Click **View Results List**.

   The Output folder window (you assigned earlier) appears.
10. Locate the newly created ***.cyhd.txt/cyex.txt** (or ***.cychp.ND.txt**) file, then double-click to open it in Microsoft™ Notepad to view it as tab-delimited text file, as you normally would.
11. Optional: If you want to view the Workflow's Log File, click **View Logs**.

    The **C:\ProgramData\Affymetrix\ChAS\Log folder** window appears.

    Locate the newly created Log.txt file, then double-click to open it in Microsoft™ Notepad to view it as tab-delimited text file, as you normally would.

Run an error checking analysis across different CytoScan™ array types

The Mendelian Error Check can also be run when the Child array differs from the Mother and Father array. Performing a Mendelian Error Check across array types is valid for Trio analysis only. For a Duo analysis, samples must be run on the same array type.

The following combinations are supported:

Child CytoScan™ array	Parent CytoScan™ array
CytoScan™ HD Array	CytoScan™ 750K Array
CytoScan™ HD Array	CytoScan™ HD Accel Array
CytoScan™ HD Array	CytoScan™ 750K Accel Array
CytoScan™ HD Accel Array	CytoScan™ HD Array
CytoScan™ HD Accel Array	CytoScan™ 750K Array
CytoScan™ HD Accel Array	CytoScan™ 750K Accel Array
CytoScan™ 750K Array	CytoScan™ 750K Accel Array
CytoScan™ 750K Array	CytoScan™ HD Array
CytoScan™ 750K Array	CytoScan™ HD Accel Array
CytoScan™ 750K Accel Array	CytoScan™ 750K Array
CytoScan™ 750K Accel Array	CytoScan™ HD Array
CytoScan™ 750K Accel Array	CytoScan™ HD Accel Array

To run a Mendelian Error Check analysis across CytoScan array types:

1. In the Analysis setup, select the array type based on the Child array type.
2. Follow the instructions for Run an error checking analysis. See "Run an error checking analysis" on page 103.

Interpret an error checking analysis

The Mendelian Error Check analysis provides 2 key points of information:

1. Are the input samples related?
   - Mother-Child
   - Father-Child

   If the samples are related, the **Role Validity** equals 1. If the samples are not related, **Role Validity** equals 0. (Figure 104) The output also indicates which CYCHP/XNCHP file is assigned as the Mother, Father and Child (Index). The analysis also can be run as a DUO analysis. (Mother-Child or Father-Child).

1	#%GroupName=Familial				
2	#%SetName=Samples				
3	#%Columns=4				
4	#%Rows=3				
5	SampleKey	CHPFilename	CHPID	Role	
6	0	A03_CytoScanHD_\	00001316-386a-4744	index	
7	1	A01_CytoScanHD_\	00006534-6a66-4722	mother	
8	2	A02_CytoScanHD_\	00006996-3b6b-4e53	father	
9	#%SetName=RelatednessTest				
10	#%Columns=5				
11	#%Rows=3				
12	AnalysisType	ReferenceSampleK	FamilialSampleKey	RoleValidity	RoleIndexScore
13	0	0	2	1	80375.99
14	1	0	1	1	65454.53
15	2	0	2	1	48592.95
16	#%SetName=MIE				

Figure 104 Trio Output Tool Validity examples.

2. Do any chromosomes have an elevated occurrence of Mendelian Errors?

   In Figure 105, chromosome 15 has a higher error rate (7.2%) compared to the rest of the chromosomes in this trio. In this example, the mother has 0% errors on chromosome 15, whereas the father has 7%, indicating that both chromosome 15 (or some portion of) alleles were inherited from the mother. It is recommended to compare the genotypes on chromosome 15 for these samples using the Graphs tab. For more information, see "Graphs table" on page 321.

19	Chromosome	Display	MarkerCount	MIE-Trio	MIE-Mat	MIE-Pat	Percent-T	Percent-M	Percent-Pat
20	1	1	56482	119	21	38	0.21	0.04	0.07
21	2	2	62283	134	32	35	0.22	0.05	0.06
22	3	3	52106	108	14	39	0.21	0.03	0.07
23	4	4	49517	101	37	25	0.2	0.07	0.05
24	5	5	46205	108	25	36	0.23	0.05	0.08
25	6	6	51944	128	29	32	0.25	0.06	0.06
26	7	7	46413	131	39	31	0.28	0.08	0.07
27	8	8	38796	84	20	29	0.22	0.05	0.07
28	9	9	30622	72	24	20	0.24	0.08	0.07
29	10	10	35472	97	23	35	0.27	0.06	0.1
30	11	11	38846	105	37	28	0.27	0.1	0.07
31	12	12	33424	65	12	20	0.19	0.04	0.06
32	13	13	27733	45	12	17	0.16	0.04	0.06
33	14	14	26983	75	20	32	0.28	0.07	0.12
34	15	15	24981	1799	1	1752	7.2	0	7.01
35	16	16	20915	48	14	19	0.23	0.07	0.09
36	17	17	17465	44	13	10	0.25	0.07	0.06

Figure 105 High Instance of Mendelian Errors Table example.

Table 14 Mendelian Errors Report table columns.

Column name	Description
Analysis Type	Provides the samples being run through the analysis based on the sample key. 0 = Proband, 1 = Mother, 2 = Father.
Familial Sample Key	Tells you the parent for which relatedness is being tested. 1= Mother only used in the analysis. 2= Father used in the analysis (may be father only (duo) or trio).
Role Validity	A logical value with 0 being False and 1 being True. If the Role Index Score is >1,000 then the Role Validity = 1 (likely related). If the Role Index Score is < 1000 then Role Validity = 0 (likely unrelated).
Role Index Score	Role Index Score: This score is basically a Log Odds score that computes the probabilities of the observed genotype calls for the trio while accounting for potential genotyping error. Assume the following hypothesis: H1 – alleged father is true H2 – alleged father is random male. For each marker, compute a likelihood ratio of H1 vs H2. Sum all markers. In theory a value of zero means equally likely probability for either hypothesis. Positive means more likely Paternity related. Negative means more likely unrelated.
Chromosome/Display	Chromosome number
Marker Count	Number of genotypeable SNPs on the chromosome.
MIE Trio	Mendelian Inheritance Error for the Trio. (Ex 119 errors of 56482 SNPs).
MIE Mat	Number of errors for Mom.
MIE Pat	Number of errors for Dad.
Percentage MIE Trio	Number of raw error turned into a percent 119/56482 *100.
Percentage MIE Mat	Percent of errors for Mom.
Percentage MIE Pat	Percent of errors for Dad.

Analysis workflow troubleshooting

1. Click the **Utility Actions** button (top right of the Analysis Workflow window)
2. Click to select **Log Collection**.

   The following dialog window appears. (Figure 106)

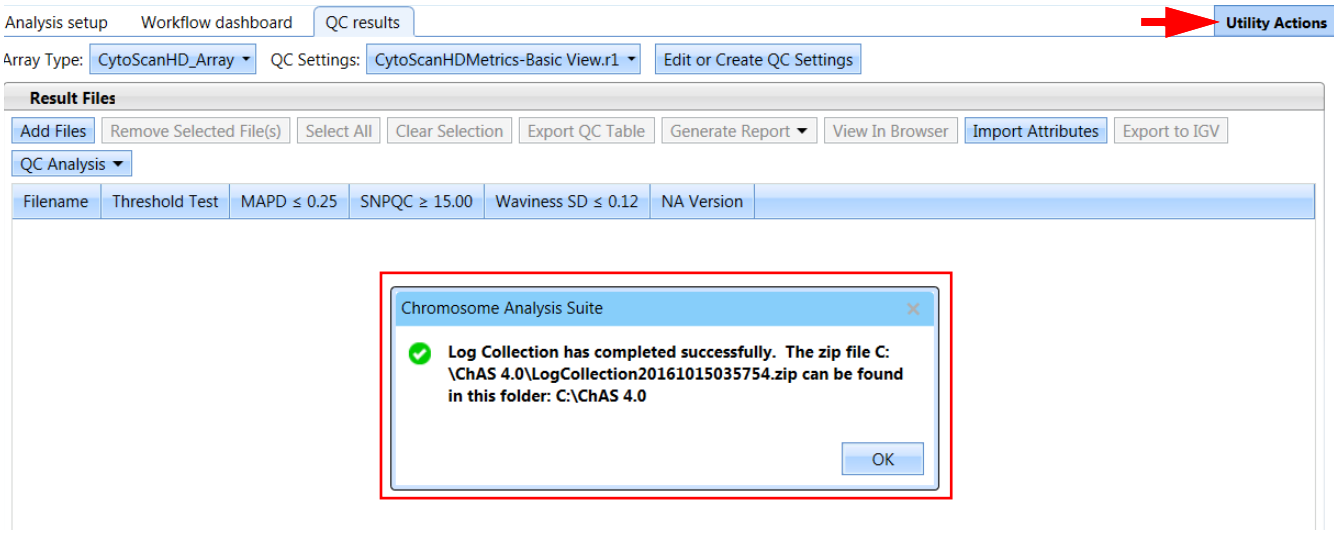


Figure 106 Log Collection dialog window.

3. Make note of the assigned ZIP folder filename and its location.
4. Use Windows™ File Explorer to navigate to the location.
   Example: **C:\ProgramData\Affymetrix\ChAS\log**
5. Locate the ZIP folder you noted earlier, then double-click to open it.

   The folder opens.

Log rollover

When the software determines that the log file for the Analysis Workflow (C:\ProgramData\Affymetrix\ChAS\log\AnalysisWorkflow.log) has reached a defined size (approximately 4 MB), the following steps are completed:

- A sub-folder is created in **C:\ProgramData\Affymetrix\ChAS\log** called '**Log***' (the * denotes the current date and time).
- A ZIP file called **RolledLogFile*.zip** is created in that folder. The '*' is the same date and time used for the folder name. The files in the **C:\ProgramData\Affymetrix\ChAS\log** folder and all files found in the currently selected QC History Log folder are included in this ZIP file.
- The Analysis Workflow files that are associated with analysis workflows that are no longer active on the Dashboard are deleted from **C:\ProgramData\Affymetrix\ChAS\log**
- A new AnalysisWorkflow.log file is created in **C:\ProgramData\Affymetrix\ChAS\log**

Log collection

When the Log Collection option is selected from the **Utility Actions** menu, a file called **LogCollection*.zip** (the * denotes the current date and time) is created in the folder C:\ProgramData\Affymetrix\ChAS\log.

This created file contains the full contents of the folder C:\ProgramData\Affymetrix\ChAS\log, including the log file for the browser (ChAS_RUO.log).

If available, the sub-folders of C:\ProgramData\Affymetrix\ChAS\log and all files found in the currently selected QC History Log folder.

Note: All log files for the ChAS Database, ChAS Browser and Analysis Workflow can be found in: **\ProgramData\Affymetrix\ChAS\Log**

6 Load data

ChAS can display data from:

- CytoScan™ Array CYCHP/XNCHP files, generated in ChAS.
- CytoScan™ HTCMA files, generated in the RHAS.
- Genome-Wide Human SNP Array 6.0 CNCHP files, generated in Genotyping Console (GTC).
- OncoScan™ OSCHP files, generated in OncoScan™ Console or ChAS.
- ReproSeq™ Aneuploidy results (.zip) from Ion Reporter™ Software.

Note: When referring to steps that apply to both CytoScan CYCHP and SNP 6.0 CNCHP data files, the CHP files are described as CxCHP files. When referring to steps that apply to CytoScan CYCHP, CytoScan XNCHP, CytoScan HTCMA RHCHP, SNP 6.0 CNCHP data, and OncoScan files, the resultant files are described as xxCHP files.

Introduction to loading data

The same steps are used to load results xxCHP files from CytoScan™ arrays, Genome-Wide Human SNP 6.0 Array, or OncoScan™ arrays.

When loading CYCHP files into ChAS for viewing, the software:

1. Loads the run-length encoded segments in the CYCHP file to display as segments.
2. Applies any smoothing or joining that would alter the length and other properties of segments.

   IMPORTANT! In a new user profile, smoothing and joining are turned on by default for CytoScan™ 750K and CytoScan™ HD Arrays. Smoothing and joining are disabled for CytoScan™ Optima Arrays. Smoothing and joining are OFF by default for both OncoScan™ and CytoScan™ HTCMA arrays. The smoothing and joining settings are specific for each array type (for more details on smoothing and joining, see "Copy number segment smoothing and joining (optional)" on page 118).

3. Displays the segments and graph data:
   - Segment Data
     - Copy Number Gain/Loss
     - Mosaicism Copy Number Gain and Loss
     - Loss of Heterozygosity (LOH)
     - Mosaic Loss of Heterozygosity (LOH)
   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Weighted Log2 Ratio
     - LOH
     - Smooth Signal
     - Allele Difference
     - B-allele Frequency
     - Genotype Calls

When loading XNCHP files into ChAS for viewing from CytoScan™ XON arrays, the software:

1. Selects the segments in the XNCHP file to display as segments.
2. Displays the segments and graph data:
   - Segment Data
     - Loss of Heterozygosity
     - Exon Region Gain/Loss
   - Graph Data
     - Log2 Ratio
     - Weighted Log 2 Ratio
     - Smooth Signal
     - Loss of Heterozygosity (LOH)
     - Allele Difference
     - B-allele Frequency (BAF)
     - Genotype Calls

When loading RHCHP files into ChAS for viewing, the software:

1. Selects the segments in the RHCHP file to displays as segments.
2. Displays the segments and graph data:
   - Segments Data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity
   - Graph Data
     - Copy Number State
     - Log 2 Ratio
     - Smooth Signal
     - LOH
     - Allele Difference
     - B-allele Frequency

When loading SNP6 CNCHP files into ChAS for viewing, the software does the following:

1. Performs segment detection by analyzing the CN and LOH graph data in the CNCHP file.

   Note: When running the Segment Reporting Tool in GTC on SNP 6 data, the software sets the end coordinate such that the segment ends at the base position of the last marker in the segment. When loading SNP 6 data into ChAS, the segment detection sets the end coordinate for a segment such that the segment ends one base after the last marker in the segment. This may result in a discrepancy between the end position for segments when comparing data analyzed in both GTC and ChAS.

2. Applies any smoothing, removing, or joining that would alter the length and other properties of segments.

   Smoothing is similar to the process applied when running the Segment Reporting Tool in GTC.

   Note: ChAS uses the median of the aberrant markers' CNStates, as the recalculated CNState of the smoothed (and/or joined) segment.

IMPORTANT! For CNCHP files from the SNP 6.0 Array, smoothing but not joining is turned on by default in a new user profile.

3. Displays the Segments and Graph Data:
   - Segment data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity (LOH)

     Note: The expected Copy Number State on the X chromosome of normal males is not constant over its entire length. This is due to the structure of the sex chromosomes. For more information see "Copy number segments on the X and Y chromosomes" on page 45.

   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Allele Difference
     - SmoothSignal
     - LOH

When loading OncoScan™ OSCHP files for viewing, the software does the following:

1. Displays segments in the OSCHP created by the TuScan Copy number algorithm. For details on this algorithm, see the *OncoScan™ Console User Guide* (Pub. No. 703195) or Appendix F.

   IMPORTANT! Smoothing and Joining are OFF by default for OSCHP files.

2. Displays the segments and graph data:
   - Segment Data
     - Copy Number Gain/Loss
     - Loss of Heterozygosity
   - Graph Data
     - Copy Number State
     - Log2 Ratio
     - Weighted Log2 Ratio
     - Allele Difference
     - B-allele Frequency
     - LOH
     - Smooth Signal
     - Variant/Somatic Mutation
     - Somatic Mutation (OncoScan FFPE Assay only)

When loading ReproSeq™ Aneuploidy data for viewing, the software does the following:

1. Displays segments from the Ion Reporter™ Software. For details, see the *Ion Reporter™ Software User Guide*: https://ionreporter.thermofisher.com/ir/
2. Displays the whole genome sequencing tiles on the Copy Number State graph.

Load files

Loading xxCHP data for viewing in ChAS involves the following steps:

1. **Optional:** Before loading: Select Segment Smoothing and Segment Joining parameters for processing the CN Gain and Loss Segment data for CxCHP files.

   IMPORTANT! Smoothing and Joining are ON by default for CytoScan™ 750K, HD, and HD Accel Arrays. Both Smoothing and Joining are OFF by default for OncoScan™, CytoScan™ HTCMA, and GenomeWide Human SNP 6.0 arrays, and is disabled for CytoScan™ Optima arrays and ReproSeq™ Aneuploidy data.

   XON Segment Merging is turned ON by default for CytoScan XON arrays. For details, see "XON segment merging" on page 123.

2. Select analysis results from:
   - CytoScan™ arrays (generated by analyzing CEL files in ChAS)
   - CytoScan™ HTCMA arrays (generated from CEL files in the RHAS.
   - Genome-Wide Human SNP 6.0 Arrays (generated by analyzing CEL files in GTC)
   - OncoScan™ arrays (generated by analyzing in ChAS)
   - ReproSeq™ Aneuploidy ZIP files (generated in Ion Reporter™ Software)

   You can also select region information files in AED and BED format for loading. Use the Open window (click the icon) to load xxCHP data files, Affymetrix Extensible Data (AED), or Browser Extensible Data (BED) annotation files. The AED and BED files that you open is automatically loaded when a new session is started with the same user profile.

   Note: You may want to edit smoothing and joining parameters. This can be done before or after loading the CxCHP data. See "Copy number segment smoothing and joining (optional)" on page 118 for more information.

3. Select **File** → **Open** on the menu bar. Alternatively, click the **File Open** icon.

   The Open window appears. (Figure 107)

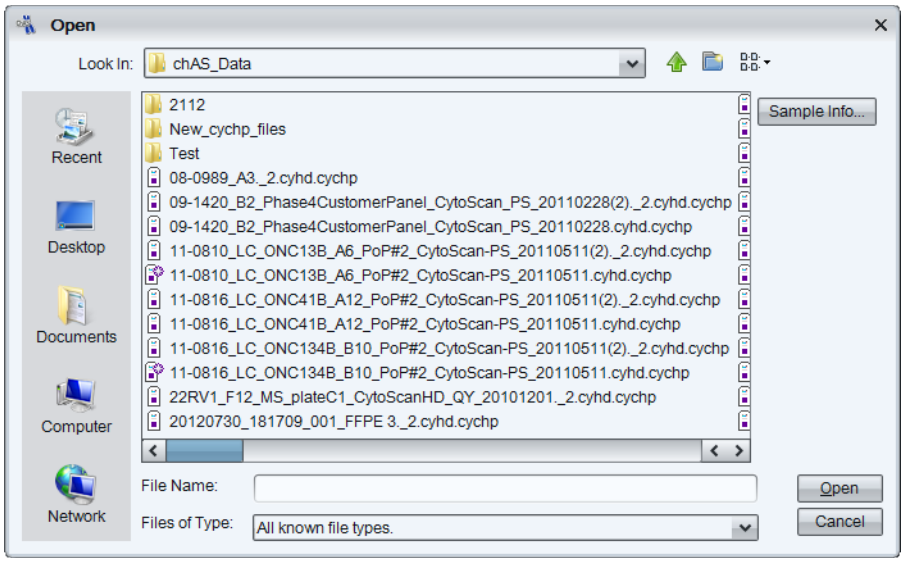


Figure 107 Open window.

4. To view information about results, select one or more files, then click **Sample Info**.

   The **Sample Info** window opens. (Figure 108)

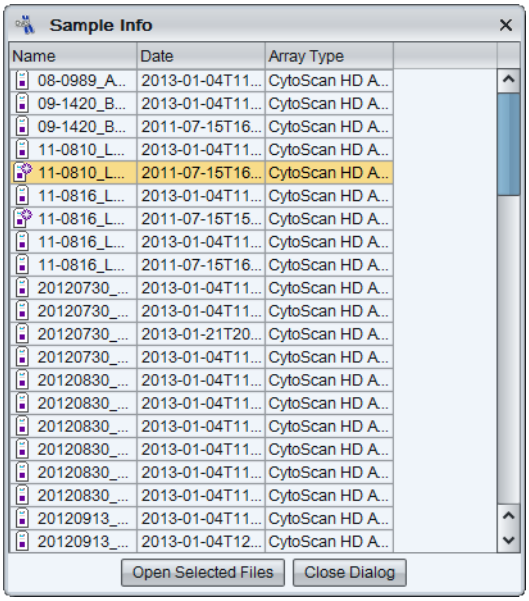


Figure 108 Sample Info window.

Note: If the xxCHP and ARR files are located in the same folder, the Sample Info window shows information about both the sample and the results. To load files from the **Sample Info** window, select the files, then click **Open Selected Files**.

Use the search feature

1. Click **Close Dialog** (Figure 108) to close the **Sample Info** window.

   The Open window appears.

2. Navigate to the folder with the files that you want to search for and load.
3. Enter a text string with an asterisk (*) **before** and **after** the search term in the **File Name** field. See example in Figure 109 on page 115.
4. Select a file type from the dropdown list. See example in Figure 109 on page 115.
5. Click **Open**.

   Files with names that include the search term are displayed in the **Open** window. See example in Figure 110 on page 115.

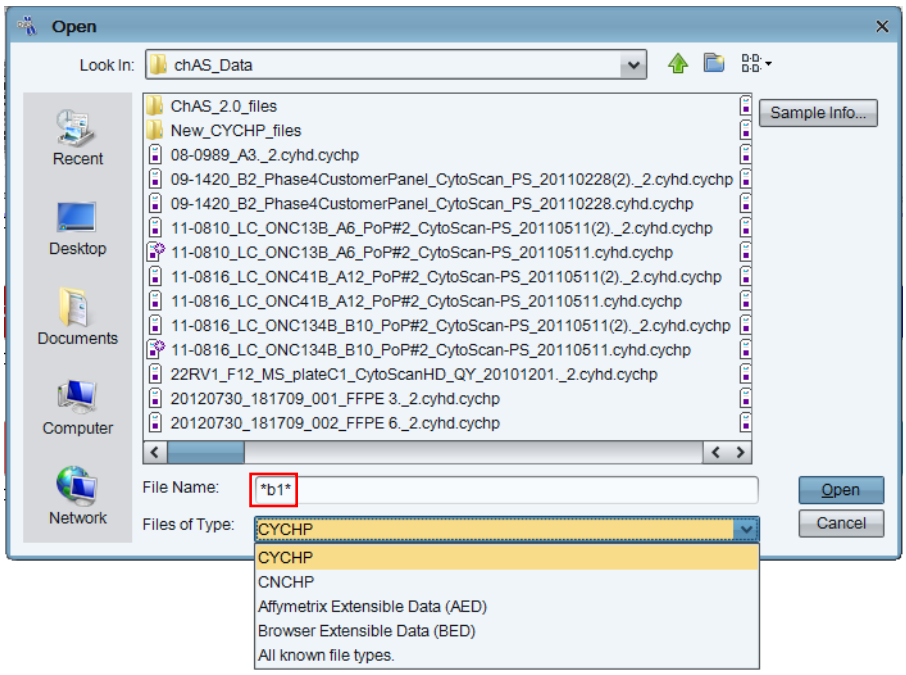


Figure 109 Enter a file name search term (*text string*) and select a file type.

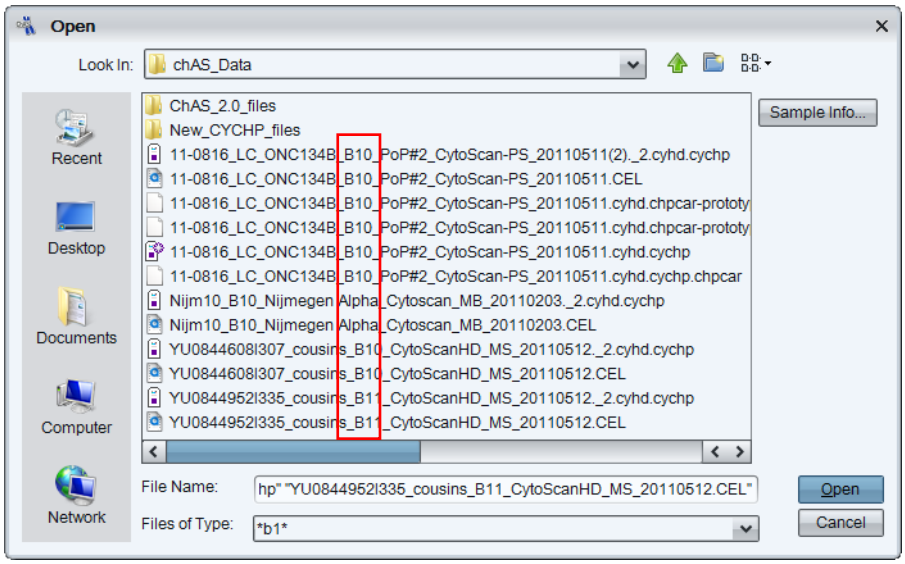


Figure 110 The Open window shows files with names that include your search term.

6. Select the files (you can use Shift+click or Ctrl+click to select multiple files)
7. Click **Open**.

   If any of the files fail the QC checks, a warning notice appears (Figure 111).

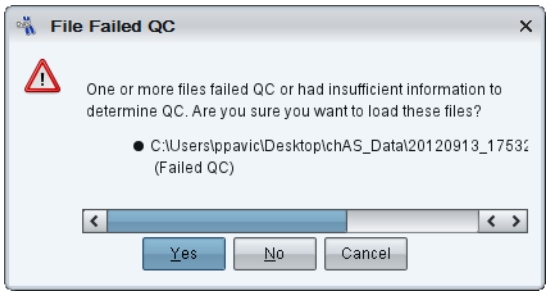


Figure 111 Warning Notice for QC failure.

You can click **Yes** to continue to load the files.

It is recommended you load no more than 3 files at one time.

If the following warning message appears (Figure 112), acknowledge it, then click **OK**.

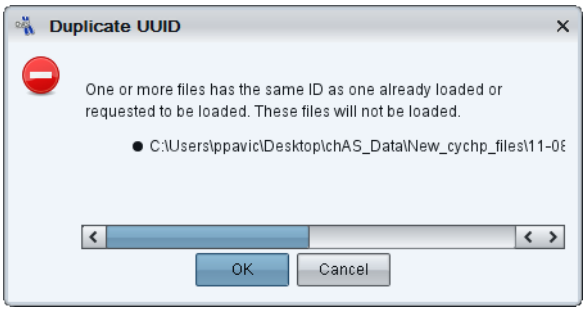


Figure 112 Duplicated files message.

If the following warning message appears (Figure 113), click **Yes** to acknowledge it.

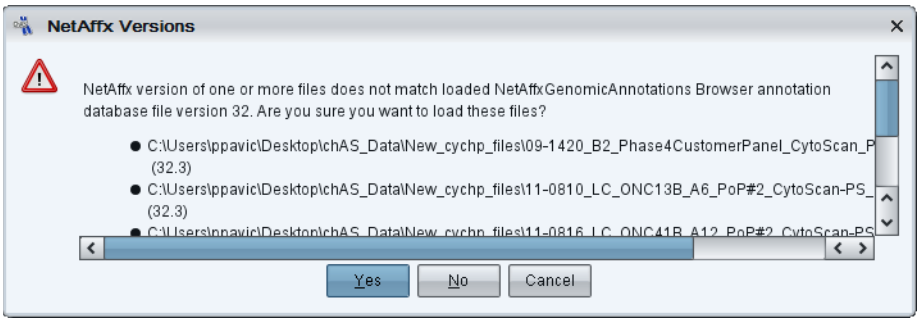


Figure 113 NetAffx™ versions message.

Note: The ChAS Browser allows loading of data from various array types as long as they were all analyzed using the same genome build such as Hg19 or Hg38.

A progress bar appears

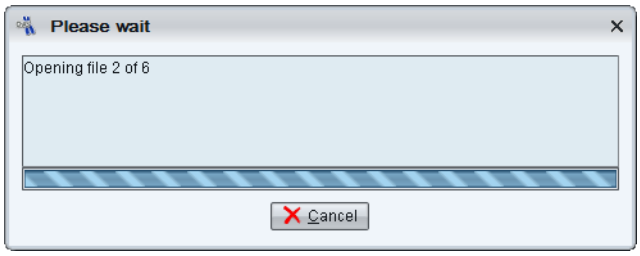


Figure 114 Progress bar.

After a few moments, the ChAS Browser featuring your selected samples appears. The loaded files appear in the Files list pane. (Figure 115).

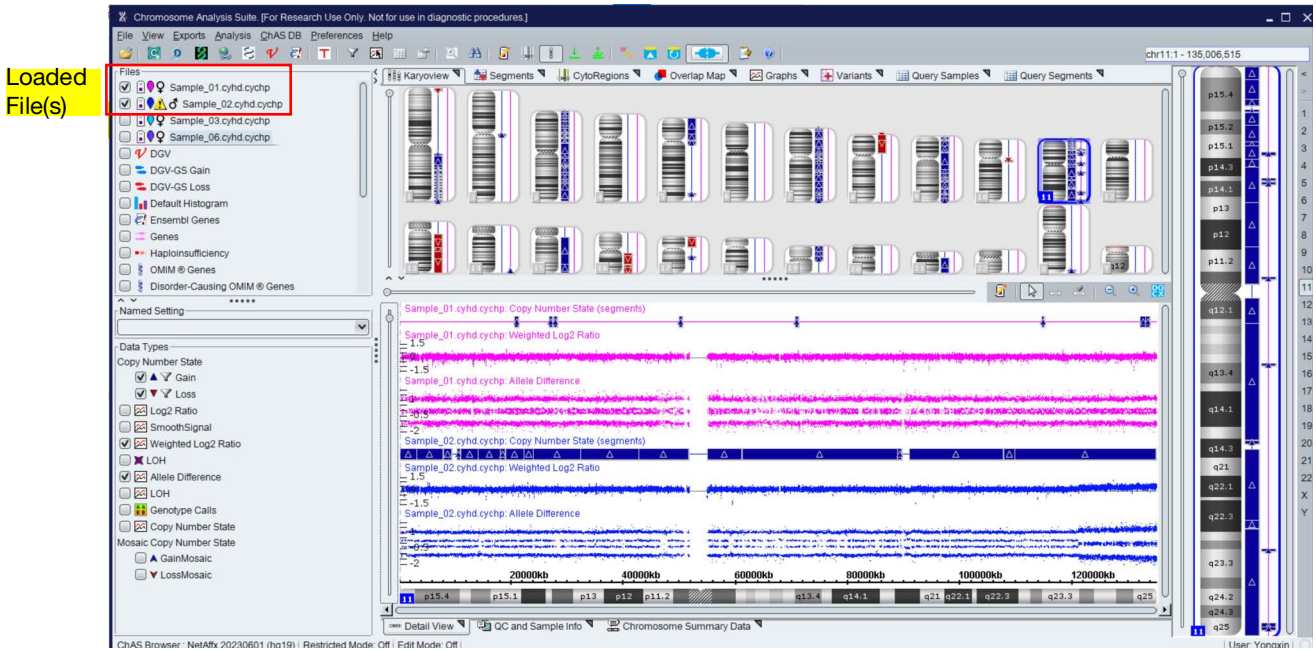


Figure 115 Files list shows the loaded data.

When files are loaded into ChAS, all files are check on by default. To uncheck multiple files at one time, highlight the filenames, right-click and choose **Unselect**. Multiple unchecked files can also be checked on by highlighting the filenames, right-click and choose **Select**.

Copy number segment smoothing and joining (optional)

"Smoothing" and "Joining" are non-destructive processes that affect the display of Copy Number segments. Smoothing and joining are performed on the Copy Number State data during the loading process, based on settings that are specified before loading. Any data filtering is applied after smoothing and joining.

IMPORTANT! Smoothing and joining are turned on by default in a new user profile for CytoScan™ 750K, HD, and HD Accel Arrays.

Smoothing and Joining are specified per array type. The processes do not affect the marker data in the CNCHP or CYCHP file. If these settings are turned off, the Copy Number segment data is displayed without smoothing or joining.

IMPORTANT! Smoothing and Joining affect only data loaded from CNCHP and CYCHP files. This ONLY applies to copy number data, NOT LOH or Mosaic types. Smoothing and Joining is OFF by default for CytoScan™ Optima, OncoScan™, and ReproSeq™ Aneuploidy files.

Segments which have been smoothed and/or joined are indicated by a blue check mark in the Smoothed/Joined column of the Segments table (Figure 116). The segment ID name indicates whether smoothing and/or joining has occurred. A red "X" indicates no smoothing or joining has been applied.

View segments that were smoothed/joined

1. Click the **Segments** tab.
2. Use the horizontal scroll bar to move the window to the far right.

   The Smoothed/Joined column appears. (Figure 116)

   For more information:

   - See "About smoothing" on page 121.
   - See "About joining" on page 122.

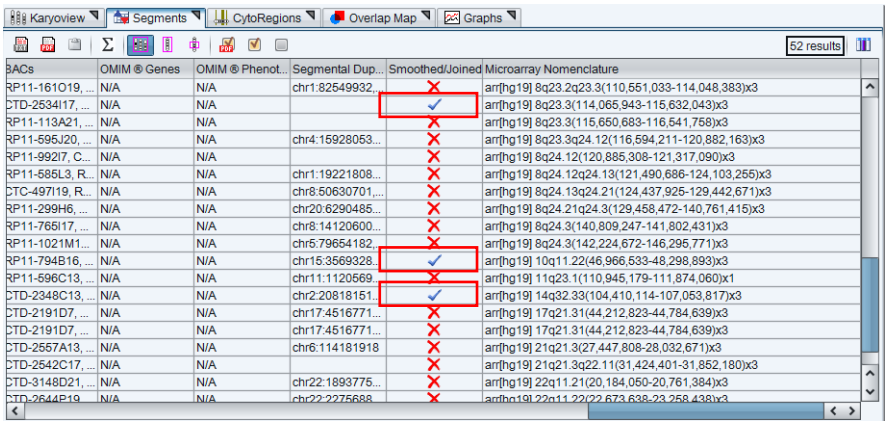


Figure 116 Example Segments table with smooth/joined segments.

Set smoothing and joining parameters

1. Click **Preferences** → **Edit User Configuration**.

   The User Configuration window appears.

2. Click the **Segment Data** tab.

3. Click the **Choose Array Type** dropdown list to select the array type to view or edit its Smoothing and Joining settings. (Figure 117 on page 119)

Note: If you change the smoothing or joining parameters, the new rules are applied to the original, raw segments of CxCHP files which have not had modifications, Calls, Interpretations, or Inheritance made. CxCHP files which have had segments modified or had Calls, Interpretations or Inheritance made have had their smoothing and joining parameters fixed and do not adopt changes made to the array type's Segment Data smoothing and joining settings.

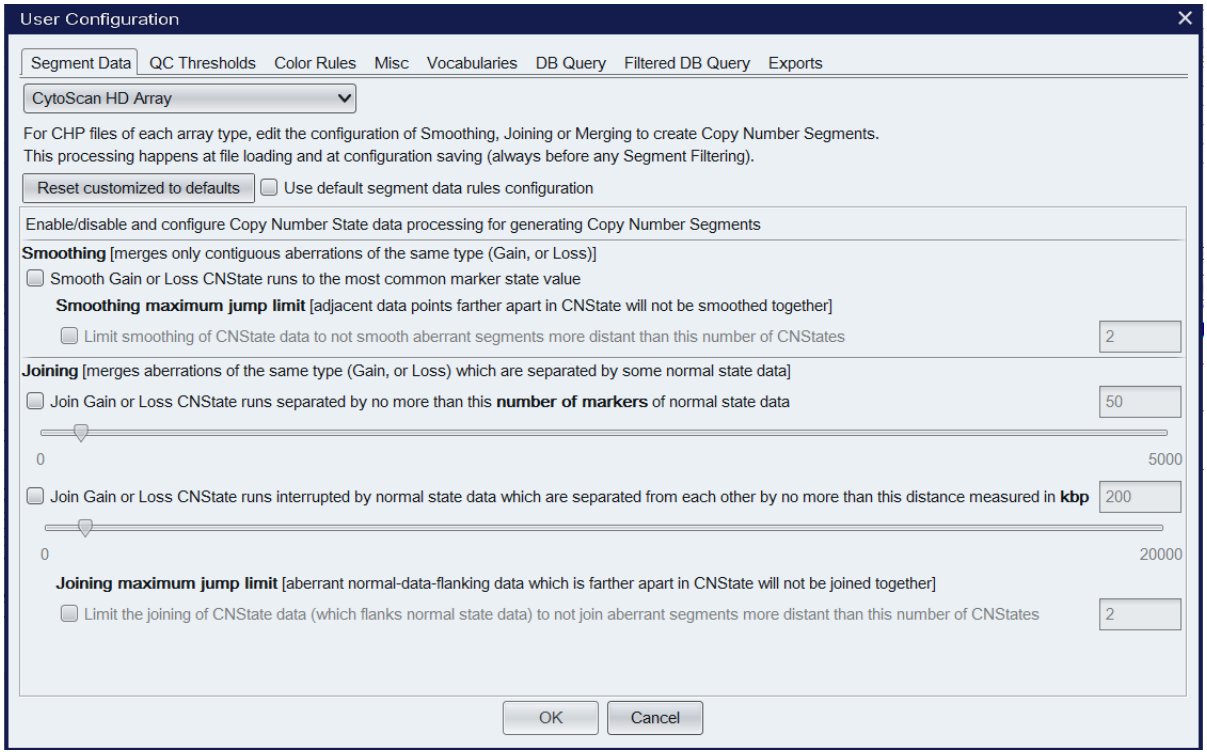


Figure 117 User Configuration window, Segment Data tab.

Segment data tab options and descriptions

Table 15

Option	Description
Use default segment data rules configuration	For the CytoScan™ 750K and HD Arrays, the default smoothing and joining rules are: • Smooth Gain or Loss CNState runs to the most common marker value, then generate segments. • Join any "split" CNState runs separated by no more than 50 normal-state markers. • Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than 200 kbp • Skip segments from the smoothing operation for all arrays that have a CN < 1. For SNP 6 arrays, the default smoothing rule: • Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.
Smooth Gain or Loss CNState runs to the most common marker value	Smoothing to the most common marker state value is only applied to contiguous CNState runs of the same type (gain or loss).
Limit smoothing of CNState data to not smooth aberrant segments more distant than this number of CNStates	If this option is chosen, CNState runs which are farther apart than the "smoothing maximum jump limit" are not smoothed. For example, if the smoothing maximum jump limit is set at 1, then adjacent segments with CNState 3 and 5 are not smoothed.
Join Gain or Loss CNState runs separated by no more than this number of markers of normal state data	If this option is chosen, only Gain or Loss CNState Runs which are separated by less than a threshold number of markers of normal state data are joined. For example, if the marker threshold is set at 50, then CNState runs separated by more than 50 markers of normal state data will not be joined.
Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than this distance measured in kbp	For the CytoScan 750K and HD Arrays, the default smoothing and joining rules are: • Smooth Gain or Loss CNState runs to the most common marker value, then generate segments. • Join any "split" CNState runs separated by no more than 50 normal-state markers. • Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than 200 kbp For SNP 6 arrays, the default smoothing rule: • Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.
Limit the joining of CNState data (which flanks normal state data) to not join aberrant segments more distant than this number of CNStates	Smoothing to the most common marker state value is only applied to contiguous CNState runs of the same type (gain or loss).

IMPORTANT! If multiple smoothing and/or joining checkboxes are selected, all criteria must be met to smooth and/or join the segments.

After smoothing and joining, the marker count, mean marker distance and confidence values get

recalculated. For more information:

- See "About smoothing" on page 121.
- See "About joining" on page 122.

About smoothing

Note: The following examples are for a case where the expected copy number is 2. Similar calculations take place for the X and Y chromosomes where the expected copy number may be 0, 1, or 2, depending on gender and whether the segment is located within or outside of the PAR region.

If you have a contiguous set of segments with gain values (for instance, of CN State values of 3 and 4), with no markers of copy number 2 or lower, **without smoothing** they are treated as a series of individual gain segments. The same rules apply to a set of segments with loss values of 0 or 1.

If you have a contiguous set of markers with gain values of 3 and 4, with no intervening markers of copy number 2 or lower, **with smoothing** they are consolidated into a single gain segment. (Figure 118)

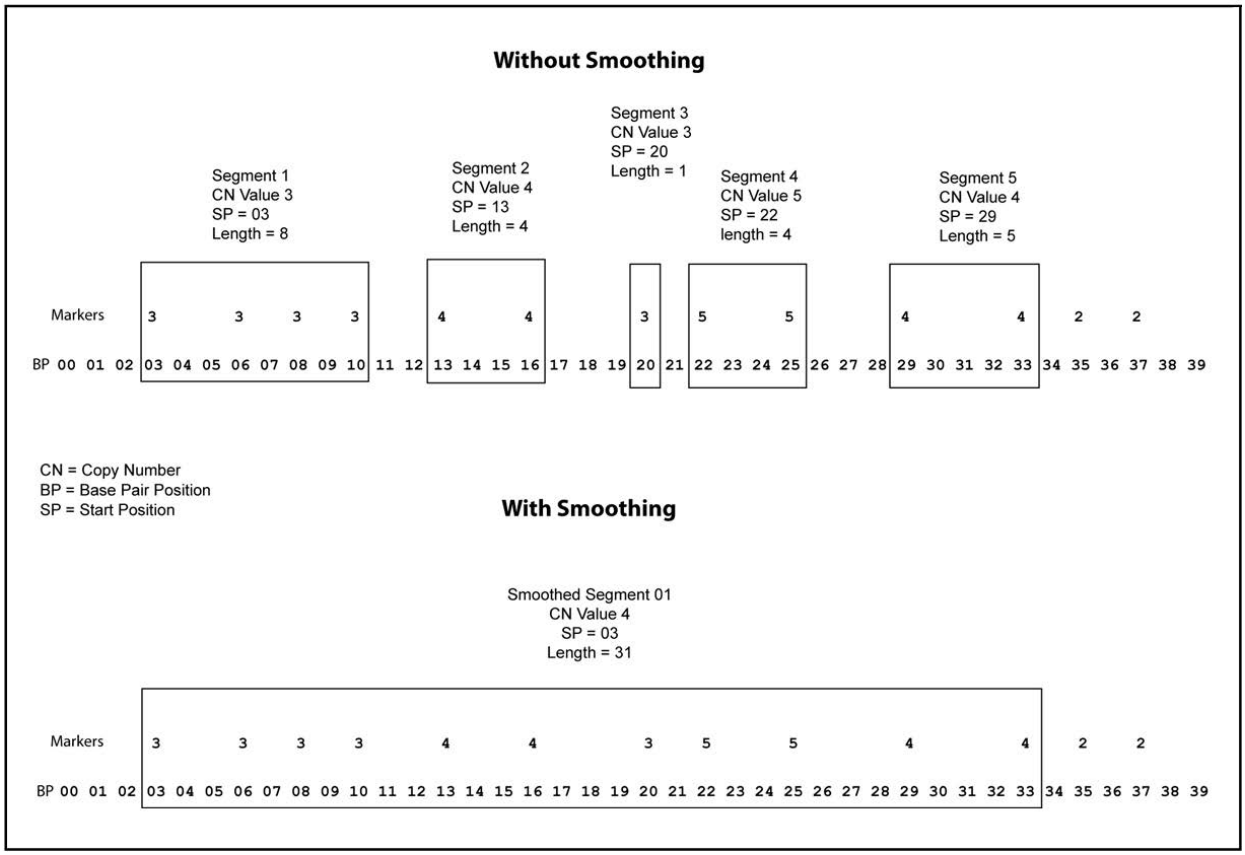


Figure 118 Simplified schematic representation of smoothing.

If you have a contiguous set of markers with loss values of zero and one, with no intervening markers of copy number 2 or higher, after smoothing, they are consolidated into a single loss segment.

The smoothing process is the same as the process automatically performed by the Segment Reporting Tool in GTC. Different methods are used to assign the CN state value and perform the confidence calculations, as described in the following section. See the GTC User Manual for more information.

Copy Number State for Smoothed Segments

The median CNState of all the markers in the Segment is assigned as the Copy Number State value for the new smoothed segment. The median will thus always be either an integer or a half integer (like 3.5).

For all the half-integer cases:

- Gains are rounded up to the next full integer (3.5 goes to 4)
- Losses are rounded down to the next full integer (0.5 goes to 0, 1.5 goes to 1).

About joining

The joining options enable you to join segments with the same type (gain or loss) aberrant CNState that are separated by no more than a specified number of normal-state markers or by no more than a specified distance of normal-state data (Figure 119).

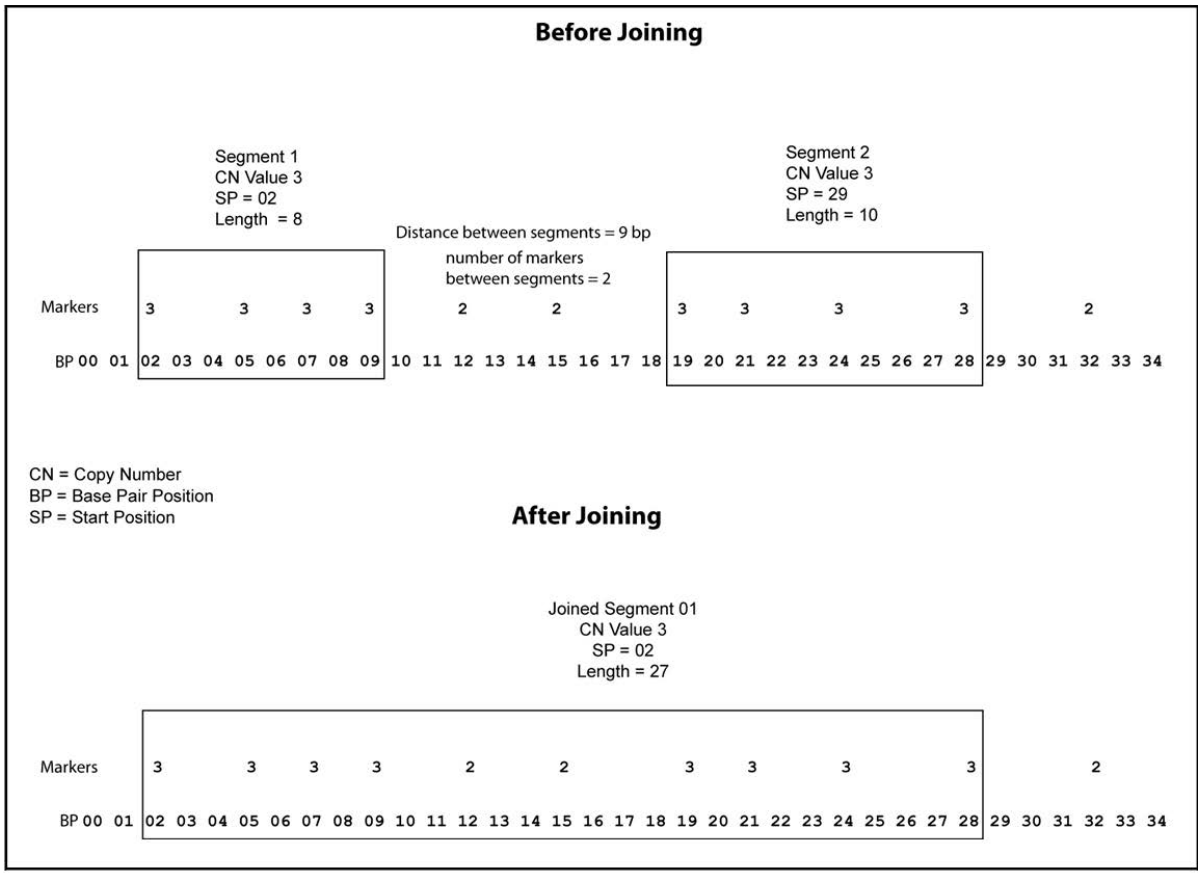


Figure 119 Simplified schematic representation of joining.

The equivalence of CNState of the segments to be joined could have happened as a result of smoothing, or been from "raw" unsmoothed segments with the same CNState.

XON segment merging

Note: Implementation of the new Whole Genome Copy Number segmentation algorithm may make XON merging no longer necessary in your workflow.

XON Merging is on by default. XON Merging combines consecutive XON segment calls of the same Type (XON Region Gains or XON Region Loss) into a larger segment. After an XON segment of a different Type (normal state or gain/loss) is encountered, the XON Merge is terminated. A Merged XON segment is represented as the larger segment in the Segments Table.

Note: The individual XON segments can still be viewed within the rectangle, provided the appropriate Levels are selected in the Filter Settings. In addition, the breakpoints of the Merged XON Segment will stop with an XON segment and may not line up exactly with the probe level data.

Note: A merged XON segment is assigned to the lowest XON annotation number within that merged segment. For example, if a merged XON segment overlaps XON Regions annotations of Level 1 and Level 3, the whole merged XON segment are considered Level 1.

The Merged XON segment is represented by the transparent rectangle. (Figure 120) If you want to turn off this feature see "Turn off XON merging".

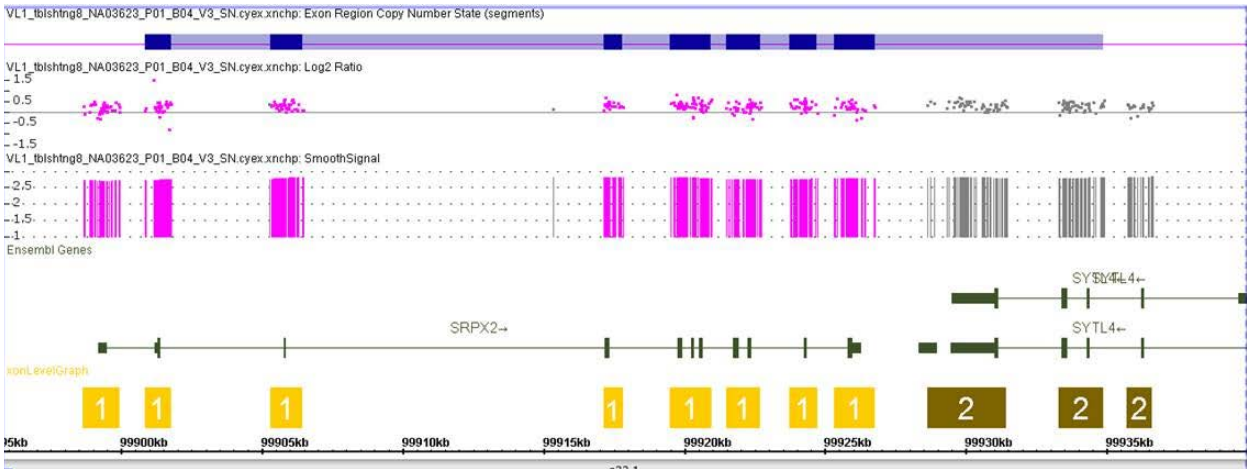


Figure 120 Merged XON segment example.

Turn off XON merging

1. To turn off XON Merging, go to **Preferences → User Configuration**.
   The User Configuration window appears. (Figure 121)

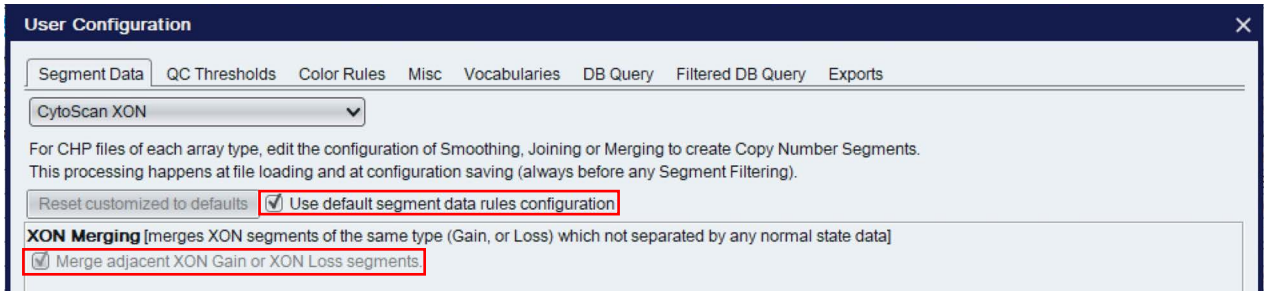


Figure 121 User Configuration window, Segment Data tab.

2. Click the Segment Data tab, then select CytoScan XON from the array dropdown list.
3. Deselect the **Use default segment data rules configuration** checkbox
4. Deselect the **XON Merging** checkbox.
5. Close the ChAS Browser, then reopen it.
   XON Merging is now off/disabled.

Set QC parameters in the ChAS Browser

ChAS checks the analysis results files for certain QC values. The software notifies you if the QC parameters do not meet the thresholds.

Note: Custom QC metrics can be viewed in both the Analysis Workflow and the Browser. However, any custom settings you wish to use, have to be entered separately in both the Analysis Workflow and the Browser. To create custom QC settings in the Browser see below. To create custom QC settings in the Analysis Workflow, see "Create your own custom QC setting" on page 54.

You can adjust the QC threshold values or select different QC metrics.

IMPORTANT! Selecting different QC thresholds is not recommended.

Note: When using custom QC thresholds for CytoScan™ HTCMA in RHAS, these custom thresholds will also need to be updated in ChAS to reflect the desired QC thresholds.

View QC thresholds

1. Click **Preferences** → **Edit User Configuration**.
   The User Configuration window appears.
2. Click the **QC Thresholds** tab. (Figure 122)
3. Select an array type from the dropdown list.

Note: QC parameters are specified per array type.

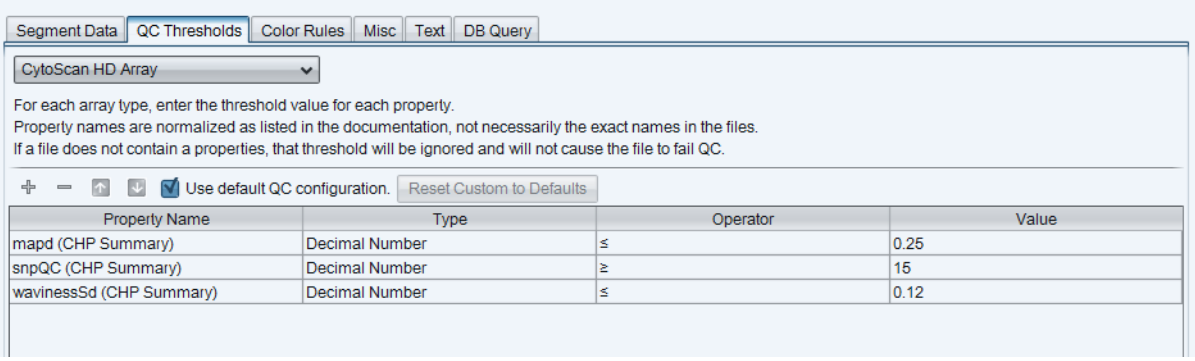


Figure 122 CytoScan HD array QC Thresholds default settings.

QC Thresholds tab options and descriptions

Table 16 QC Thresholds tab options.

Option	Description
Property Name	**SNPQC** is a QC metric for SNP probes that is derived from polymorphic (SNP) probes **MAPD** is a QC metric for all probes used to determine copy number that is derived from both polymorphic (SNP) and non-polymorphic (CN) probes **Waviness SD** is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. **nd SNP QC** is a QC metric for SNP probes that is derived from polymorphic SNP probes in normal diploid regions. **nd Waviness SD** is the same measure as Waviness SD, but only calculates in those regions that are identified as normal diploid. **Cel Pair Check** is a test that inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel. (OSCHP only) **DQC (DishQC)** measures the amount of overlap between 2 homozygous peaks created by non-polymorphic probes. DQC of 1 is no overlap, which is good. DQC of 0 is complete overlap, which is bad. **QC Call Rate** is the percentage of autosomal SNPs with a call other than NoCall (measured at the Sample QC step). **SMN MAPD** is a QC metric for all probes used to determine copy number that is derived from both polymorphic (SNP) and non-polymorphic (CN) probes calculated during SMN analysis. **SMN WavinessSD** is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation calculated during SMN analysis. **Note:** The property names are from the header information of the xxCHP file. QC thresholds are not established for ReproSeq™ Aneuploidy files, but QC metrics are displayed in the QC and Sample Info tab.
Type	Value or algorithm used for that type of QC.
Operator	The type of comparison performed.
Value	Value assigned to the threshold.

Table 17 Default Copy Number QC thresholds.

Array type	QC parameter				
	MAPD	snpQC	Waviness SD	ndsnpQC	ndWavinessSD
CytoScan™ 750K, 750K Accel, HD and HD Accel Array	≤0.25	≥15.0	≤0.12	–	–
CytoScan™ XON Arrays	≤0.20	≥10.0	≤0.08	–	–
CytoScan™ (Normal Diploid Analysis)	≤0.25	≥15.0	≤0.12	≥15.0	≤0.12
CytoScan™ HTCMA	≤0.28	≥10	= 0.07	–	–
CytoScan™ Optima Array	≤0.29	≥8.5	–	–	–
Genome-Wide Human Array SNP 6.0	≤0.35	–	–	–	–

Table 18 Default Genotyping QC thresholds.

Array type	QC parameter	
	DQC	QC Call Rate
CytoScan™ HTCMA Arrays	≥0.88	98.5%

Table 19 Default SMN QC thresholds.

Array type	QC parameter	
	SMN MAPD	SMN WavinessSD
CytoScan™ HTCMA Arrays	≤0.35	≤0.1

Table 20 Default QC metrics for OncoScan™ arrays.

Array type	QC parameter		
	MAPD	ndSNPQC	CelPairCheckStatus
OncoScan™ arrays	≤0.3	≥26	Pass

Note: The waviness SD metric is applicable to blood and cell line data. The waviness SD metric is not intended for alternative sample types such as solid tumor or FFPE samples in which the results may vary as a result of the biological complexity. For these sample types, it is recommended using **nd Waviness SD**.

Effect of SNPQC value (for a Single Sample Analysis) on the Allele Difference Track. (Figure 123)

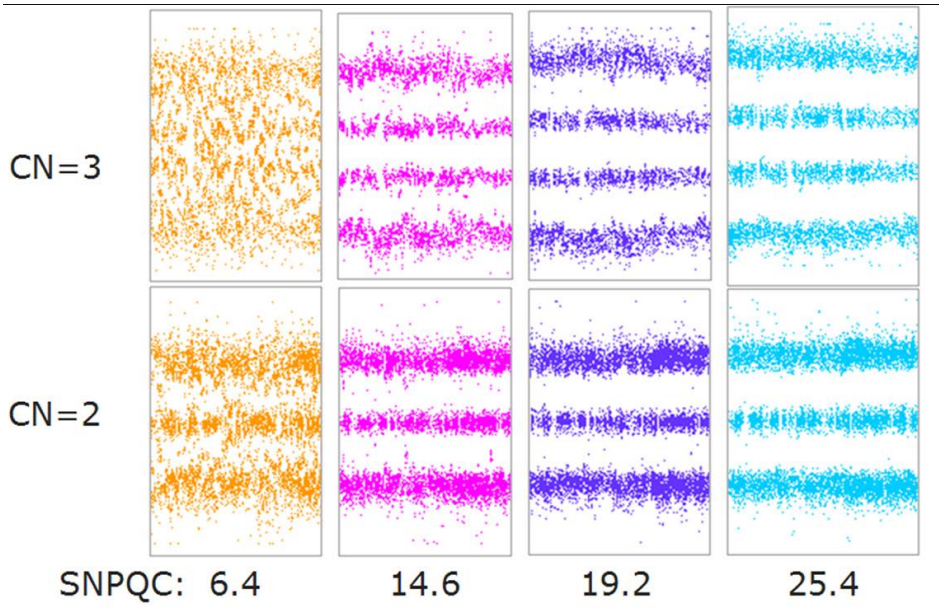


Figure 123 SNPQC Metric vs. Track Quality.

SNPQC is one of the CytoScan™ within-array QC metrics which provides insight into the overall level of data quality from a SNP perspective. When evaluating the SNPQC values, the key consideration is to ensure that the threshold is exceeded. The quality of the SNP allele data is compromised, from an interpretation perspective, when the SNPQC values are below the recommended acceptance threshold as illustrated by the 2 left most graphs representing the 2 and 3 copy allelic state.

For the CytoScan™ HD Array, when the SNPQC value is below 15, (as illustrated by the data in the 2 graphs above), the noise within the array is higher than expected. This in turn, compromises the overall data quality and clarity of the results. However, when the SNPQC value is above 15, the consideration is whether the SNPQC value is above or below the threshold value and not the absolute magnitude.

As long as the SNPQC value exceeds the threshold, there is a retention in the data quality as illustrated by the graphs which demonstrate clear allelic data across a broad range of SNPQC values that exceed the recommended threshold. The threshold was determined from thousands of arrays processed across multiple reagent lots, operators, and sample aberration types. SNPQC is one of the metrics used to assess array quality and should be helpful in determining which experimental data sets are of satisfactory quality to continue with subsequent interpretation.

Add a QC property

1. Click **Preferences** → **Edit User Configuration**.

   The User Configuration window appears.
2. Click the **QC Thresholds** tab. (Figure 124)

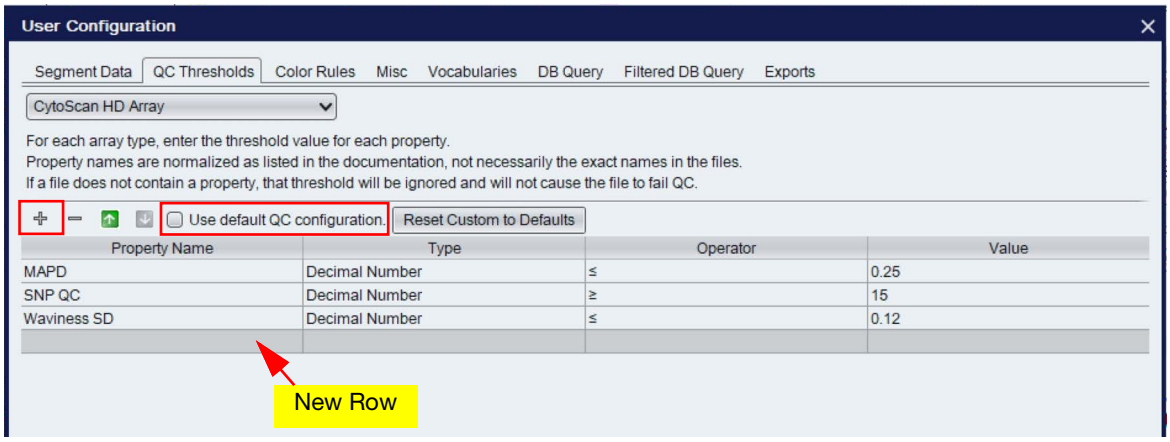


Figure 124 Adding a new row to the QC Thresholds table.

3. Select an array type from the dropdown list.
4. In the QC Thresholds tab, deselect the **Use default QC configuration** checkbox.
5. Click the **Add** icon .

   A new row appears in the table.

   To delete a property row, select the row, then click the **Remove** icon.
6. Click the **Property Name** field, then enter a new QC property name.
7. Click the **Type** field to select **Decimal Number** or **Whole Number**.
8. Click the **Operator** field to select an operator from the dropdown list.
9. Double-click the **Value** field to enter the threshold value for the newly added QC property.
10. To add another QC property, repeat steps 5–9.
11. Click **OK** to apply the newly added QC threshold(s).

Edit an existing QC threshold

1. Click an existing **Property Name**, then edit its QC property name.
2. Click the **Type** field to edit its **Decimal Number** or **Whole Number**.
3. Click the **Operator** field to choose a different operator from the dropdown list.
4. Double-click the **Value** field to edit the current threshold value.
5. To edit another existing QC property, repeat steps 1–4.
6. Click **OK** to apply the newly edited QC threshold(s).

Histogram data

Load histogram data

IMPORTANT! You must be logged into the ChASDB to view Histogram data.

The histograms load by default. If they are not currently displayed, click **ChAS DB → Refresh ChAS DB data** to view them in the ChAS Browser.

Note: The histograms are only available for NetAffx™ Genomic annotation files for genome build Hg19. The browser produces an error message, if you try to load Hg19-based histograms while a hg18 or hg38 based NetAffxGenomeAnnotation is currently displayed.

Change the default histogram filters

1. From the File tree (upper left column), locate the Default Histogram entry.
2. Right-click **Default Histogram**.

   A menu appears.
3. Click **View/Edit Properties**.

   The File Properties window appears. (Figure 125)

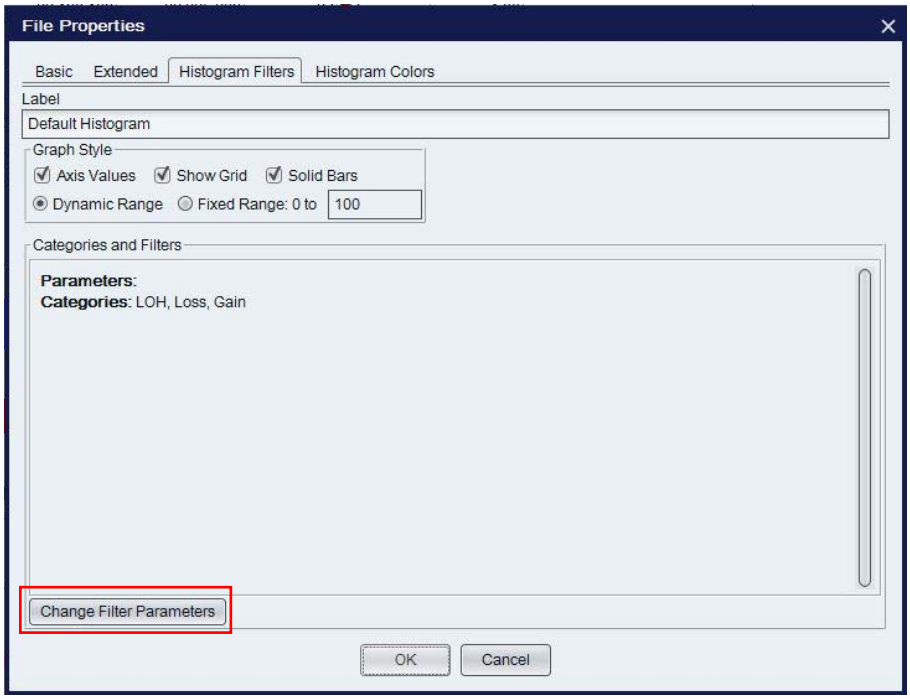


Figure 125 File Properties window and default **Histogram Filters** tab.

4. Click the **Histogram Filters** tab, then use its checkbox(es), selections, and radio buttons to modify the default Histogram's factory settings.
5. Click the **Change Filter Parameters** button to select new filter settings.
6. Optional: To change filter parameters, click **Change Filter Parameters**.

   The Set Filter Parameters window appears. (Figure 126)

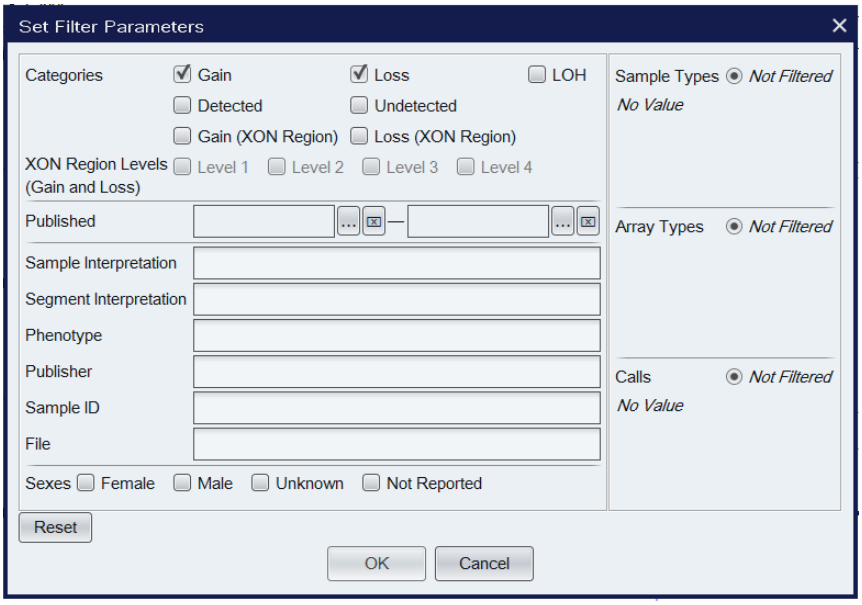


Figure 126 Set Filter parameters window.

7. Use this window's checkboxes, pre-populated entries, and/or the provided text fields (to enter the filter parameters you want).
8. Click **OK** to save your changes.

Note: If using the aDGV containing segments from both HD and XON arrays, use the Categories filters to limit the histogram data to only HD (check Gain and Loss) or only XON (Gain XON Region and Loss XON region).

Change the default histogram colors

1. From the File tree (upper left column), locate the Default Histogram entry.
2. Right-click **Default Histogram**.

   A menu appears.
3. Click **View/Edit Properties**.

   The File Properties window appears.
4. Click the **Histogram Colors** tab. (Figure 127)

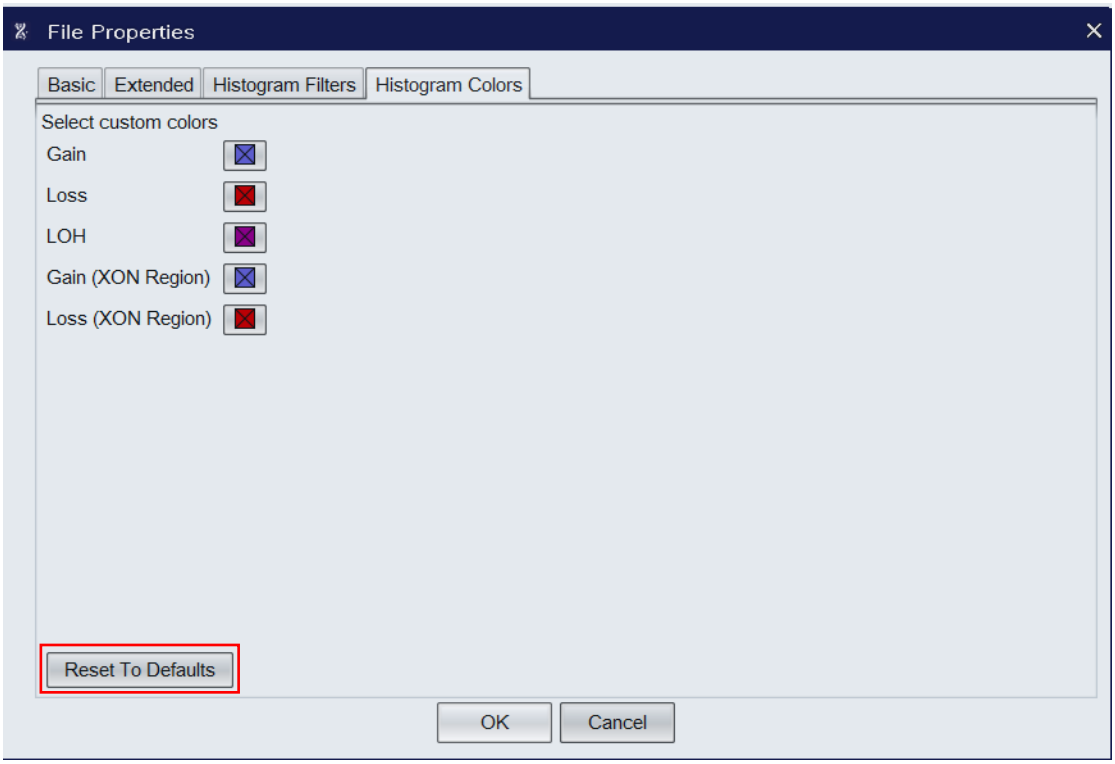


Figure 127 File Properties window and Histogram Colors tab.

5. Click the color square representing the histogram track you want to change.

   A change color window appears.
6. Select a new color, then click **OK**.
7. Repeat steps 5–6 as needed.
8. Click **OK** to save your color changes or click **Reset to Defaults** to return to the default color settings.

Add filtered histogram data

You can add additional histograms filtered on certain properties for viewing in the Detail View.

1. Go to **ChASDB → Add Histogram**

   The **Add a Histogram** window appears. (Figure 128)

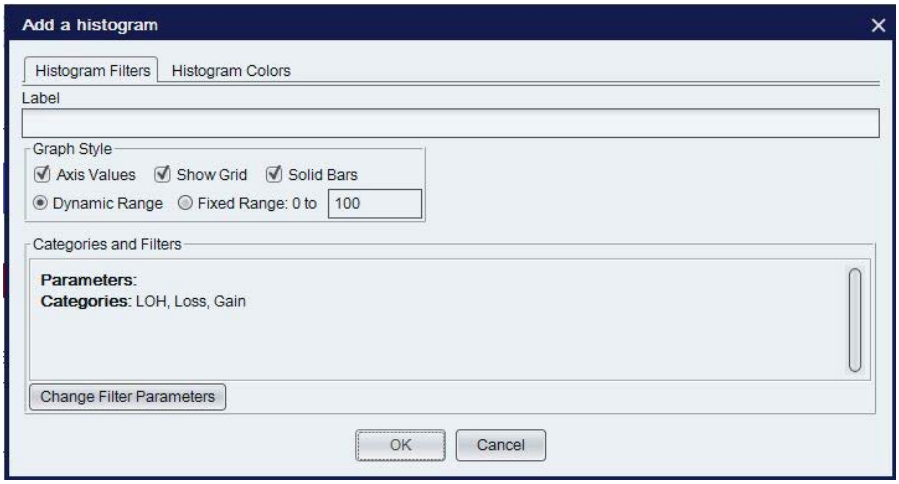


Figure 128 Add a Histogram window.

2. In the **Label** text box, enter a name for the histogram.
3. In the **Graph Style** pane, select the appropriate checkbox(es) and/or radio button(s) to define your filtered Histogram's graphic style.
4. Use this window's checkboxes and radio buttons to create the desired graph style. Click the **Change Filter Parameters** button to select filters to be applied to your new histogram. Click the **Histogram Colors** tab to select colors for your new histogram tracks. See "Change the default histogram colors" on page 131 for details.
5. Click **OK**.

   The histogram is added to the bottom of the File tree list. The histogram can be moved to a different position in the Detail View by clicking the name of the histogram, then dragging it to the desired location within the File tree.

You can access what segments are in a particular bin of the histograms by right-clicking the histograms and selecting, **Show Histograms items in bin**. For more details, see "Segment intersections" on page 366.

Note: When right-clicking to Show Histograms in bin, the percentages for DB Coverage Count and DB Overlap Count columns in the Segment Intersections window represent the percentage intersection with the query region (the light blue vertical bars) shown in the Segment Intersections window. For more details on this Segment Intersection window, see Figure 423 on page 367.

Remove a histogram

1. Locate the Histogram you want to remove in the Files window pane. (Figure 129)
2. Right-click it, then click **Close**.

Note: Closing a Histogram removes the Histogram from the Detail View. To view this Histogram again, you must recreate it using the Add Histogram steps listed above. See "Add filtered histogram data" on page 132.

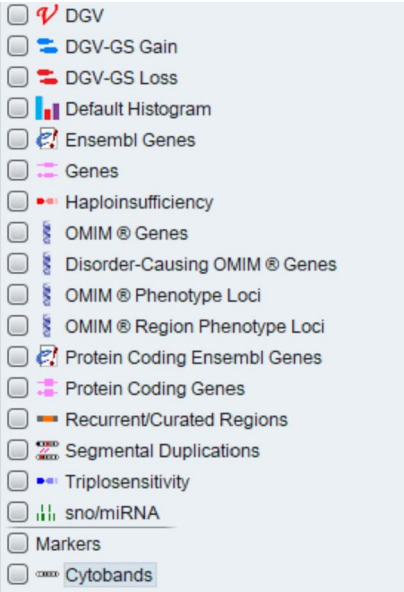


Figure 129 Right-click a Histogram to remove it.

7 View data

Display options of analysis results data

- Graphic Displays

  See “Display data in graphic views” on page 142.
- Tables

  See “Display data in table views” on page 305.

After the data is loaded, you can:

- Filter the segments by Segment Parameters to hide segments that do not meet your requirements for significance. See “Filter segments” on page 205.
- Select a region information file for use as a CytoRegion file and:
  - Perform differential filtering for segments in CytoRegions and in the rest of the genome.
  - Display only segments that appear in CytoRegions using Restricted Mode. See “Use CytoRegions” on page 251.
- Select a region information file for use as an Overlap Map and use the Overlap filter to conceal segments that overlap with the Overlap Map items. This functionality may be helpful for tracking or filtering out benign copy number change regions. See “Use the overlap map and filter” on page 262.
- Add selected features of the genome to new or existing Region (AED) files, and edit annotation data on existing annotations. See “Create and edit AED files” on page 269.
- Prepare reports on your findings by exporting graphics and table data in PDF and other formats. See “Export results” on page 386.
- Save setups of ChAS for different tasks in user profiles and named settings. See “User profiles and named settings” on page 408.

Overview of ChAS window components

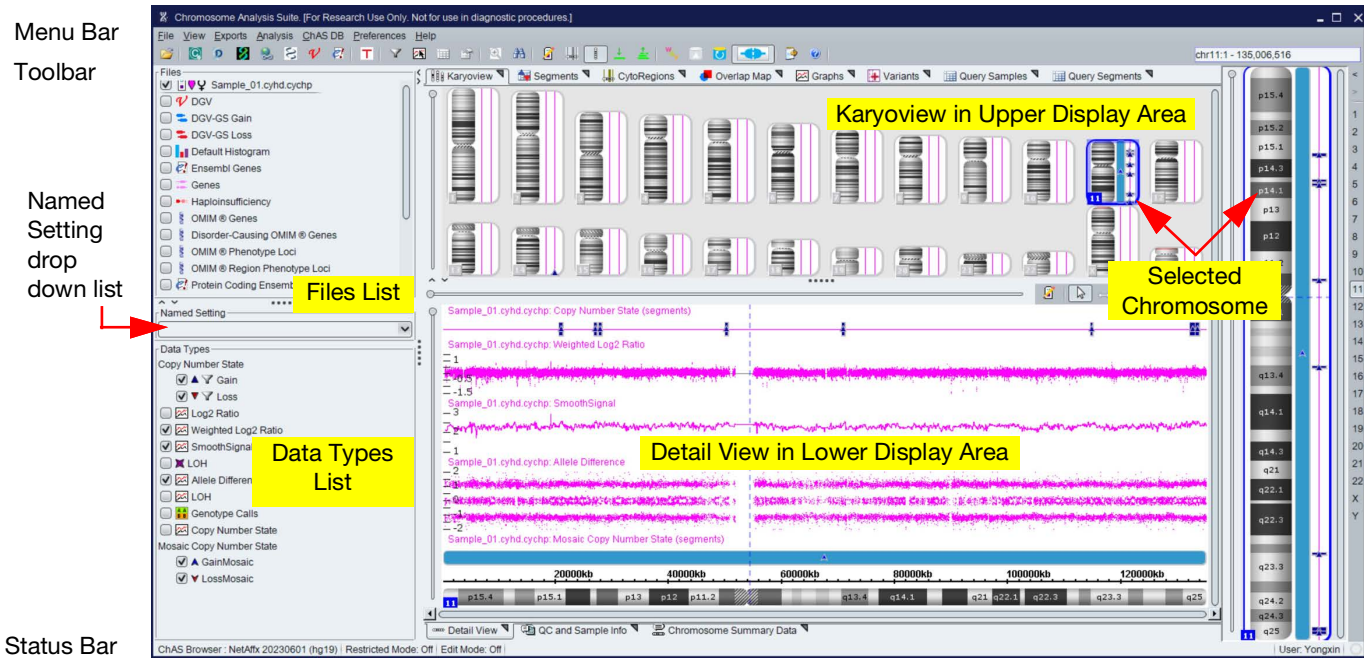


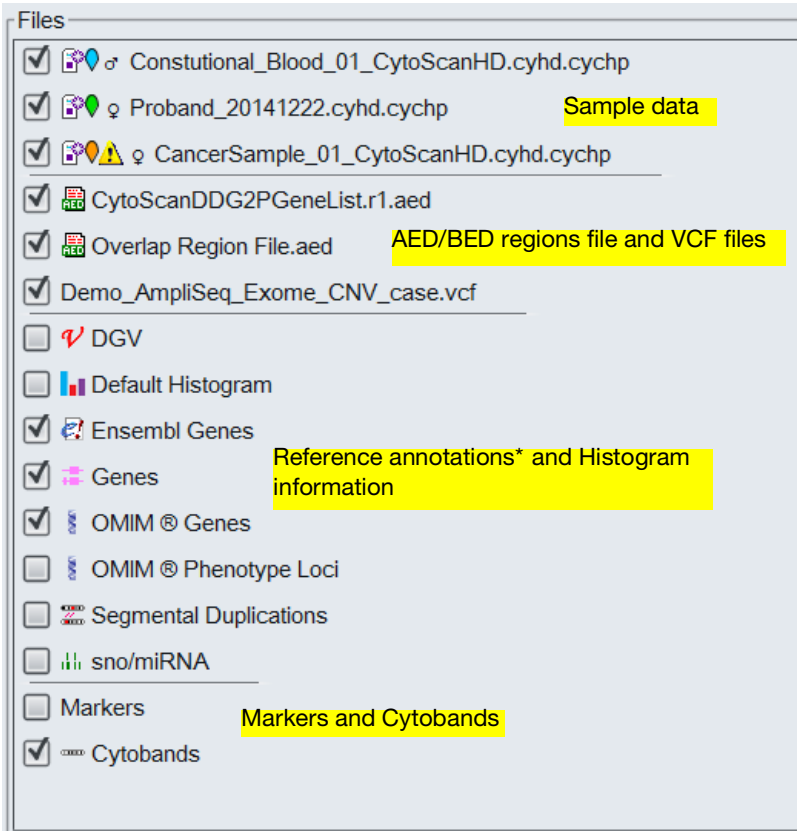
Figure 130 ChAS window.

The ChAS window components (Figure 130) include:

- **Menu Bar**: Access to the functions of the software.
- **Toolbar**: Quick access to commonly used functions.
- **Files List**: Displays data and annotation files that can be displayed. (page 136)
- **Data Types List**: Displays the type of data available in the files. (page 137)
- **Named Settings**: Displays a list of the previously saved display settings for ChAS. (page 138)
- **Status Bar** (page 139): Displays:
  - Software status
  - ChAS Browser's NetAffx™ Genomic Annotation file version.
  - hg version
  - The annotation or probe that your mouse pointer is nearest (in the Detail View).
- **Display Area** (page 139): Displays the following data in graphical and table formats:
  - Analysis results graph data.
  - Detected Segments
  - Histograms
  - Regions
  - Reference Annotations

Files list

The **Files** list (Figure 131) displays the different sources of data and annotations that are loaded in the Chromosome Analysis Suite. Files are grouped by type in the Files list.



*Reference annotations displayed may be different than shown above depending on the NetAffx™ Genomic Annotation file loaded.

Figure 131 Files list.

Displayed files grouped by type:

- Sample Data

  Colored nibs display the color used for the data lanes for that sample in the Karyoview, Selected Chromosome View, and Detail View. The appropriate gender symbol is displayed to the right of the colored nib.

  If a loaded file has a QC parameter that is out of range, an alert symbol appears next to the file name. (Figure 132)

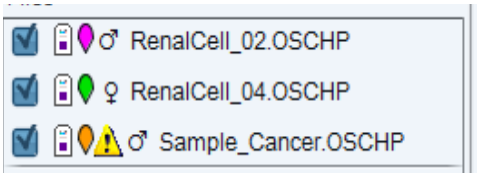


Figure 132 QC alert for Sample Data files.

- Region Information Files
- Icons indicate the file type (AED or BED) and whether the loaded files have been selected as a CytoRegion file or Overlap Map file.

- Reference Annotations (loaded during software start-up). **Note:** Icons indicate the annotation type.
- Histogram information (loaded during software start up if connected to ChAS DB).
- Cytobands (Separated from other reference annotations because cytobands cannot be moved in the displays)

See "Select data for display" on page 178. for information on using the Files list to select loaded data and region information files, and reference annotation for display.

You can export the feature information in these files to a new AED region information file. See "Export information in AED or BED format" on page 296.

Data Types list

The **Data Types** list (Figure 133) shows the types of data available for display in the Karyoview, Selected Chromosome View, and Detail View. The available data types may vary, depending upon the type of sample data available. See "Introduction to loading data" on page 110.

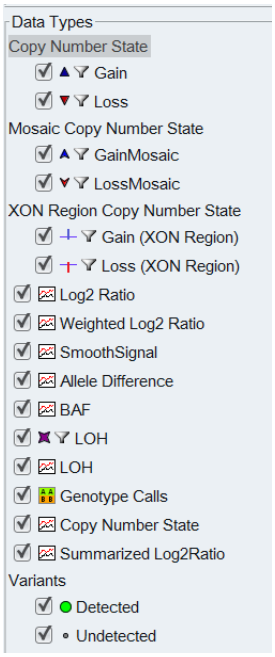


Figure 133 Data Types list.

The Data Types list enables you to select from Segments data and Graph data.

The Segments data is displayed graphically in:

- Karyoview
- Selected Chromosome View
- Detail View

If filtering is applied to a segment type, a funnel icon appears next to the segment symbol in the list.

Graph data, indicated by the icon, is displayed only in the Detail View. See "Detail view" on page 158 for more information.

Unselected data is also concealed from the different tables and graphs.

See "Select Data Types for display" on page 180. for information on using the Data Types list to select different data types for display.

Named Settings

The **Named Settings** dropdown list (Figure 134) enables you to apply a previously created setting for ChAS. The settings may include things like:

- Segment Filter and Overlap Map Filter settings
- Types of data to be displayed
- Restricted Mode ON/OFF status. See “Use restricted mode” on page 259.

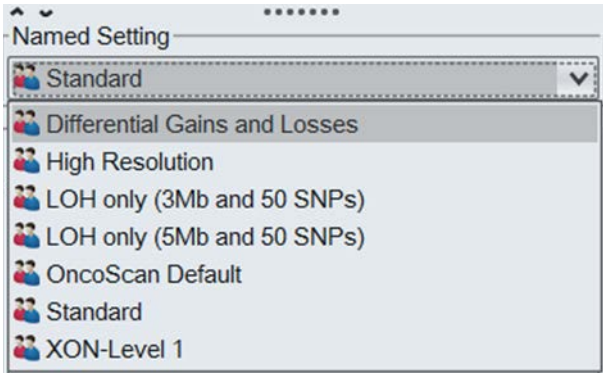


Figure 134 Named Settings.

Note: Default Named Settings (indicated by the icon) should not be deleted from the system because they are shared by all user profiles.

See Chapter 20, "User profiles and named settings" on page 408 for information on creating and using Named Settings.

Note: The OncoScan Default Named Setting has no filters applied so all segments called by the TuScan algorithm can be viewed. Users can create their own appropriate custom filter Named Setting for their data, see "User profiles and named settings" on page 408.

Status bar

The status bar (Figure 135) (very bottom of browser) displays information on:

- NetAffx™ Genomic Annotation database and its hg version
- Restricted Mode status (See "Use restricted mode" on page 259.)
- Edit Mode status (See "Use edit mode" on page 212.)
- Cursor (Mouse Over) Position
- User Profile ID

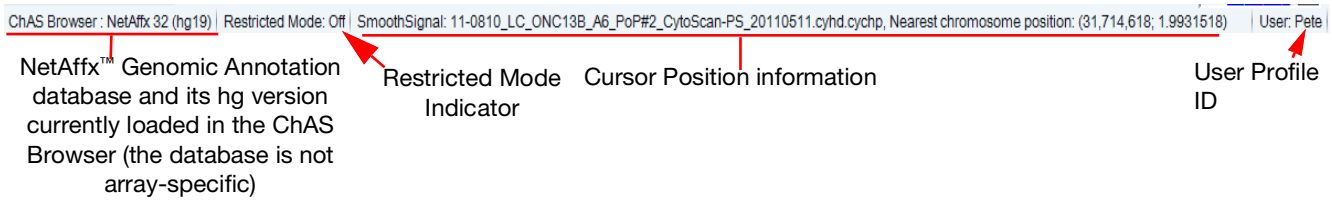


Figure 135 Status Bar.

Display area

The Display Area (Figure 136) is divided into 3 panes:

- "Upper panes" on page 140.
- "Selected chromosome view" on page 140.
- "Lower panes" on page 140.

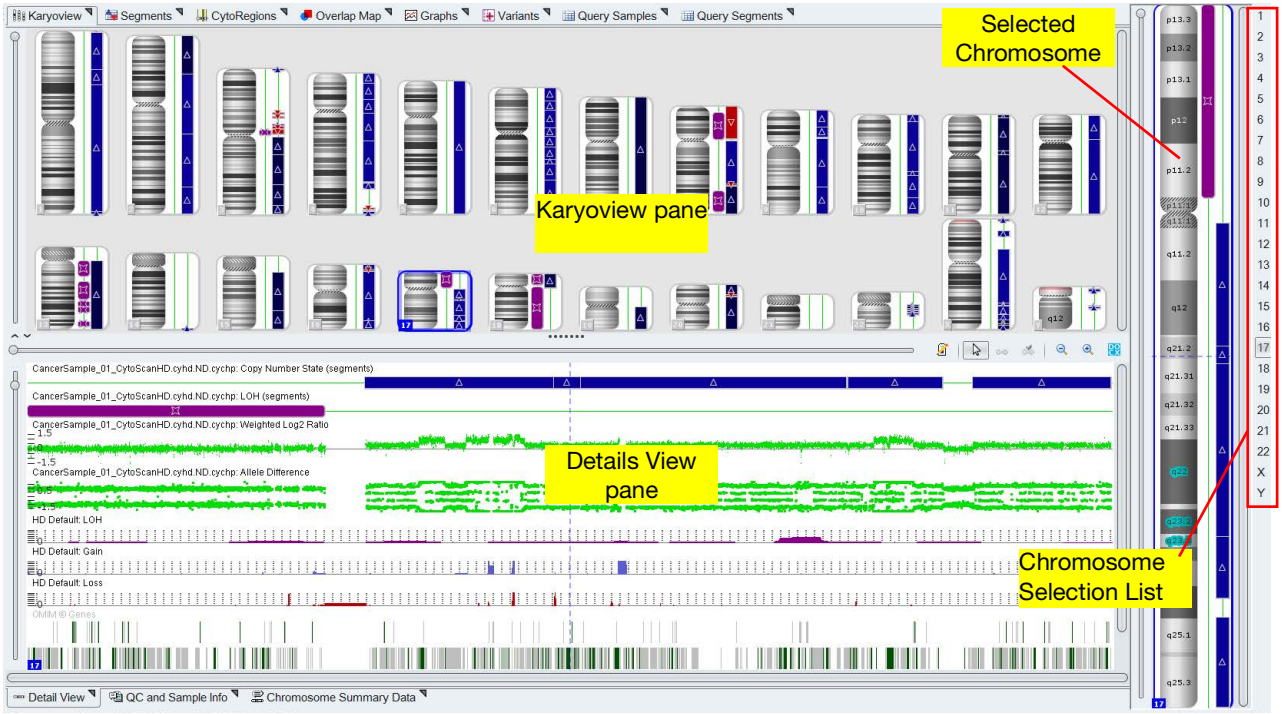


Figure 136 Display Area showing Segments Table and Detail View.

The tabs in the upper and lower panes display different types of data, in both graphical and table formats. Data from the same sample files is displayed in all 3 panes.

You can display a pane in a separate window by clicking the icon on the tab.

To close the window and return the information to the tab panel, click the icon in the window.

Upper panes

The Upper pane displays the following data in graphics and table formats:

- "Karyoview" on page 143: Displays selected segment types for selected sample files for all chromosomes.
- "Segments table" on page 314: Displays a list of the detected segments in the selected sample files.
- "CytoRegions table" on page 256: Displays a list of the Regions in the AED/BED file selected as the CytoRegion file. Includes information on detected segments which lie in CytoRegions.
- "View the overlap map table" on page 266: Displays a list of the Regions in the AED/BED/Reference Annotation file selected as the Overlap Map. Includes information on detected segments that are overlapped by Overlap Map Items.
- "Graphs table" on page 321: Displays marker data for the loaded and selected xxCHP files.
- "Variants table" on page 326: Displays somatic mutation data from OncoScan CNV Plus and Carrier Variant data from CytoScan HTCMA arrays.
- Query Samples Table: Displays sample level results from searches performed on the ChAS DB.
- Query Segments Table: Displays segment level results from searches performed on the ChAS DB.

Selected chromosome view

The Chromosome View displays detected segments in selected sample files for the chromosome selected in the Karyoview, while the Chromosome Selection List (far right column) displays its number.

See "Selected chromosome view" on page 156 for more information.

Lower panes

The Lower Pane displays:

- "Detail view" on page 158: Displays the selected section of the chromosome displayed in Selected Chromosome View and includes:
  - Detected segments, variants, and graph data in selected xxCHP files.
  - Histograms, AED/BED file regions and annotations.
  - Reference annotation files
- "QC and sample info tab" on page 330: Displays QC metric information as well as information about the loaded Sample and Region (AED) files.
- "Chromosome summary data" on page 337:
  - Summarizes particular data across each chromosome in the loaded sample data files (for example, proportion of each chromosome found to be in the state of LOH).
  - Calculated Properties: Calculates the Percent Autosome LOH in the sample based on the LOH filter setting.

Change the NetAffx™ genomic annotation file version

1. At the top menu bar, click **File** → **ChAS Browser NetAffx Genomic Annotation file version**.
2. From the **Select NetAffx Database** dropdown list (Figure 137), click to select the NetAffx™ build version you want, then click **OK**.

Note: If there are loaded xxCHP files with hg version different from the selected NetAffx™ database, a message appears stating that these data files will be closed (in both the ChAS Browser and the MSV) *before* the NetAffx™ annotations are loaded.

Note: More current NetAffx™ Genome Annotation files have the date the file was generated embedded into the file name. To load the most current file from your library folder, select the file with the most recent date.

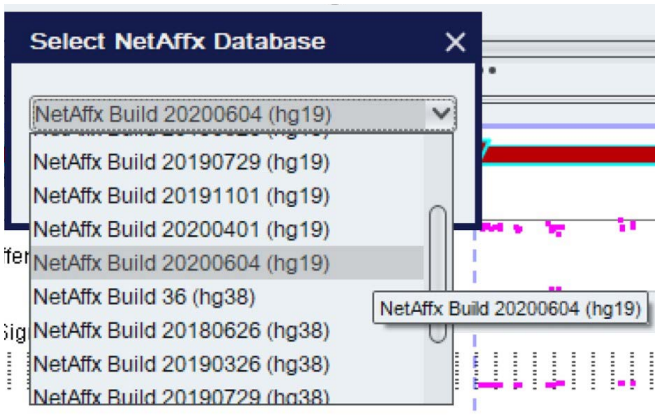


Figure 137 Select a NetAffx™ Genomics Annotation file version.

For more information on what types of data is in the database files and how this information varies in different versions, see "ChAS Browser NetAffx™ Genomic Annotations" on page 444.

8 Display data in graphic views

ChAS provides multiple graphic views for detected segments and other data.

Use these graphic views to:

- Get an overview of the detected segments.
- Get an overview of segments stored in the ChAS DB.
- Compare segments between samples.
- View sample data for the whole genome.
- Drill down to examine areas of interest in more detail.
- View the graph and marker information used to generate the detected segments.
- Take advantage of reference annotations and external web sites.
- Create your own Affymetrix Extensible Data (AED) files with regions of interest and annotations.

Graphic views

Data can be displayed in a Karyoview, Whole Genome View, Selected Chromosome View, and a Detailed View, as shown in Figure 138.



Figure 138 Graphic display views.

Data from the same sample files is displayed in all the views at different scales.

If an item in any of the views is selected, the icon for that item is enlarged or highlighted in the views.

Karyoview

The Karyoview (Figure 139) displays a genome-wide view of the detected segments and other data.

In the Karyoview:

- Click a chromosome in the Karyoview to select it.
- Press Ctrl+Left/Right arrow keys to move between chromosomes.
- To jump to chromosome 1, press Ctrl+Home
- To jump to chromosome Y (last chromosome in the Karyoview), press the Ctrl+End.

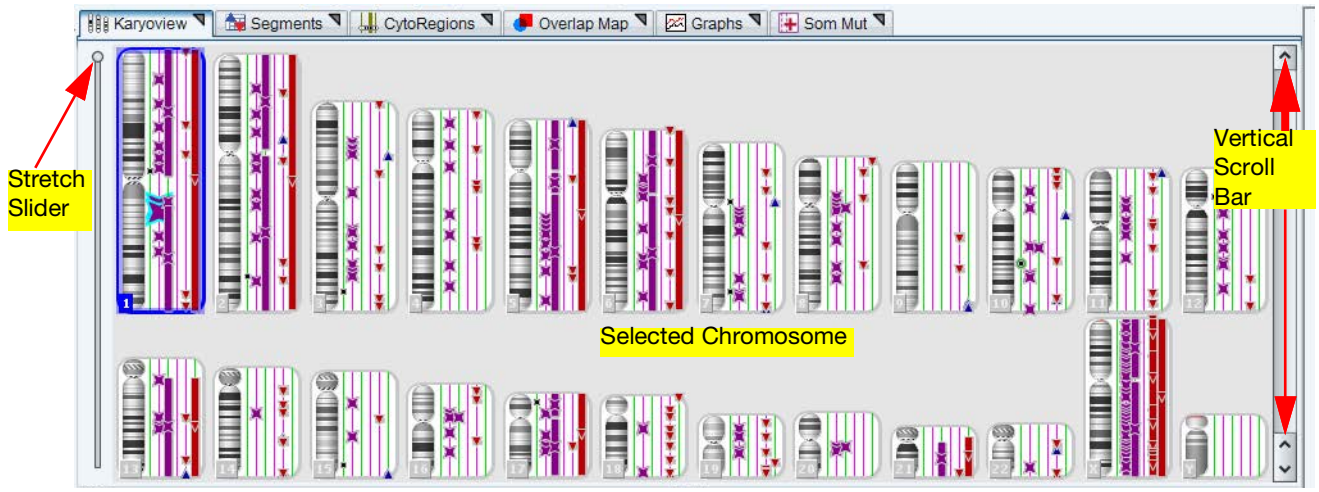


Figure 139 Karyoview Controls.

Using the mouse, click and drag on a selected chromosome to select an area for display in the Detail View. This area is highlighted in the Selected Chromosome View. (Figure 140)

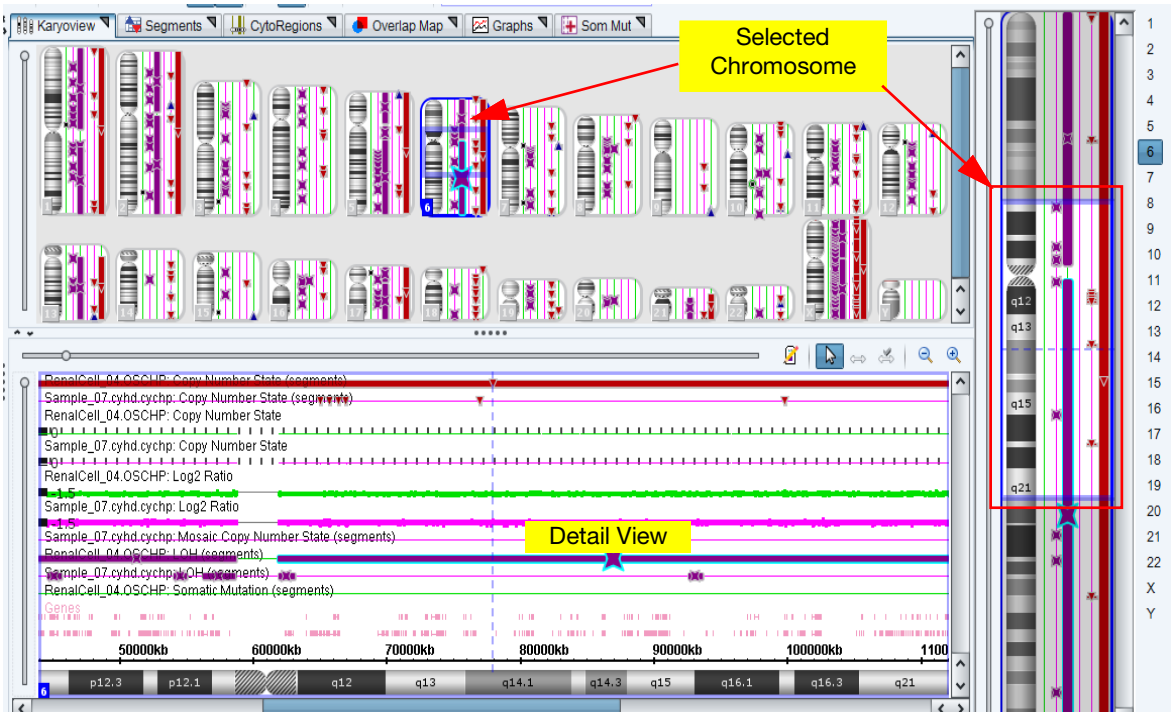


Figure 140 Karyoview, Selected Chromosome View, and Detail View.

Note: To easily remove the blue highlight surrounding the selected chromosome for image captures, go to **View → Hide Karyoview Highlights**.

Use the Stretch Slider and Vertical Scroll Bar to zoom in on a section of the Karyoview. You can also use the mouse wheel as detailed below:

- Alt+mouse wheel stretches the display.
- Mouse wheel scrolls up and down.

The following information is displayed for each chromosome: (Figure 141)

- Chromosome number
- Cytobands
- Segment Data - Uses separate lanes for each sample file and each displayed segment type. Each sample is assigned a unique color in the display that is used for the lane. You can:
  - Change the grouping of samples and segment types. See "Change the grouping of samples and data types" on page 181.
  - Change the position of samples. See "When the lanes are grouped by sample, the different segment types for each sample are kept together in the Karyoview and Selected Chromosome View (Figure 183) and in the Detail View." on page 182 and "Lanes grouped by sample" on page 182.
- Variant Data (only available for OSCHP and RHCHP)
- CytoRegions in selected CytoRegion File.
- Items in selected Overlap Map file.

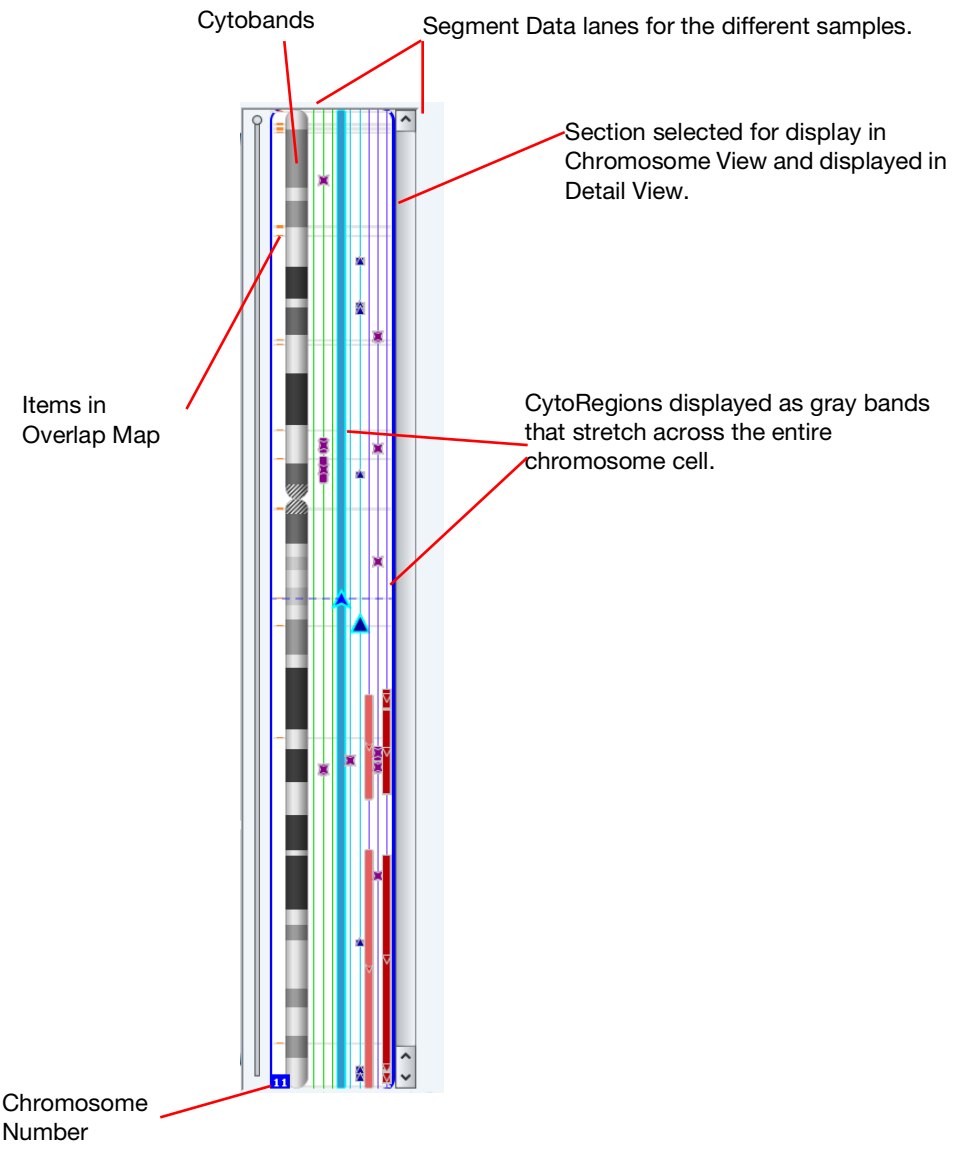


Figure 141 Detail of a highlighted chromosome.

Click a chromosome in the Karyoview to select it for display in the Selected Chromosome View and the Detail View. The selected Chromosome is highlighted in the Karyoview.

The Stretch Slider and the vertical scroll bar controls the vertical stretch of the Display area.

At higher magnifications, more details of the Cytobands are displayed in the Karyoview. You can see Cytoband labels if the display has room.

The portion of the chromosome selected in the Selected Chromosome View and displayed in the Detail View is highlighted in the Karyoview.

If you have selected a CytoRegions file, the CytoRegions are displayed as gray bands that stretch across the entire chromosome cell, from right to left of the Cytobands.

If you assigned an Overlap Map file, the overlap map items are displayed as small rectangles to the left of the cytobands. Its color is the same color used in the Details View.

Note: You can mouse over a feature in the Karyoview, Selected Chromosome View, or Detail View to display a pop-up with information about the feature. Also, you can right-click a feature in the Karyoview or Selected Chromosome View to open a shortcut menu of options, as shown in Figure 142.

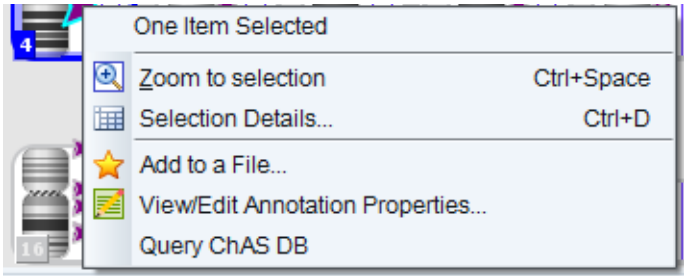


Figure 142 Karyoview shortcut menu.

Whole genome view

The Whole Genome View (Figure 142) displays the following data in a single display from Chromosome 1 to Y for:

- **CYCHP**: Log2 Ratio, Weighted Log2 Ratio, Copy Number State, Smooth Signal, LOH Allele Difference, and B-allele Frequency.
- **XNCHP**: Log2 Ratio, Weighted Log2 Ratio, Smooth Signal, LOH, Allele Difference, and B-allele Frequency.
- **CNCHP**: Log2 Ratio, Copy Number State, Smooth Signal, Allele Difference
- **RHCHP:** Log2Ratio, Smooth Signal, Copy Number State, LOH, Allele Difference, and B-allele Frequency.
- **OSCHP**: Log2 Ratio, Weighted Log2 Ratio, Copy Number State, Smoothed Signal, Allele Difference, and B-allele Frequency.
- **ReproSeq Aneuploidy .zip:** Copy number state data provides the sequencing tile data.

Note: When opening ReproSeq™ result files for the first in the Whole Genome View, the graphs may appear empty. Use **Choose Data** to select the Copy Number Graph type to view the data.

Note: The Y axis on the left represents the data points in that graph (Log2/Weighted Log2 Ratio). The Y axis on the right represents the line graph data (Smooth Signal/Copy Number).

Display the Whole Genome View for a sample(s)

1. Right-click the Sample name in the File Tree (Figure 142), then select **Show WGV**.

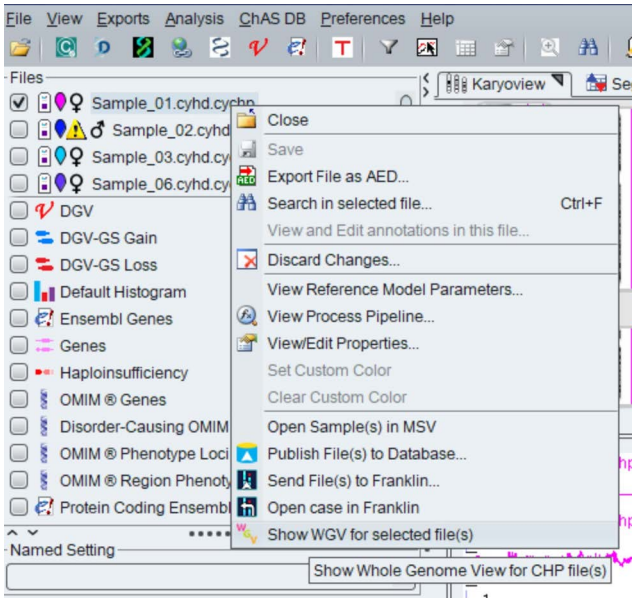


Figure 143 Right-click menu - File Tree.

2. The Whole Genome View for the selected file(s) opens. (Figure 144)



Figure 144 Whole Genome View.

3. To select a different sample(s) to display:
   - Select **File → Choose File**.
   - Click a different file name, then click **OK**.

Change graph types

1. Click **View → Choose Data**.

   The Choose Data window appears. (Figure 145)

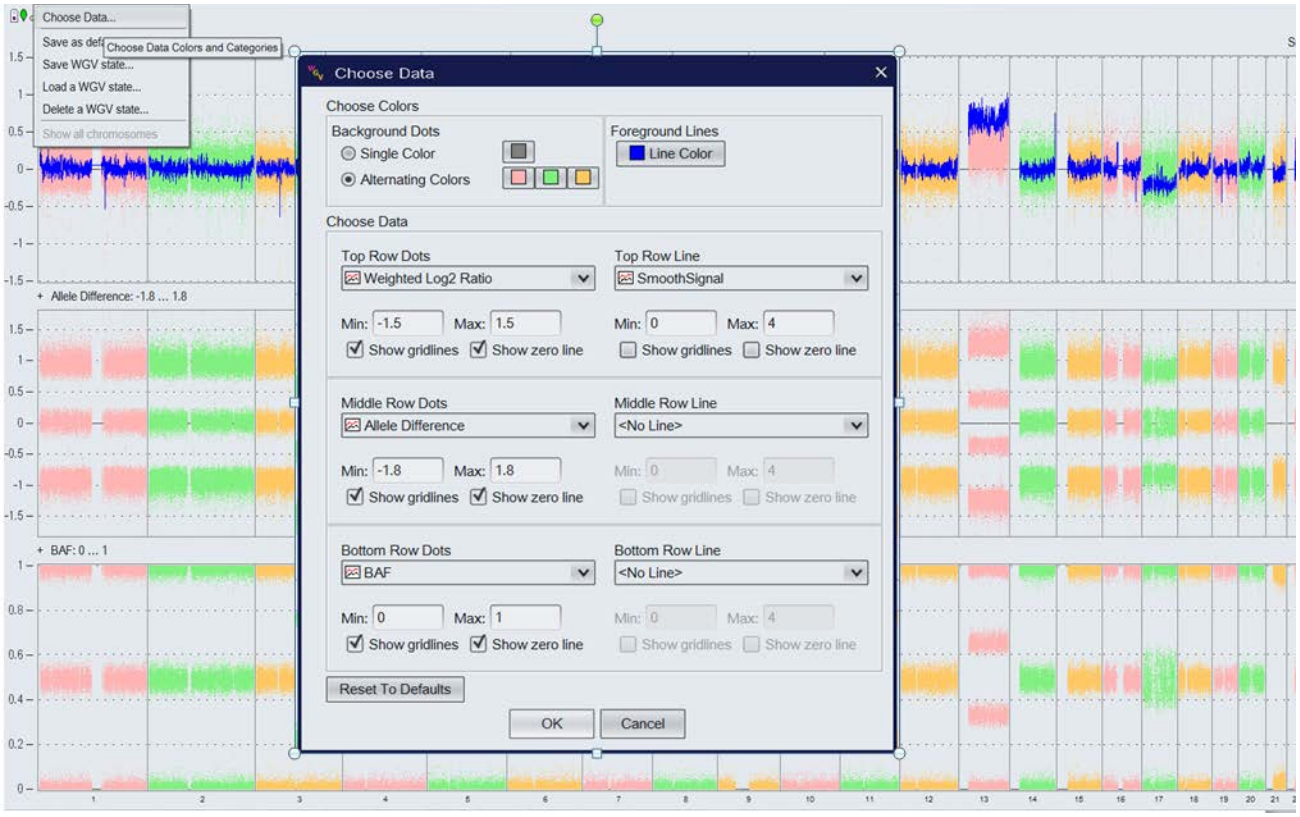


Figure 145 Choose Data window.

- Up to 3 rows of data can be viewed simultaneously for each sample.
- Use the Row Data and/or Row Line to assign which data tracks to view.
- Select **<no Dots>** or **< no Line>** to disable the display (of any data) in the row. Figure 146 is an example of no data being assigned to the Bottom Row, therefore only 2 rows of data are displayed in the Whole Genome View.

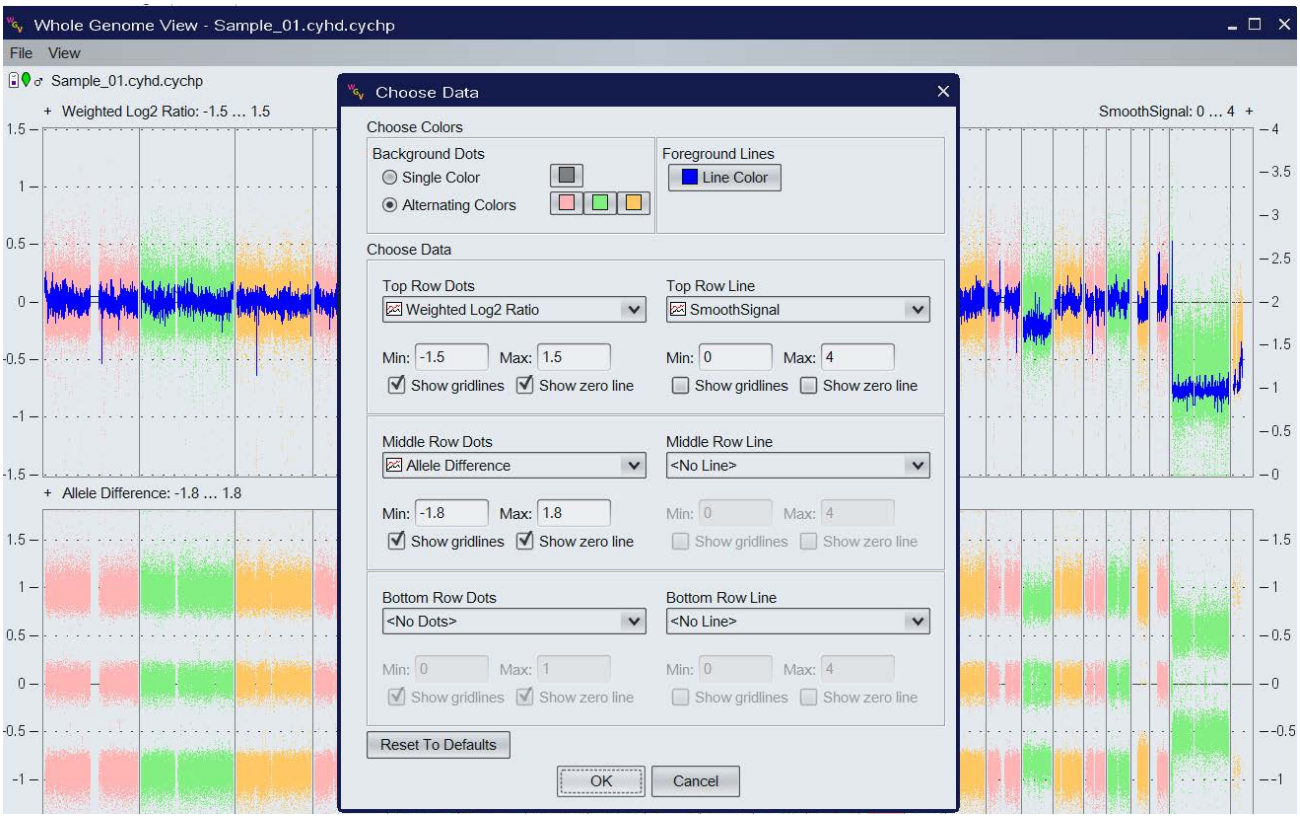


Figure 146 Choose Data window.

Note: The Y axis settings for the Whole Genome View are initially determined from the Y axis settings set in the Detail View Graph Settings. The Y axis on the left of the WGV pertains to the Row Data. The Y axis on the right of the WGV pertains to the Line data. Also, not all graphs are available for a given xxCHP file. If a graph type is selected in which data is not available, the graph will appear with no data points.

Change colors of the data points or line data

Graphs can be viewed in a single color or alternating colors every 3 chromosomes.

1. Click **View → Choose Data**. (Figure 146)

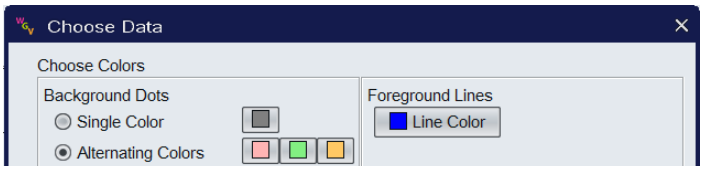


Figure 147 Choose Colors pane.

2. Select the option to change to either **Single Color** data points or **Alternating Colors** (3) data points.
3. Click the colored square(s) to open a **Color Selection** palette. Select new colors for the Whole Genome View.
4. Click **OK** to return to the Whole Genome View with your new selection.

Note: Changing Data display and/or Colors affects the current sample only. To save these settings as default setting (whenever a file is opened), save these settings as the Default WGV State. (See "Set a default WGV state display and colors".)

Set a default WGV state display and colors

1. Select the Data and Colors to be displayed as your Default settings when opening files in the WGV, as described in "Change colors of the data points or line data" on page 150.
2. Select **View → Save as default WGV State**. (Figure 146)

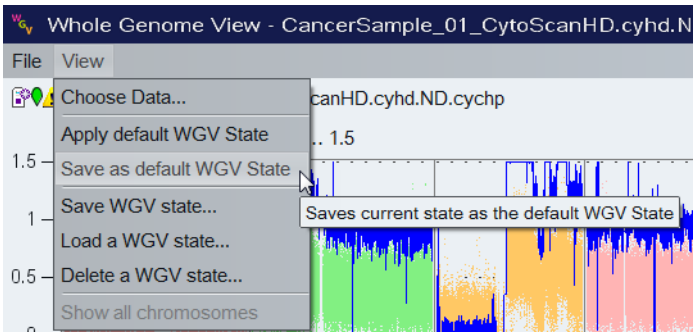


Figure 148 Save as default WGV State.

A confirmation message appears.

3. Acknowledge the message, then click **OK** to save these settings as the Default WGV display.

Note: If changes have been made to colors, data or Y axis values, you can return to this WGV Default State by clicking **View → Apply default WGV State** or click **Load a WGV State → Default**.

Create WGV states

Multiple WGV States can be created, then saved for a quick selection of different graph/color settings.

1. Select the Data and Colors to be displayed as your Default settings when opening files in the WGV.
2. To save these selections as a WGV State, click **View → Save WGV State**.
3. Enter a name in the dialog box, then click **OK** to save this new WGV State.

Apply a saved WGV state(s)

1. Select **View → Load a WGV State**.
2. Choose a saved WGV state from the dropdown list.
3. Click **OK** to apply the WGV State or click **Cancel** to keep the current display.

Delete a saved WGV state

1. Click **View → Delete a WGV State**.
2. Select the saved WGV state you want to delete from the dropdown list, then click **OK**.

   A message appears asking if you are sure you want to delete it.
3. Click **OK** or click **Cancel** to keep the WGV State.

Use the WGV zoom feature

1. Click the chromosome number that appears along the bottom row, as shown in Figure 149.

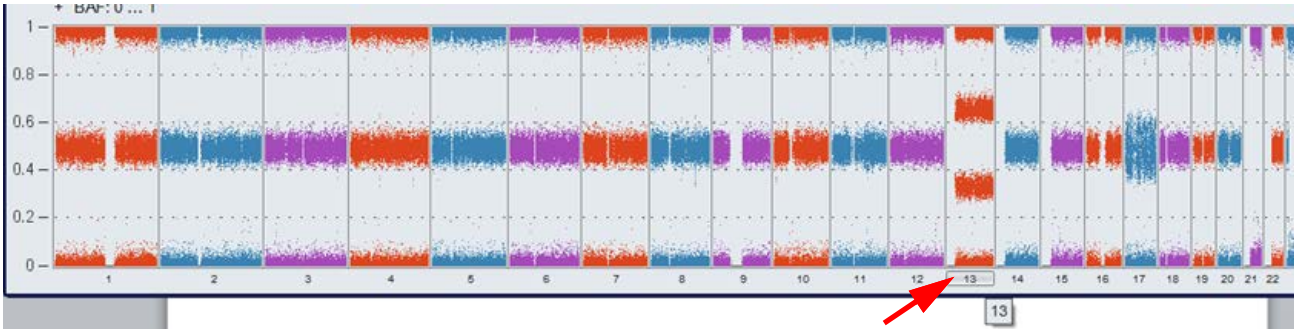


Figure 149 Zoom in.

2. The following "zoomed in" WGV window appears. (Figure 149)

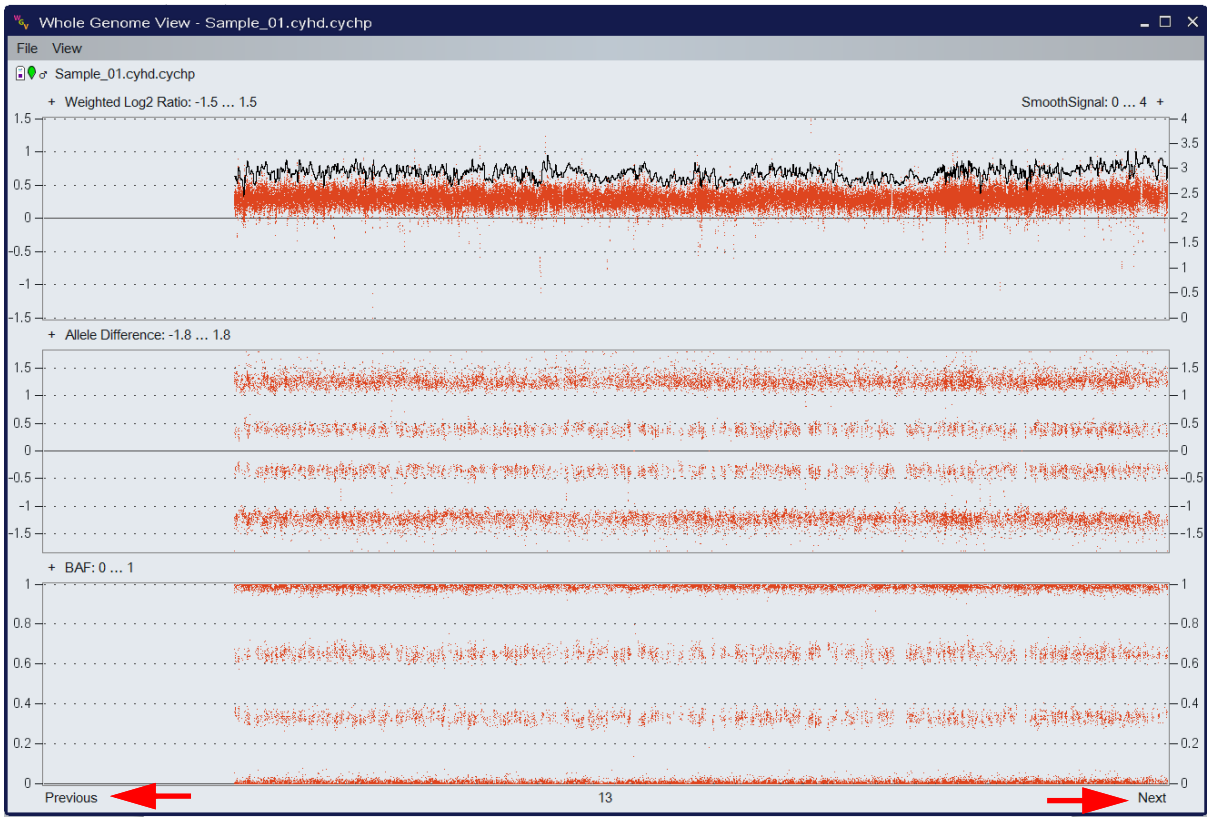


Figure 150 *Zoomed in* WGV window.

3. Click **Previous** or **Next** to view adjacent chromosomes in this window.
4. To return to the Whole Genome View (Figure 149), click the chromosome number again or click **View→ Show all chromosomes**.

Add a reference line to the WGV

1. Click the **+** (above the panel) to add a reference line to that panel.

   Note: When a row of data has both Dots and Line data, click the + sign next to the data type to which you would like to add the Reference Line.

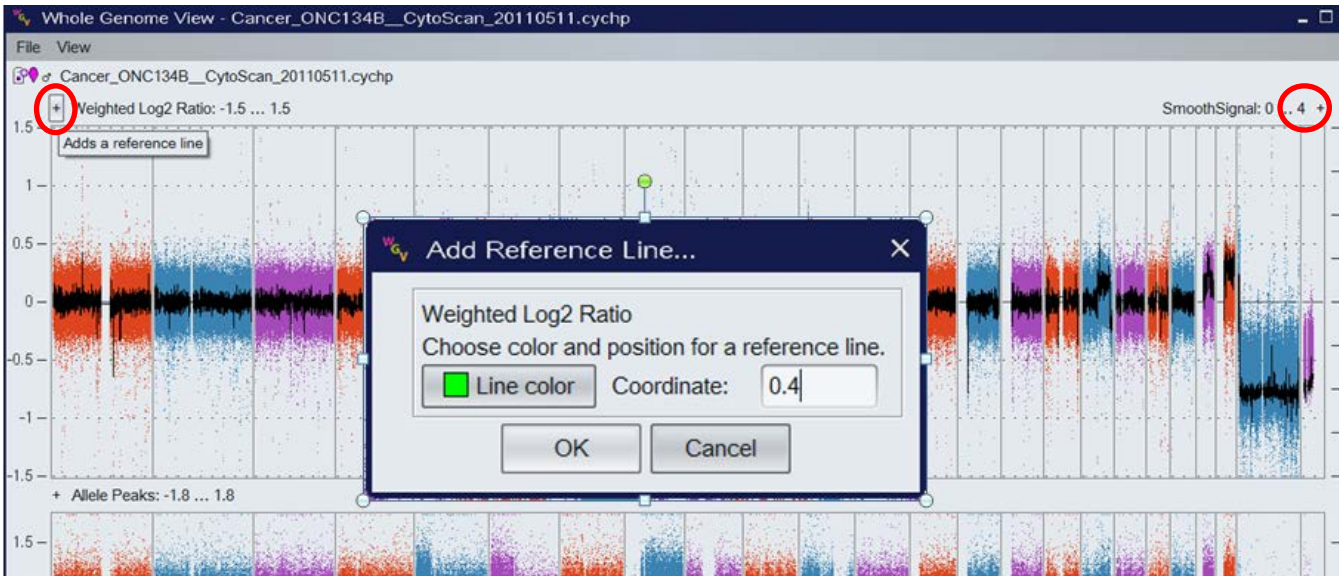


Figure 151 Add a reference line.

2. Click the **Line color** button to select a color for the Reference Line.
3. In the **Coordinate** text field, enter an approximate coordinate based on the Y axis where you want the Reference Line placed.

   Note: The Reference Line can be dragged and dropped to a different location after placed onto the graph.

4. Click **OK**.
5. Repeat steps 2–4 to place additional Reference Lines onto the graph.

   Note: Additional Reference Lines can also be added by right-clicking in the graph and selecting **Add Reference Line for...** (Figure 152).

6. Delete a single Reference Line by right-clicking the Line and selecting **Delete Selected Line**. Remove all Reference lines by right-clicking in the graph and selecting **Delete All Lines**. (Figure 152)
7. To change the color or coordinate of a Reference Line, right-click the selected Reference Line, then select **Edit Selected Line**. (Figure 152)



Figure 152 Add a reference line - right-click menu.

Add a comparison file

Use this feature to view 2 samples in the same WGV window.

Note: Before using this feature, both analysis files MUST BE loaded and available in the ChAS Browser.

1. Open the first file in the Whole Genome View, as you normally would.
2. Click **File → Add Comparison File**.

   A Comparison File window opens with available analysis results files loaded into ChAS Browser.
3. Click to highlight the file to be viewed with the first file (already open in the WGV), then click **OK**.

   The data for the files are loaded in the same window (Figure 153) displaying the following alternating tracks:

   - Track 1: Weighted Log 2 and Smooth Signal for File 1
   - Track 2: Weighted Log 2 and Smooth Signal for File 2
   - Track 3: Allele Difference for File 1
   - Track 4: Allele Difference for File 2
   - Track 5: B-allele Frequency for File 1
   - Track 6: B-allele Frequency for File 2



Figure 153 Example of the displayed alternating tracks.

Note: To remove a comparison file, click **File → Remove Comparison File**.

Select a new comparison file

1. Click the second of the filenames to open the file selection window
2. Highlight a new file from the **Choose a comparison file** window (Figure 153), then click **OK**.

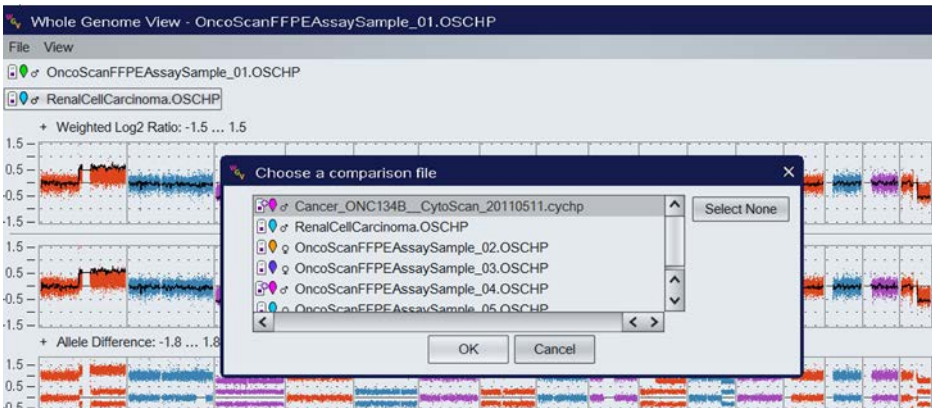


Figure 154 Selecting a new comparison file.

The newly selected file and its data is displayed.

Export a WGV

1. Click **File** → **Export window PNG**.

Selected chromosome view

The Selected Chromosome View (Figure 155) is similar to the Karyoview, but it displays a single selected chromosome at higher magnification. Click, then drag in the Chromosome View to select an area for display in the Detail View.

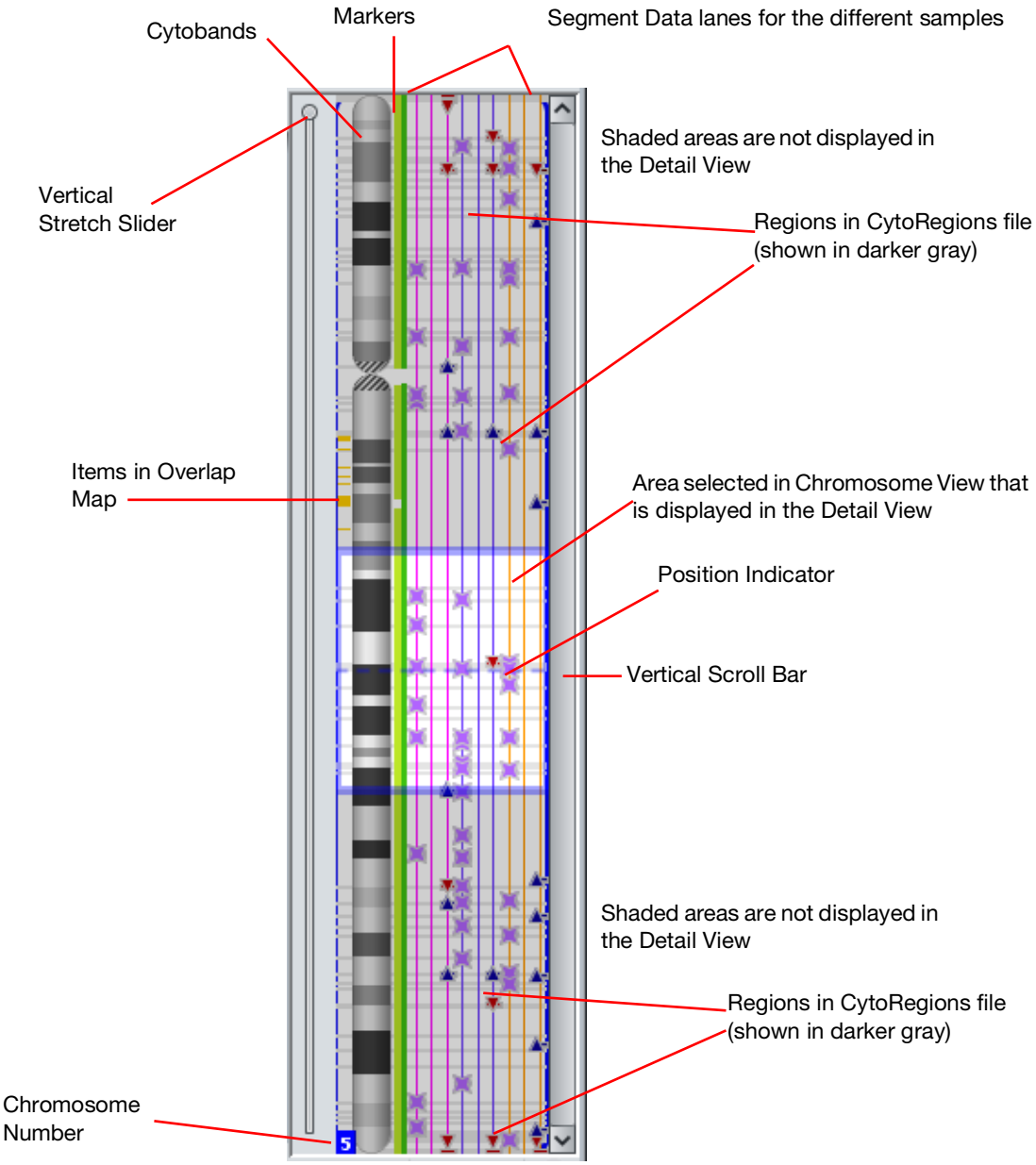


Figure 155 Selected Chromosome View.

Use the Stretch Slider and Vertical Scroll Bar (Figure 155) or press the **Alt** key and turn the mouse wheel to zoom in on a section of the **Selected Chromosome View**.

If you have selected a CytoRegions file, the CytoRegions are displayed as gray bands that stretch across the entire chromosome cell (from right to left of the Cytobands).

If you have selected an Overlap Map file, the overlap map items are displayed as small rectangles (Figure 155) to the left of the Cytobands. Its color is the same color used in the Details View.

The Position Indicator is a dashed horizontal blue line. Click in the Selected Chromosome View to set the position of the indicator. The position is highlighted in the graphs table and used as the center point when zooming.

The marker positions are displayed to the right of the Cytobands. When zoomed out, they appear as green ribbons. When zoomed in, the markers and their positions can be seen, as shown in Figure 156.

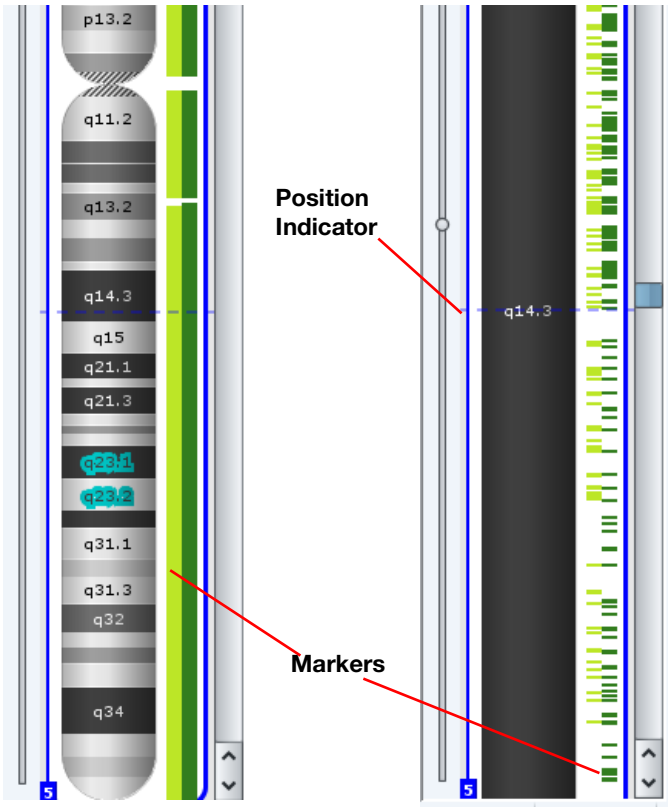


Figure 156 Selected Chromosome View, markers in zoomed-out view (left) and zoomed-in view (right).

- SNP markers are displayed in the light green band nearest the cytobands. The SNP marker/probe names in CytoScan™ arrays with the letter 'S'.
- There is one marker track for every distinct array type that is loaded.
- Copy Number markers are displayed in the dark green band nearest the detected segments. The non-polymorphic copy number probe names in the CytoScan™ arrays start with the letter 'C'.
- You can mouse over a marker to learn more about it.
- Segments selected in any view are highlighted in the Selected Chromosome View.
- For information on the other features of the Selected Chromosome View, see "Selected chromosome view" on page 156.

Detail view

The Detail View (Figure 157) enables you to look in detail at the detected segments, marker data, regions, and reference annotations for the loaded files.



Figure 157 Detail View.

View CytoScan™ XON region segments in detail view

CytoScan™ XON generates both whole genome segments and XON region segments. Whole genome segments capture the larger copy number events while XON region segments capture copy number aberrations at the exon level.

CytoScan™ XON regions segments and probe level data are categorized into 4 different levels.

Note: The 4 levels do not apply to Whole Genome segmentation for CytoScan™ XON.

- Level 1: Includes genes with the highest level of evidence: developmental delay, epilepsy, ASD, XLID, Metabolic disorders, hereditary cancer OMIM™ Morbid genes.
- Level 2: ClinVar genes not covered in Level 1.
- Level 3: Other OMIM™ genes.
- Level 4: Other Ref Seq, UCSC, Ensembl genes, LOVD.

In the Filters tab, when only Level 1 is selected and Levels 2–4 remain unchecked (as shown in Figure 158 on page 160) then:

XON Region segments that overlap regions of the genome designated as Level 1 is visible in the XON Region Segment Track and in the Segments Table. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 1 regions is colored the same color as the color nib of the sample. XON Region segments that overlap regions of the genome designated as Levels 2–4 are not visible on the XON Region segment track or in the Segments Track. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 2–4 regions is colored gray.

In the Filters tab, when Levels 1 and 2 are selected and Levels 3 and 4 remain unchecked (as shown in Figure 159 on page 160) then:

XON Region segments that overlap regions of the genome designated as Level 1 or Level 2 are visible in the XON Region Segment Track and in the Segments Table. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Levels 1 or 2 regions are colored the same color as the color nib of the sample. XON Region segments that overlap regions of the genome designated as Levels 3 or 4 will not be visible on the XON Region segment track or in the Segments Table. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 3 and 4 regions are colored gray.

If selected Level 3, then XON Region Segment calls are displayed on the XON Region Segment track and the marker level data are colored the same color as the color of the color nib of the sample.

When all 4 Levels are selected in the Filters Tab, all XON Region segment calls for the sample are displayed and all marker level data is colored the same color as the color of the color nib of the sample.

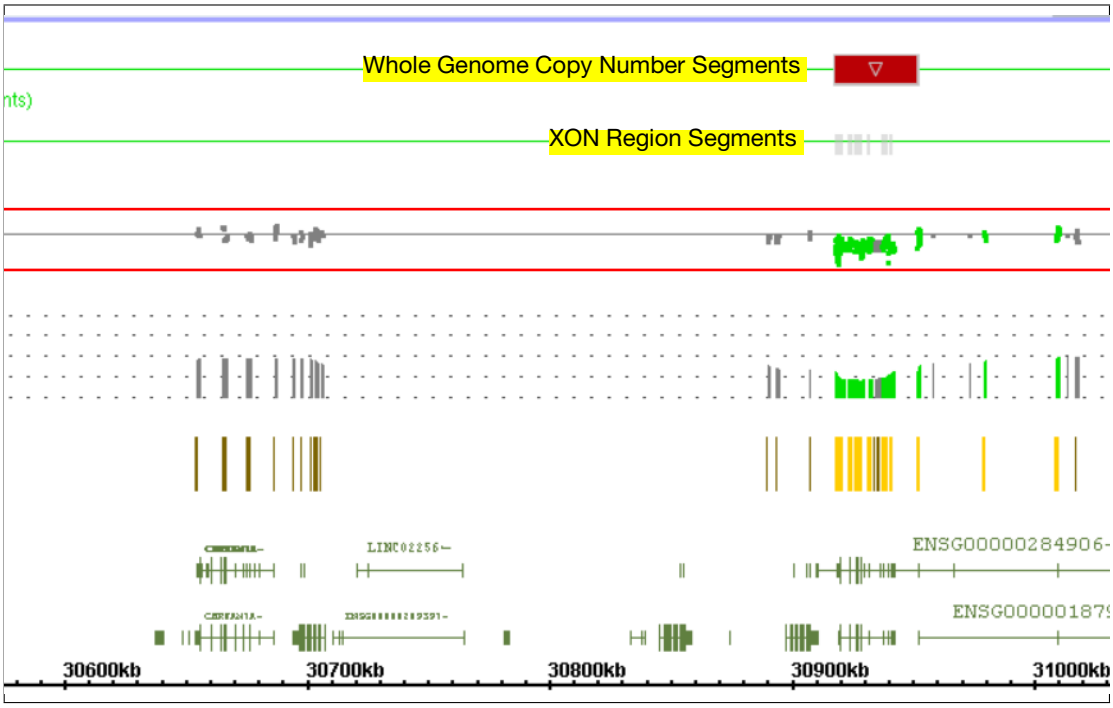


Figure 158 Example: Level 1 check only.

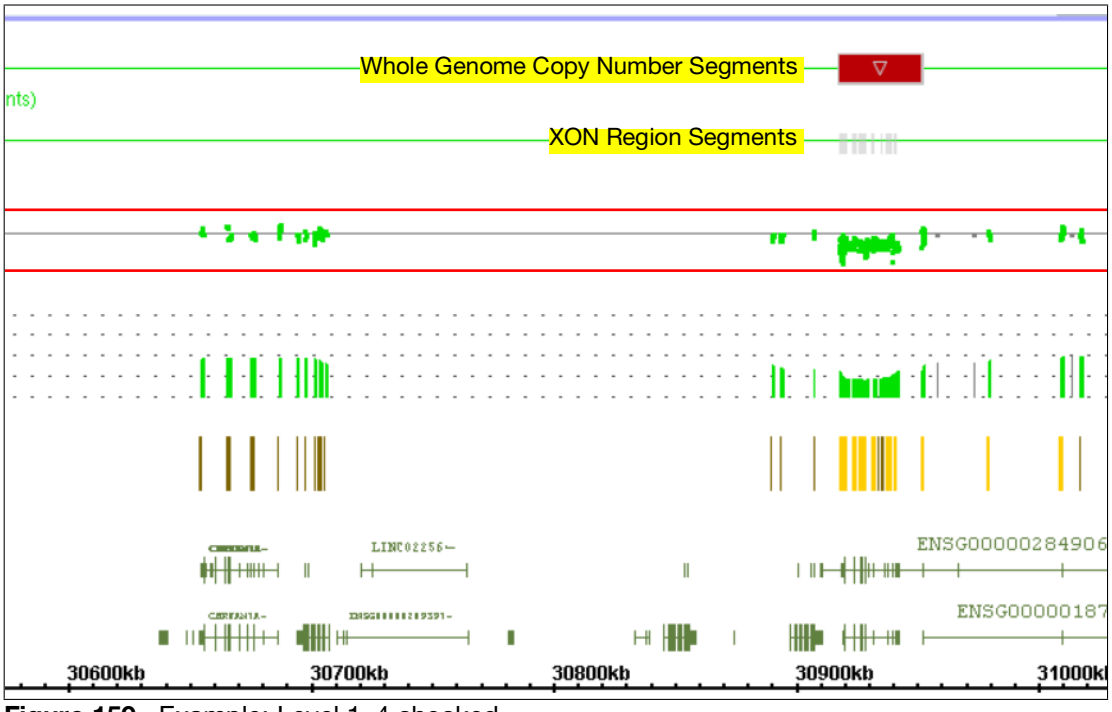


Figure 159 Example: Level 1–4 checked.

View CytoScan™ XON whole genome segments in the detail view

The CytoScan™ XON array offers whole genome segmentation for larger duplications and deletions. These segments appear on the Copy Number State Gain and Loss track. The track can be turned on/off using the Copy Number State Gain/Loss checkboxes in the Data Types File tree.

Viewing CytoScan™ XON whole genome segments and xon region segments simultaneously in the Detail View.

Whole genome segments and xon region segments maybe be viewed at the same time in the Detail View by turning on both the Copy Number State and XON Regions tracks. When both tracks are turned on, any xon region segment that overlaps a larger whole genome segment will appear grayed out and will not be displayed in the Segments Table or published to the ChAS Db. Using the whole genome segment to represent the copy number event eliminates redundant segment calls in the Segment Table. To view only the xon region segment calls, turn off the Copy Number State Gain/Loss track.

XON merging can still be used on the XON region segments but is no longer necessary due to the more robust whole genome segmentation algorithm. Any merged XON regions that overlap the whole genome segments in the Copy Number State track will appear grayed out and will not appear in the Segment Table. To enable grayed out XON region segments, turn off the Copy Number Gain/Loss Segment Track.

XON regions that overlap a larger Whole Genome Copy Number segment that is below the filter thresholds are enabled and will appear in the Segment table.

Grayed out segments will not appear in the Segments Table as the loss is already represented by the larger loss in the Copy Number State Track, as shown in Figure 160.

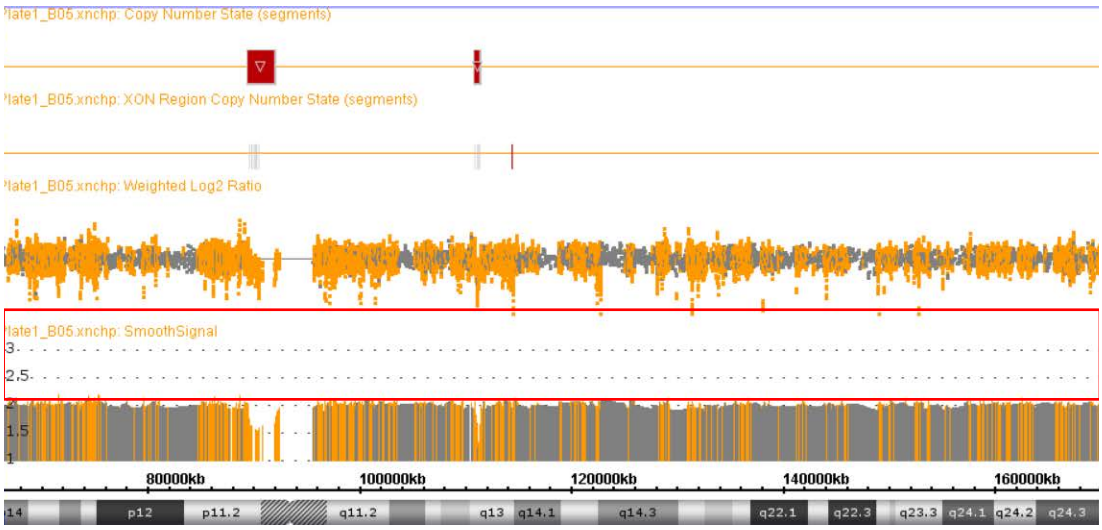
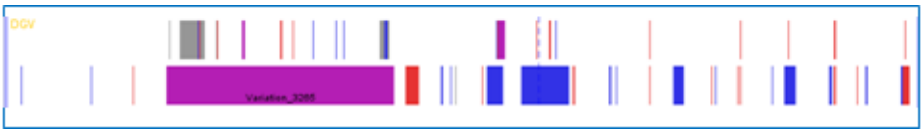


Figure 160 Grayed out segments example.

Annotation color codes

In the Detail View, DGV annotations are color-coded to indicate association with gain or loss.



- **Purple** – Gain and loss are associated with the region
- **Red** – Only loss is associated with the region
- **Blue** – Only gain is associated with the region
- **Gray** – Copy number variation is associated with the region, but information regarding the number of times gains or losses were observed is not present in the annotation record in the DGV database

Annotation OMIM™ color codes

In Browser annotation files version NA32.3 and higher, the following OMIM™ colored gene entries were generated by genome.ucsc.edu and are based on the associated OMIM™ phenotype map key. For more information on OMIM™ display conventions, go to: www.genome.ucsc.edu

- Lighter Green for phenotype map key 1 OMIM™ record - the disorder has been placed on the map based on its association with a gene, but the underlying defect is not known.
- Light Green for phenotype map key 2 OMIM™ records - the disorder has been placed on the map by linkage; no mutation has been found.
- Dark Green for phenotype map key 3 OMIM™ records - the molecular basis for the disorder is known; a mutation has been found in the gene.
- Purple for phenotype map key 4 OMIM™ records - a contiguous gene deletion or duplication syndrome; multiple genes are deleted or duplicated causing the phenotype.
- Light Gray for Others - no associated OMIM™ phenotype map key info available.

Annotation MANE Transcripts color codes

In the Detail View, MANE Transcripts annotations are color-coded to indicate association with different types.

Blue-MANE Select transcript

RED-MANE Plus Clinical transcript

Change an annotation color

1. Right-click an annotation type in the Files window pane and select **Set Custom Color** on the shortcut menu, as shown in Figure 161.

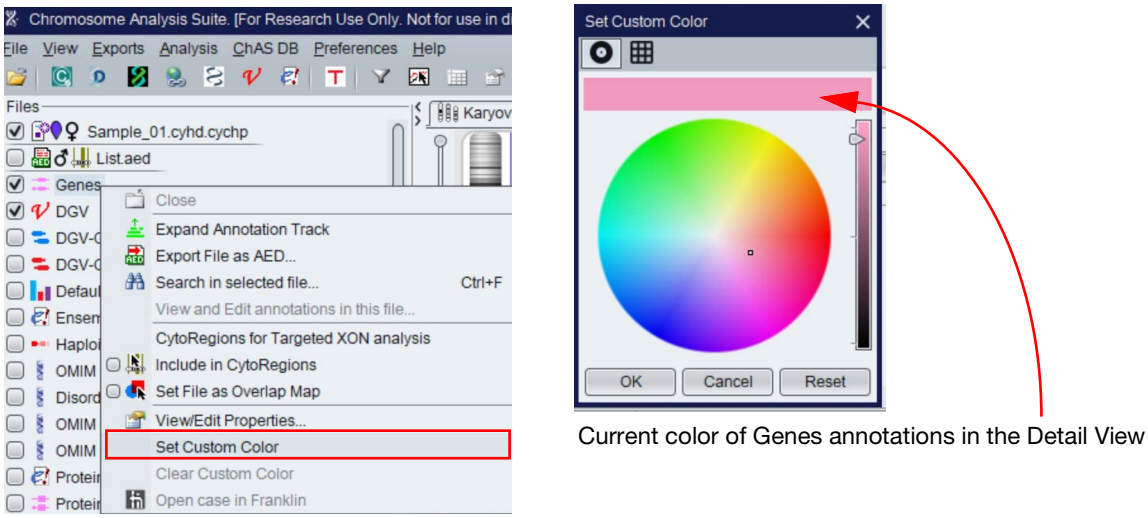


Figure 161 Open the color palette.

2. Specify a color for the selected annotation type using the color controls in the color palette (Figure 160), then click **OK**.

   The new color is applied to the annotations in the Details View.

3. To return to the default annotation color, right-click the annotation in the Files windowpane, and select **Clear Custom Color** on the shortcut menu.

Data in the detail view

The Detail View displays the following types of data for CytoScan (CYCHP):

Table 21 Data for CytoScan™ array.

Data Types	Definition
Detected Segment Types	
GainMosaic	Non-integer amplifications or duplications
LossMosaic	Non-integer hemizygous or homozygous deletions
Gain	Amplifications or duplications
Loss	Hemizygous or homozygous deletions
LOH	Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple) **Note:** In Dark Scheme (page 183), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple.
LOHMosaic **Note:** This segment type is not available for CytoScan™ Optima	A percentage of cells in a genomic region loses one of the two different copies (alleles).

Table 21 Data for CytoScan™ array. (Continued)

Data Types	Definition
Probe array data (displayed as graph data)	
Copy Number State	HMM-derived integer Copy Number State
Log2 Ratio	Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.
Weighted Log2 Ratio	Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator. These processed values are input to the CNState algorithm HMM.
LOH	Loss of Heterozygosity
Allele Difference	Filtered and smoothed values for individual markers. Nonparametric estimation is used to understand possible regional peak structure towards which the data is smoothed. The amount of filtration and smoothing is dynamically adapted based on sample quality. Allele difference is computed based on differencing A signal and B signal, then standardizing based on reference file information.
Genotype Calls	SNP genotype calls (single sample, BRLMM-P-plus algorithm)
Smooth Signal	Gaussian Smoothed Calibrated Copy Number Estimate
B-allele Frequency	Number of B alleles/number of A+B alleles used to show allelic imbalances.

The Detail View displays the following types of data for a CytoScan™ HTCMA Array:

Table 22 Data for CytoScan™ HTCMA Array.

Data Types	Definition
Detected Segment Types	
Gain	Amplifications or duplications
Loss	Hemizygous or homozygous deletions
LOH	Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple) **Note:** In Dark Scheme (page 183), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple.
Probe array data (displayed as graph data)	
Copy Number State	HMM-derived integer Copy Number State
Log2 Ratio	Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.
LOH	Loss of Heterozygosity
Allele Difference	Filtered and smoothed values for individual markers. Nonparametric estimation is used to understand possible regional peak structure towards which the data is smoothed. The amount of filtration and smoothing is dynamically adapted based on sample quality. Allele difference is computed based on differencing A signal and B signal, then standardizing based on reference file information.
Genotype Calls	SNP genotype calls (single sample, BRLMM-P-plus algorithm)

Table 22 Data for CytoScan™ HTCMA Array. (Continued)

Data Types	Definition
Smooth Signal	Gaussian Smoothed Calibrated Copy Number Estimate
B-allele Frequency	Number of B alleles/number of A+B alleles used to show allelic imbalances.
Variants	Location and detection of variants.

Note: There is a subset of ~55,000 SNP probes which are used for allelic information analysis but which are not used for Copy Number analysis (on the CytoScan HD Array).

For these SNP probes, LOH and Allele Peaks data are displayed, but these SNP probes will not have Log2 Ratio, Weighted Log2 Ratio, SmoothSignal, or Copy Number State data displayed, nor will they be used for ascertainment of Mosaicism.

The calculation of Segment data for all the various Segment types takes this into account. All non-polymorphic (copy number) and the vast majority of SNP probes are NOT affected by this change, and will continue to display all graphs and their data points from the CytoScan™ HD Array CYCHP files.

Table 23 Data for CytoScan™ XON Array.

Data Types	Definition
Detected Segment Types	
XON Region Gain	Amplifications in XON regions.
XON Region Loss	Hemizygous or homozygous deletions in XON regions.
Gain	Amplifications or duplications.
Loss	Hemizygous or homozygous deletions.
LOH	Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple) **Note:** In Dark Scheme (page 183), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple.
Probe array data (displayed as graph data)	
Log2 Ratio	Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.
Weighted Log2 Ratio	Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator. These processed values are input to the CNState algorithm HMM.
LOH	Loss of Heterozygosity
Allele Difference	Filtered and smoothed values for individual markers. Nonparametric estimation is used to understand possible regional peak structure towards which the data is smoothed. The amount of filtration and smoothing is dynamically adapted based on sample quality. Allele difference is computed based on differencing A signal and B signal, then standardizing based on reference file information.
Genotype Calls	SNP genotype calls (single sample, BRLMM-P-plus algorithm)
Smooth Signal	Gaussian Smoothed Calibrated Copy Number Estimate
B-allele Frequency	Number of B alleles/number of A+B alleles used to show allelic imbalances.

The Detail View displays the following kind of data for Genome-Wide SNP Array 6.0 Array data (CNCHP):

Table 24 Data for Genome-Wide SNP Array 6.0 (CNCHP).

Data Types	Definition
Detected Segment Types	
Gain	Amplifications or duplication
Loss	Hemizygous or homozygous deletions
LOH	Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple) **Note:** In Dark Scheme (page 183), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple.
Probe array data (displayed as graph data)	
Copy Number State	HMM-derived integer Copy Number State
Log2 Ratio	Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.
LOH	Loss of Heterozygosity
Allele Difference	Difference of A signal and B signal, each standardized with respect to their median values in the reference.
Smooth Signal	Smoothed Calibrated Copy Number Estimate

Table 25 Data for OncoScan™ array (OSCHP).

Data Types	Definition
Detected Segment Types	
Gain	Amplifications or duplication
Loss	Hemizygous or homozygous deletions
LOH	Loss of Heterozygosity
Probe array data (displayed as graph data)	
Copy Number State	TuScan derived Copy Number State.
Log2 Ratio	Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.
Weighted Log2 Ratio	Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator. These processed values are input to the CNState algorithm HMM.
LOH	Loss of Heterozygosity.
Allele Difference	Difference of A signal and B signal, each standardized with respect to their median values in the reference.
Smooth Signal	Smoothed Calibrated Copy Number Estimate.
Variants	Location and detection of Somatic Mutation. (OncoScan FFPE Assay only)
B-allele Frequency	Number of B alleles/ number of A+B alleles, used to show allelic imbalances.

Table 26 ReproSeq™ Aneuploidy data.

Data Types	Definition
Detected Segment Types	
Gain	Amplifications
Loss	Hemizygous or Homozygous deletions
Graph Data	
Copy Number State	The copy number state of sequence tiles

See "Change graph appearance" on page 184 for more information about controlling the display of graph data.

In addition, the Detail View displays:

- Regions: Features in the various region files loaded into ChAS, including CytoRegions and Overlap Map items.
- Annotations: Indicate the known or suspected locations of features, such as mRNAs, exons, structural variants, and so forth.

You can expand or contract the annotations. See "Expand and contract annotations" on page 183.

- Database Display
  - Default Histograms: displays all segments in the database.
  - Filtered Histograms: displays all segments meeting the filter criteria set by the user. For information on how to create a filtered histogram, see "Add filtered histogram data" on page 132.
- Chromosome info, with:
  - Coordinate scale
  - Marker position information
  - Chromosome number
  - Cytoband information

Selected segments are displayed with enlarged icons; selected regions or annotations are outlined and highlighted. (Figure 162)

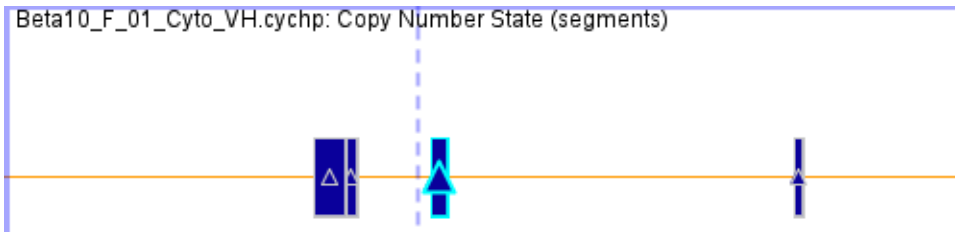


Figure 162 Selected Segment.

Navigation controls in detail view

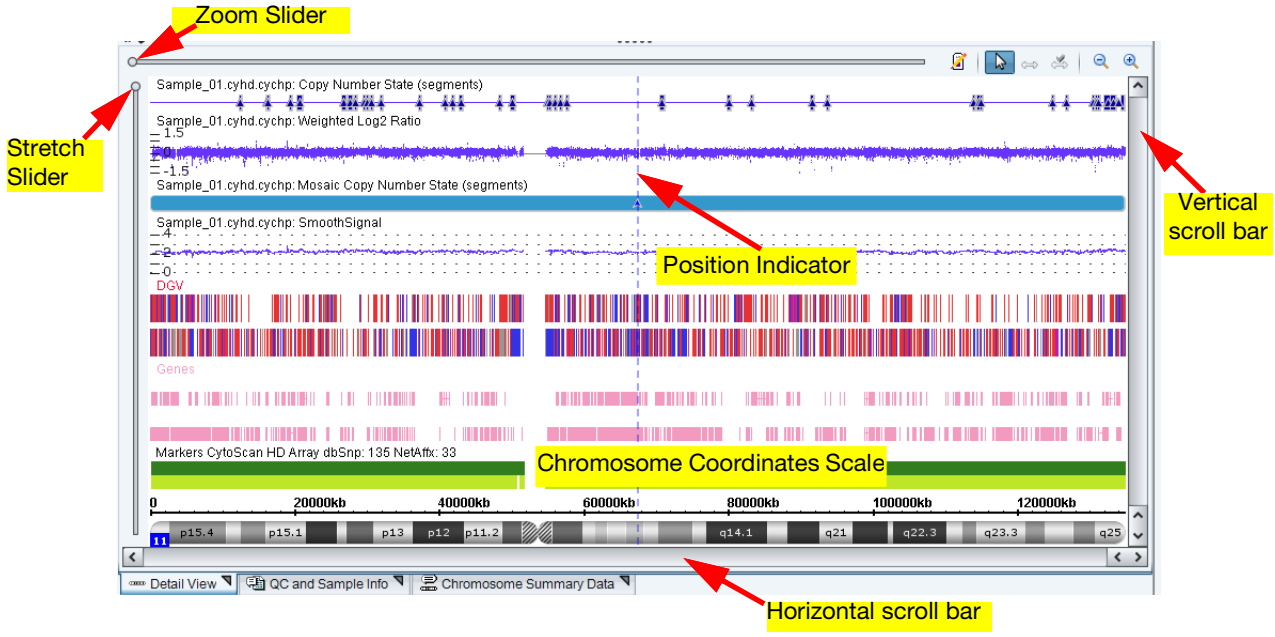


Figure 163 Detail View navigation controls.

Table 27 Navigation controls.

Control	Function
Chromosome Coordinates scale	Shows the position along the genome.
Zoom Slider	Controls the horizontal zoom and the area of the chromosome displayed.
3x Zoom In	Press Ctrl+Plus to view up to 3 preset Zoom In settings.
3x Zoom Out	Press Ctrl+Minus to view up to 3 preset Zoom Out settings.
Zoom in Using Click and Drag	Place your mouse cursor over a point of interest. Press Shift while holding down the left mouse button. Drag the mouse cursor to frame/zoom in on your point of interest.
Stretch Slider	Controls the vertical stretch of the Display area.
Scroll bars	Used to select the area displayed after zooming or stretching the vertical or horizontal scale.
Position Indicator	Dashed vertical blue line. Click in the view to set the position of the indicator The position that is highlighted in the graphs table. The position that is used as the center point when zooming.

Use Stretch Slider and Vertical Scroll Bar to zoom in on a section of the Detail. You can also use the mouse wheel as shown below:

- Alt+mouse wheel stretches the display
- Ctrl+mouse wheel zooms in on the horizontal scale
- Mouse wheel scrolls up and down

Obtain summary metrics for a zoomed in region

You can obtain summary metrics from the data tracks displayed in the Detail View after you have zoomed in to a region of interest.

1. In the Detail View, zoom in to a region of interest using any of the techniques described above.
2. Hover the mouse over a data point on the data track for which you would like summary metrics. For example, log 2 ratio, weighted log 2 ratio, Allele Difference, etc.

   A pop-up appears and displays the following metrics for markers currently displayed in the Detail View:

   - Region Size
   - Number of Markers
   - Min Marker Value
   - Max Marker Value
   - Mean Value
   - Standard Deviation
   - Median Value

Select a chromosome section for display

Data from the same sample files is displayed in all 3 views, at different scales.

You can select a particular chromosome, or a section of the chromosome, for detailed study using:

- "Karyoview and selected chromosome view"
- "Coordinate range box" on page 170
- "Zoom to a selected item" on page 171
- "Navigation controls in detail view" on page 168

You can also double-click an item in a table to zoom to the region of the chromosome where that item is located.

Karyoview and selected chromosome view

Select a chromosome for detailed examination

1. Click a chromosome in the Karyoview.

   The chromosome is displayed in the Selected Chromosome View and the Detail view.

Examine a section of the chromosome

1. Click and drag on the section in the Karyoview or the Selected Chromosome View.

   The selected section is displayed in the Detail View. (Figure 164)

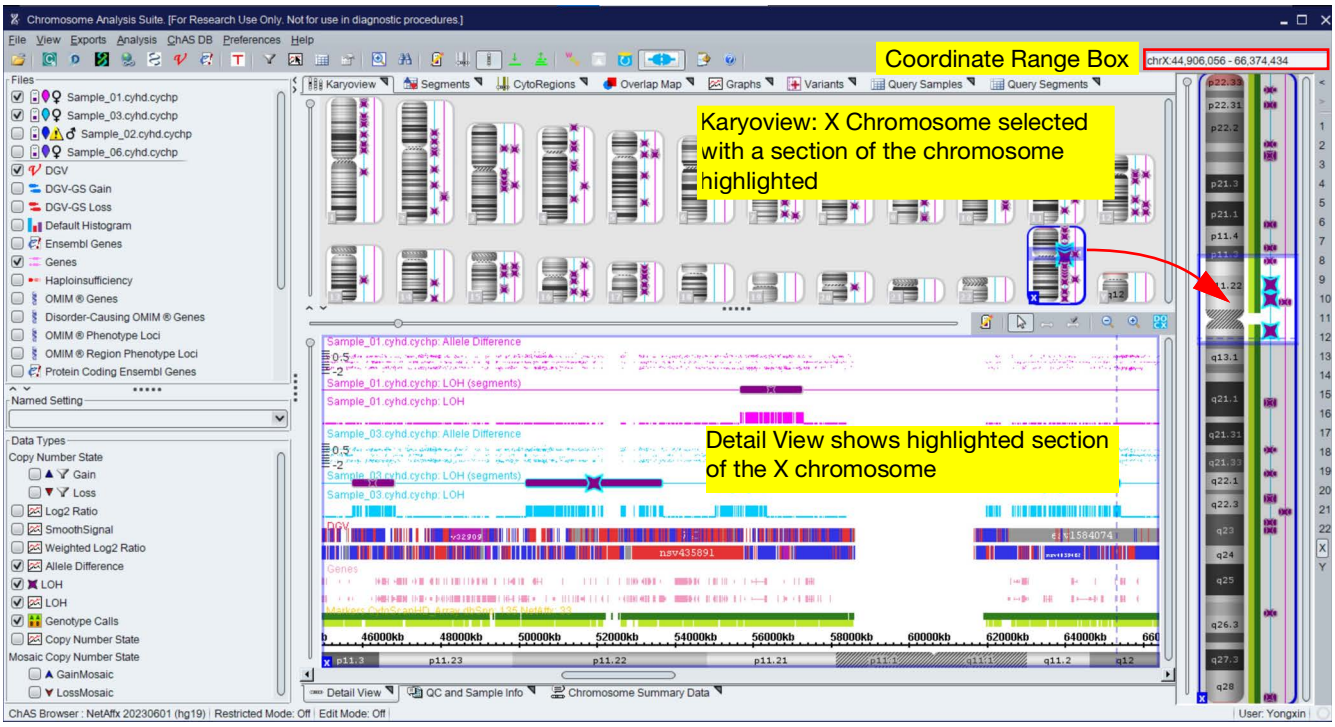


Figure 164 Areas displayed in Karyoview, Selected Chromosome View, and Detail View.

Coordinate range box

The Coordinate Range box (Figure 165) is located in the ChAS main toolbar. It shows the selected chromosome and the start and stop positions displayed in the Detail View. You can enter coordinates in the box to update the Detail View.

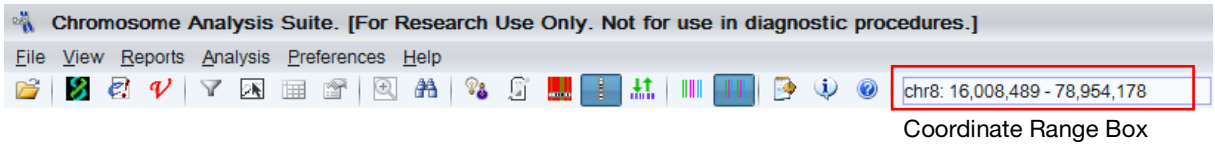


Figure 165 Coordinate Range Box in Main toolbar.

Go to a specific coordinate or coordinate range

- Enter the desired location in any one of these formats then press the <Enter> key:
  - "**chromosome number: start - end**": sets the view to the given start and end coordinates on the given chromosome.
  - "**start: end**" or "**start - end**": sets the view to the given start and end coordinates of the current chromosome.

Return to a previous location

- To return to previous genomic coordinates, click the < > buttons above the chromosome number field (far right), as shown in Figure 166.

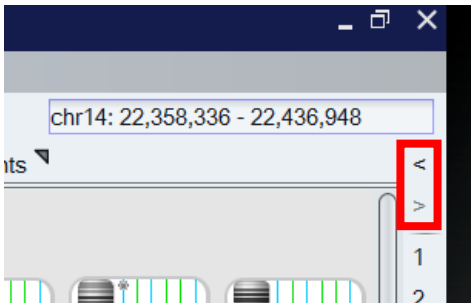


Figure 166 Previous coordinate buttons.

Zoom to a selected item

There are several ways to zoom in on a feature.

- Click a segment in the Segment table, CytoRegions table, or Overlap Map. Clicking items auto-zooms to your configured zoom buffer (15% of item size is default), To edit the zoom buffer, see "Zoom Buffer" on page 174.
- Double-click a feature in the **Details View**, **Karyoview** or **Selected Chromosome View**.
- Press **Ctrl+Space** to zoom all the way in to the selected item's start and end coordinates, with no buffer.
- In the Karyoview, Selected Chromosome View, or Details View, you can use the **Zoom to selection** option in the feature right-click menu (Figure 165) to go to the start and stop coordinates of the selected feature.

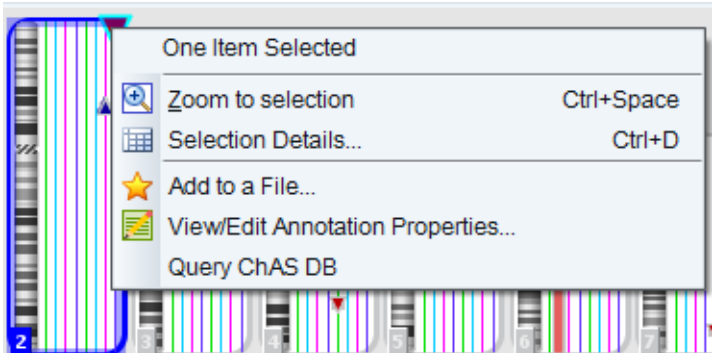


Figure 167 Right-click menu options.

Set a vertical highlight

Use this feature to set a vertical highlight on a selected segment(s) or annotation(s) to view its breakpoints across other data types and/or annotations. This highlighting option can be useful when aligning a segment/annotation of interest with other samples or annotations.

- Right-click a selected segment(s) or annotation(s), then click **Set highlight region from selection** to apply a vertical yellow highlight through the Detail View, as shown in Figure 168.



Figure 168 Vertical highlight example.

Edit configurations in the Misc tab

The **Misc** tab contains:

- Autosave
- Coordinate Box Format
- Zoom Buffer
- Chromosome Sorting Order
- CHP File Colors
- Remapping Segment patterns
- Microarray Nomenclature configuration

Access the Misc tab

1. Click **Preferences** → **Edit User Configurations** or click  on the upper toolbar.
   The **User Configuration** window appears.
2. Click the **Misc** tab. (Figure 168)

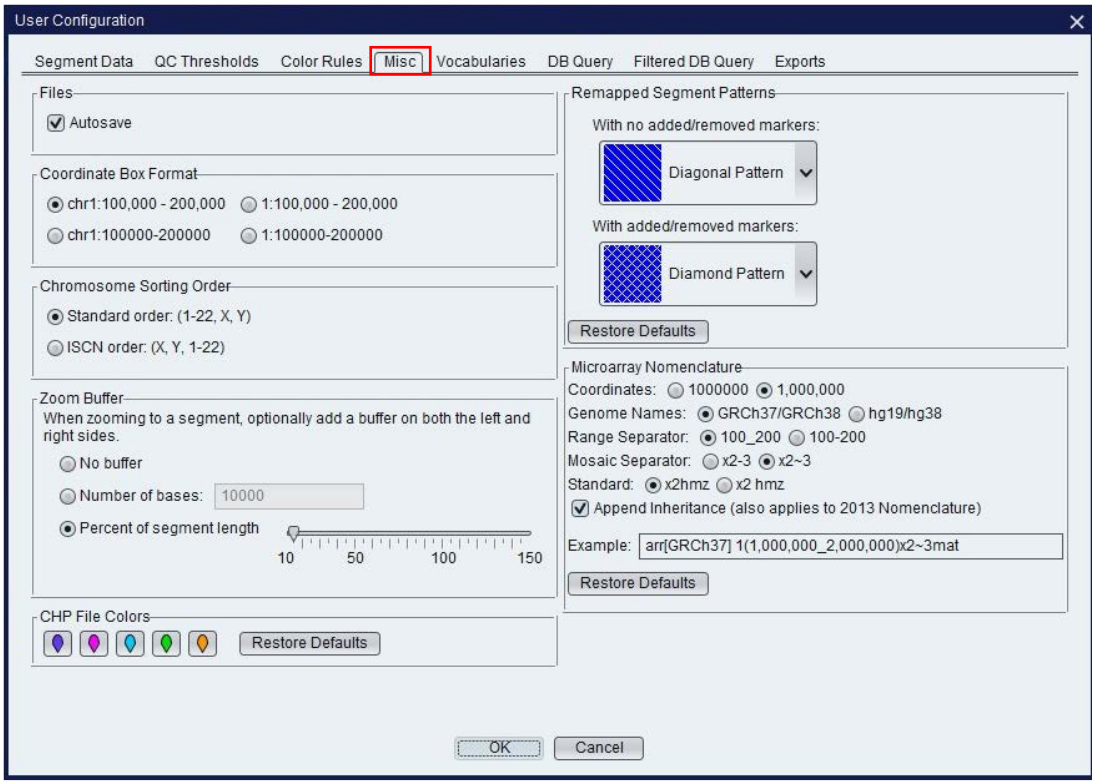


Figure 169 User Configuration window - Misc tab window.

Autosave

- Select the **Autosave** checkbox to automatically save your files as they are edited. Deselect the checkbox to disable auto-save. (Figure 168).



Figure 170 Autosave.

Coordinated Box Format

- Click the appropriate option to choose the format of your displayed data. (Figure 168)

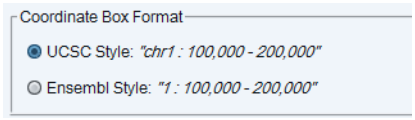


Figure 171 Coordinate Box Format.

Chromosome Sorting Order

- Click the appropriate option to sort the segments in the Segment Table. (Figure 168)

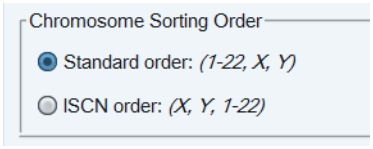


Figure 172 Chromosome Sorting Order.

Zoom Buffer

By default, the zoom percentage is set to 15% in new user profiles.

The Zoom Buffer feature offers 3 settings:

- **No Buffer**: Click this option to turn off the Zoom Buffer feature.
- **Number of bases**: Click this option, then manually enter the number of bases you want.
- **Percent of segment length**: Click, then drag the slider bar (Figure 168) to the zoom percentage you want.

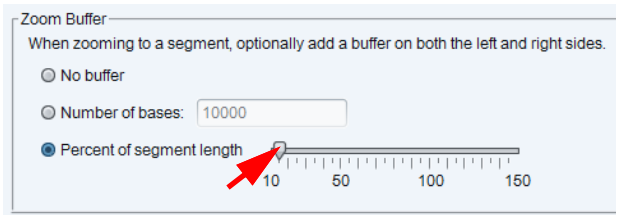


Figure 173 Zoom Buffer.

CHP File Colors



Figure 174 CHP File Colors.

There are 5 preset CHP file colors assigned to your CHP data (Figure 174), but each default color, can be changed to a different color.

Change a CHP file color

1. Click the colored icon you want to change.

   A color wheel appears. (Figure 174)

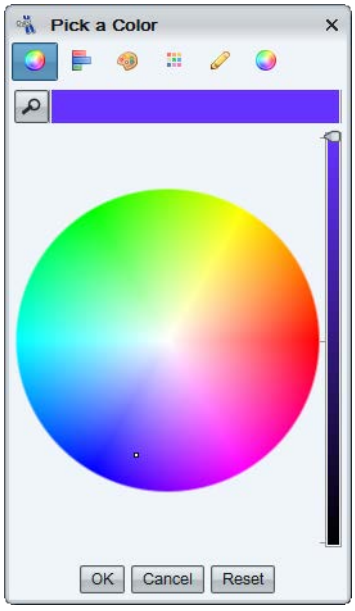


Figure 175 Pick a Color window.

2. Use the color wheel to locate the specific color you want or click one of the several coloring options  (including a website-safe color pallet).
3. Click **OK**.
4. Repeat steps 1–3 to change additional default colors.

   At anytime, click **Reset to Default** to return the 5 CHP file colors back to their default colors. (Figure 174)

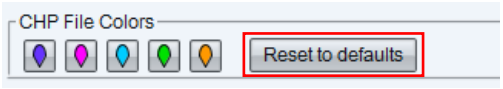


Figure 176 CHP File Colors.

   To return a single CHP file color back to its default color, click the CHP file color, then click the Color Wheel's **Reset** button.

5. Click the User Configuration window's **OK** button to save your changes and exit.

Note: Samples that are currently loaded while a color change is made, may not reflect your new color scheme. To remedy this, close, then re-open ChAS to ensure your new color choices are reflected throughout all your samples.

Remapped segment patterns

Choose how segments (remapped from a hg19 ChAS db to a hg38 ChAS db) are represented. See "Additional segment intersection information" on page 370 for information on default patterns.

- Click the **With no added/removed markers:** dropdown to select a pattern to represent segments in a hg38 ChAS db that were mapped from a hg19 ChAS DB, in which all the markers in the original segment mapped to the new genome version.
- Click the **With added/removed markers:** dropdown to select a pattern to represent segments in a hg38 ChAS db that were mapped from a hg19 ChAS DB, in which one or more markers in the original segment no longer map to the new genome version.

Microarray nomenclature configuration

Define how the ISCN Microarray nomenclature are represented in the segments table and exports. Any format from ISCN 2013, 2016, 2020, and 2024 can be configured

- Coordinates: Choose this option to display genomic coordinates with or without commas.
- Genome names: Choose this option to display genome versions using either GRCh or hg.
- Range separator: Choose this option to display either a dash (-) or an underscore (_) between genomic positions.
- Mosaic separator: Choose this option to display either a dash (-) or a tilde (~) between copy number values on mosaic segments.
- Standard: Choose whether or not to include a space between the copy number and suffix.
- Optional: Select the checkbox to automatically have information in the Inheritance field appended to the Microarray nomenclature field.

Control the display of data

ChAS (Figure 177) provides controls for:

- "Select data for display" on page 178
- "Select Data Types for display" on page 180
- "Change the grouping of samples and data types" on page 181
- "Select display schemes" on page 183
- "Expand and contract annotations" on page 183
- "Change graph appearance" on page 184

Later chapters explain other options for filtering data, how to specify certain regions for extra attention or ignoring, and how to create Region files with region information and annotations.

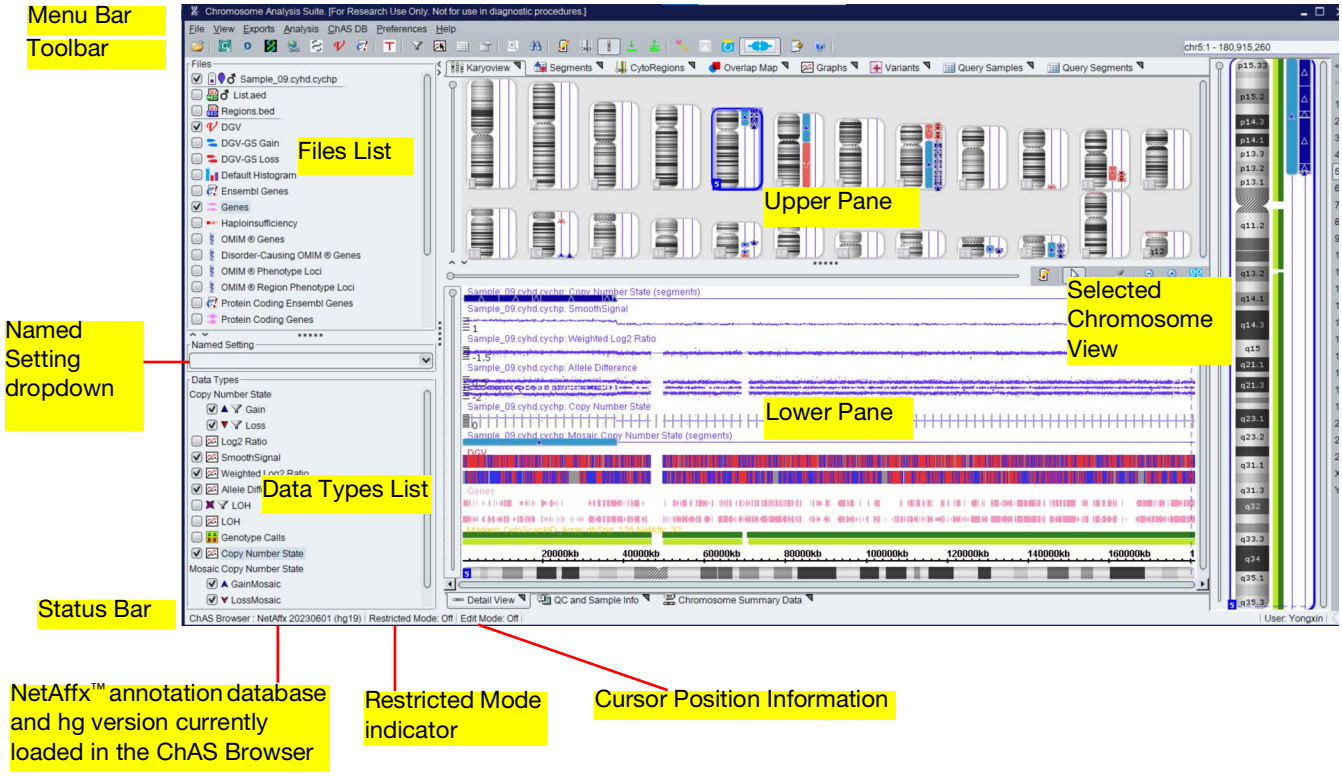


Figure 177 ChAS with data loaded.

Select data for display

The **Files** list (Figure 178) enables you to select sample data, region files, histograms, and reference annotations for display in the graphic view.

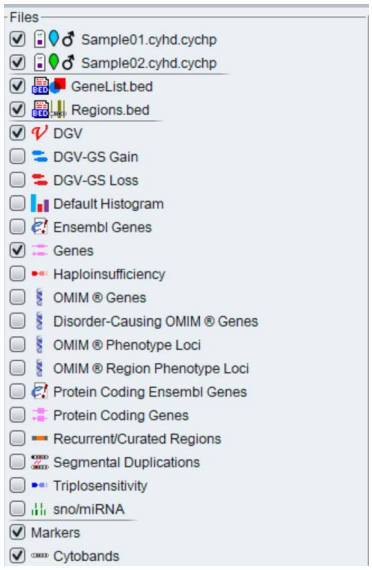


Figure 178 Files list.

The Files list displays the files grouped by:

- **Sample Data**
  - Colored nibs display the color used for the data lanes for that sample in the Karyoview, Selected Chromosome View, and Detail View.
  - The appropriate gender symbol is displayed to the right of the colored nib. GenomeWide SNP 6.0 CNCHP file data may also contain a "**?**" if the gender was determined to be "unknown".
  - If a loaded file has a QC parameter that is out of range, an alert symbol appears next to the file name.
- **Region Data Files**

  Icons indicate the file type (AED or BED) and whether the loaded files have been selected as a CytoRegions file or Overlap Map file. VCF files are also loaded here.
- **Histograms**: Displays Gains, Loss, and LOH segments stored in ChAS DB. The display is loaded in the Detail View upon software start up or by selecting **ChAS DB → Load Histograms** (when connected to the ChAS DB).
- **Reference Annotations**: Loaded during software installation and startup. Only displayed in Detail View.
- **Cytobands**: Separated from other reference annotations because they cannot be moved in the displays, and because they are also displayed in the Karyoview and Selected Chromosome View.

Select and deselect files for display

1. Select the checkbox next to the file name.

   The order in the Files list determines the order of display of the lanes in the Karyoview, Chromosome view, and Detail View.

Change the order of the Sample lanes or reference annotations

1. In the Files list, click a file name and drag it to a new position.

View data properties

1. Right-click a file and select **View/Edit Properties** on the shortcut menu.

   The **Properties** window appears.

   Optional: Use the Filter "Property Name" search text field (Figure 179) to quickly locate a property of interest.

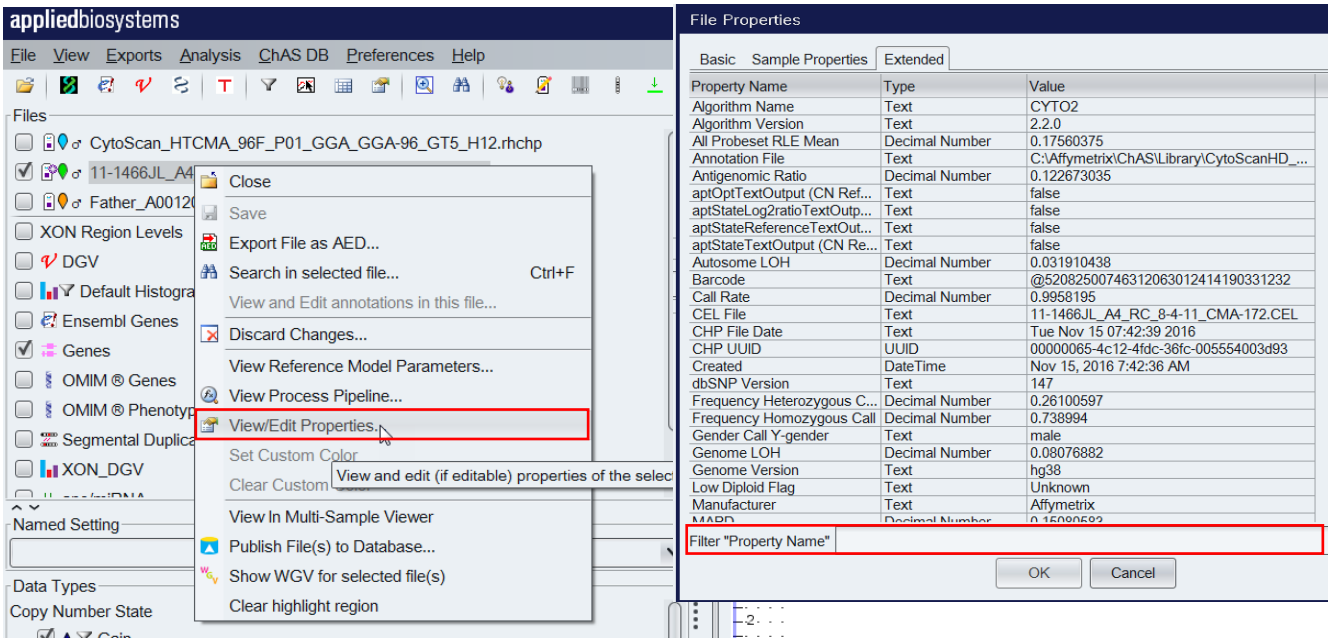


Figure 179 View data properties - Filter "Property Name" field.

Close a file

1. Right-click the file you want to close.
2. Select **Close** from the menu. (Figure 179)

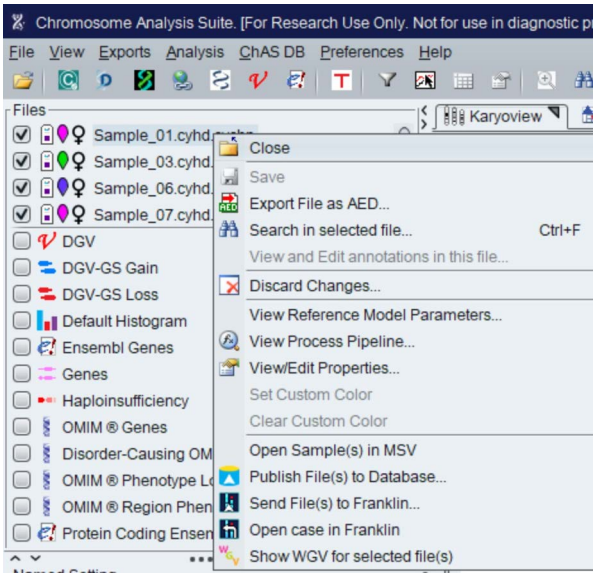


Figure 180 Closing a file.

The file is removed from the Files list and the data is no longer displayed.

Select Data Types for display

The **Data Types** list (Figure 181) shows the data types that can be displayed in ChAS.

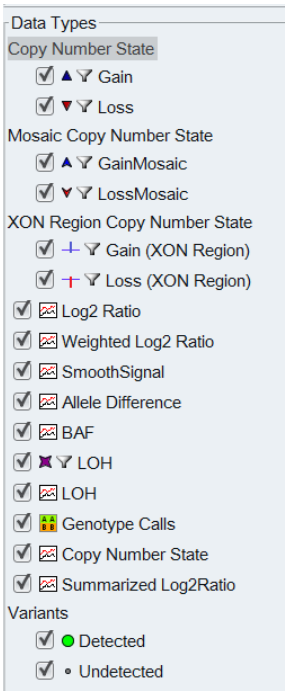


Figure 181 Data Types list.

Figure 181 displays a list of data that can be displayed in the Karyoview, Selected Chromosome View, Detail View, and tables. The available data types may vary, depending upon the type of sample data available.

It enables you to select from Segments data and CN/LOH discrete graph data.

The Segments data is displayed in:

- Karyoview
- Selected Chromosome View
- Detail View

If segment parameter filters have been applied to a segment type, a funnel symbol appears next to the segment type name, as shown in Figure 182.

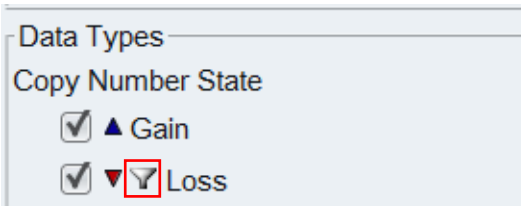


Figure 182 The funnel graphic denotes filters have been applied.

The Graph data is displayed only in the Detail View.

Select and deselect data types for display

- Select the checkbox next to the Data Type name.

Change the order of the data types:

- In the Data Types list, click a file name and drag it to a new position.

Note: The selections made here can be saved with a Named Setting (see "Named settings" on page 409).

Turn the symbols used for segments on or off:

- From the View menu, select or de-select Segment Symbols.

Change the grouping of samples and data types

A unique color is assigned to each sample and used for the lanes in the Karyoview, Selected Chromosome View, and Detail View.

Each segment type is assigned to its own lane and has its own symbol.

Change the grouping

1. From the View menu, select Group by Sample or Group by Type or in the toolbar, click the Group by Sample or Group by Type icon.

   This enables you to perform different types of comparisons between samples and segment types.

Lanes grouped by sample

When the lanes are grouped by sample, the different segment types for each sample are kept together in the Karyoview and Selected Chromosome View (Figure 183) and in the Detail View.

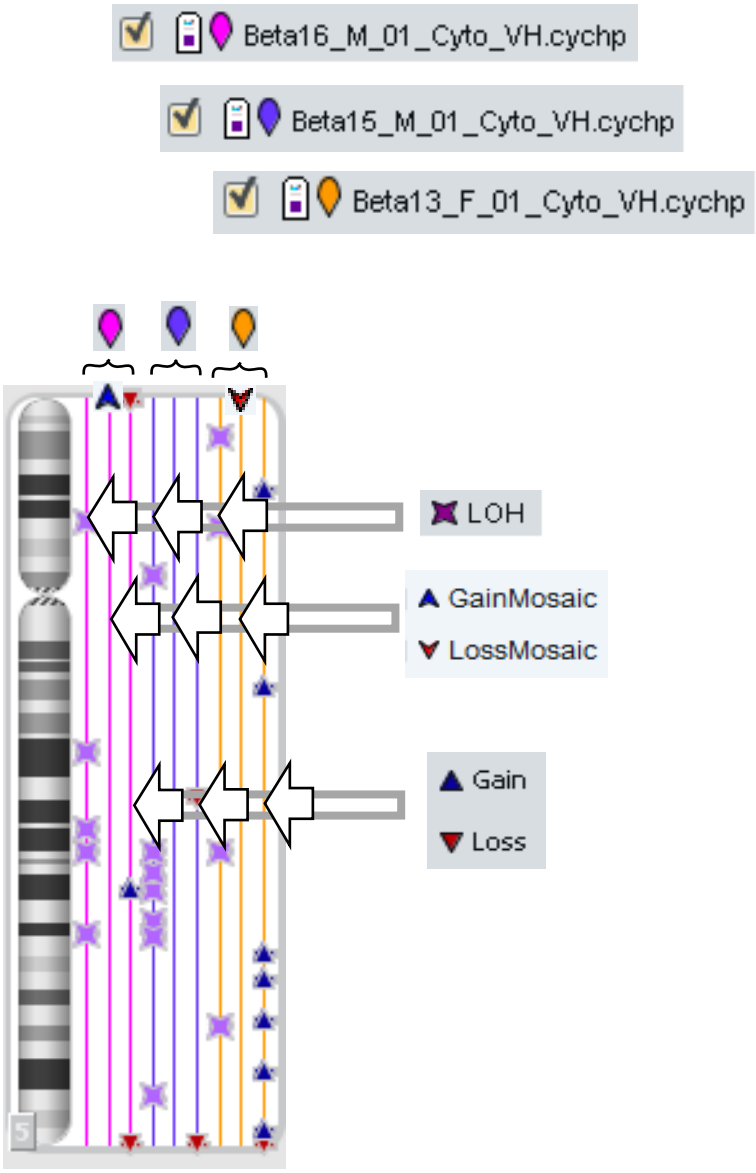


Figure 183 Lanes grouped by sample in Karyoview.

When the lanes are grouped by data type, the lanes for different samples are kept together for each segment or graph type in the Karyoview and Selected Chromosome View and in the Detail View.

Note: You can change the order of samples and Data types in the views by clicking and dragging in the Files and Data Types list.

Select display schemes

You can switch to a dark background.

1. From the View menu, select **Switch Color Scheme**.

   The color scheme changes, as shown in Figure 184.

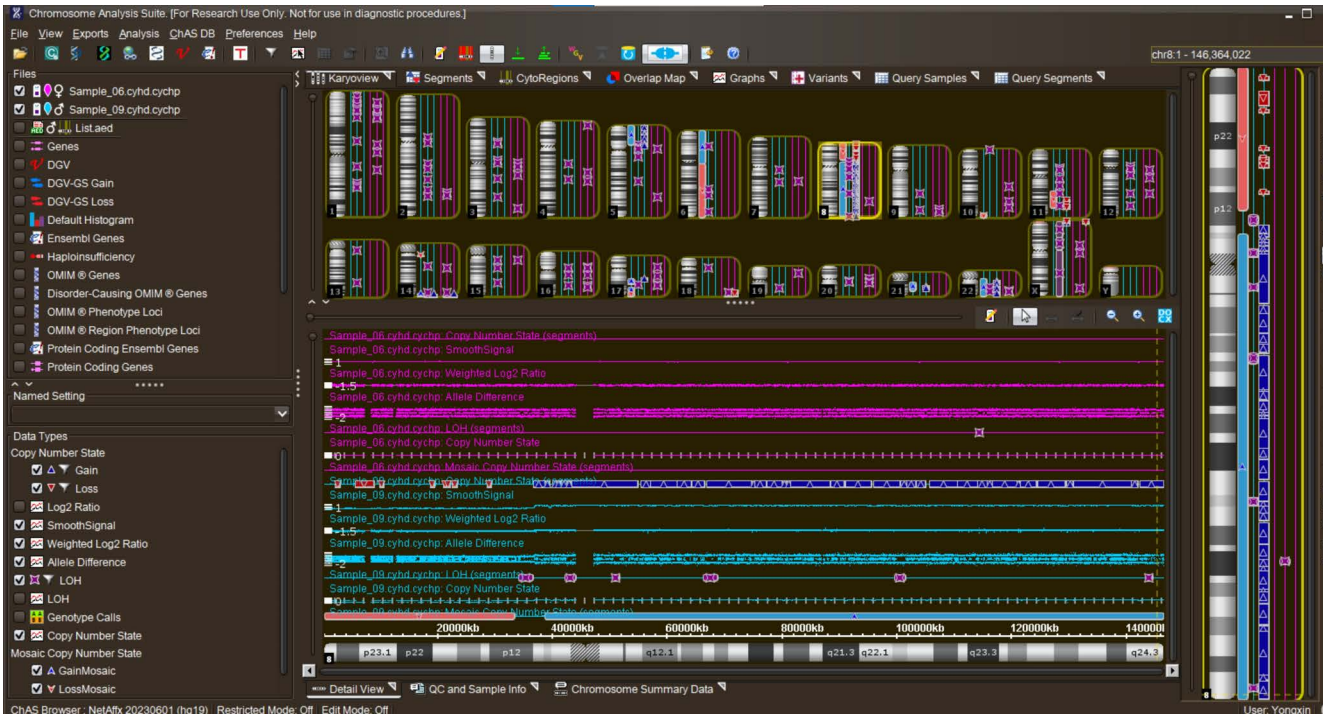


Figure 184 ChAS with Dark Scheme selected.

Expand and contract annotations

For tracks containing multiple rows of annotations, collapsing tracks consolidates all rows within a track into 2 rows. Any annotations after the first one are placed in the second row (Figure 185).

When there are multiple annotations of one type at the same coordinate, the separate annotations are shown on separate rows.

Collapsing tracks is useful if you don't need to see all the details. However, be aware that in collapsed tracks larger annotations may obscure smaller ones; annotations with introns may be obscured by annotations that don't show the intron.

To expand or collapse just a single annotation track, right-click the track name in the Files tree and choose Expand (or Collapse) Annotation track. The Annotation track checkbox must be selected to see this option in the right-click menu.

Note: The maximum number of tracks that can be displayed for any reference annotation is 25.

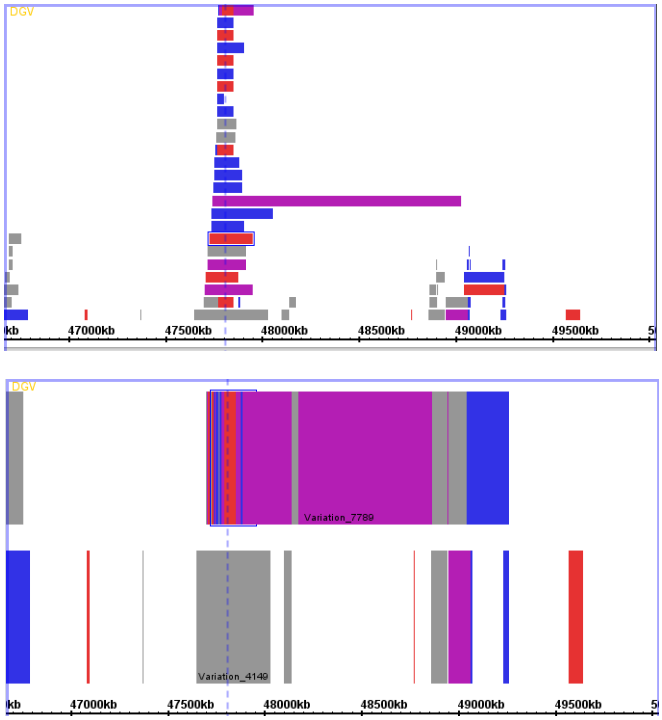


Figure 185 DGV annotations view examples: Expanded (top) Collapsed (bottom).

Toggle between collapsed and expanded display of annotations:

- From the **View** menu, select **Expand/Collapse Annotations**

  Or
- Click the **Expand/Collapse** icon on the main toolbar.

Change graph appearance

You can modify many properties of the graphs in the Detail View. ChAS provides options for:

- "Select different graph styles" on page 185
- "Change graph attributes" on page 189
- "Change scale" on page 190

Settings and adjustments that are specific for graphs can be made using the **Graph Settings** window.

Open the Graph Settings window

1. From the **View** Menu, select **Graph Settings** or click the **Graph Settings** icon in the Graphs Tab toolbar.
   The Graph Settings window opens. (Figure 185)

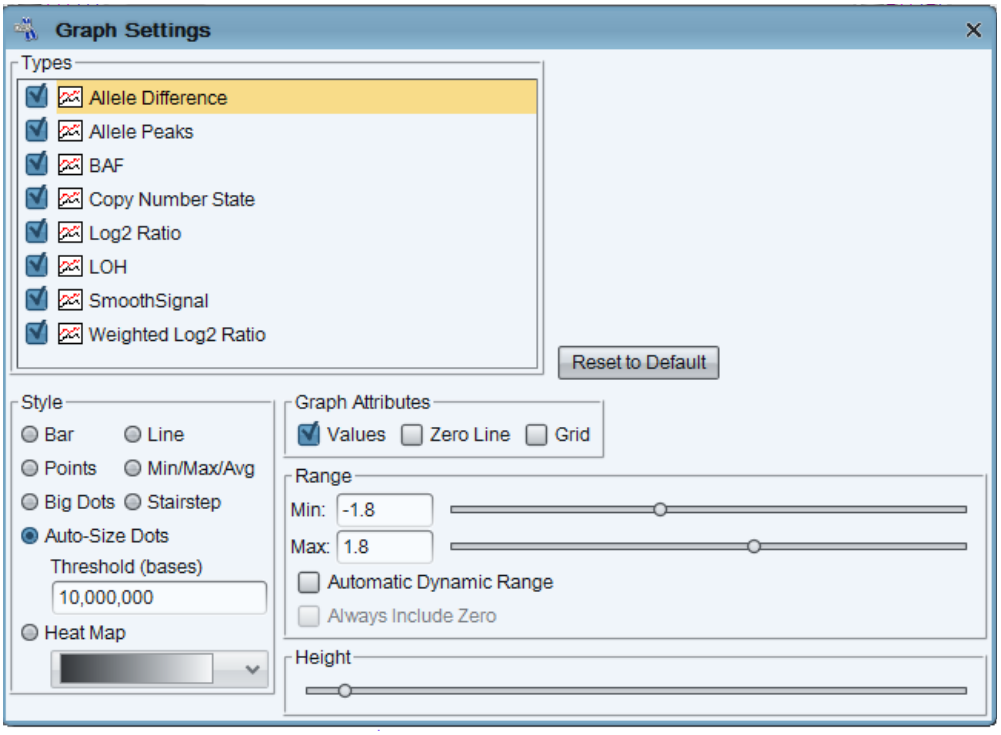


Figure 186 Graph Settings window.

The Types box displays the graph data types being displayed in the Detail View.

Change the settings for a graph type

1. Click a data type to change the settings for that type.
2. Make changes to the graph settings by typing in new values or by operating sliders in the Graph Adjuster panel. For details, see:

- "Select different graph styles" on page 185
- "Change graph attributes" on page 189
- "Change scale" on page 190

Note: Any changes you make to the values in the Graph Settings window will apply to all currently selected graph types.

Select different graph styles

Graphs can be shown in various representational styles. The type of graph that is most appropriate depends on the type of question being asked about the data. For example, when comparing trends and patterns, it is very useful to use the line graph display method. The user is encouraged to experiment with the different display types to find out which method works best for specific purposes and at specific zoom in magnifications.

Change the graph style

1. In the **Style** section choose one of the options. (Figure 185)

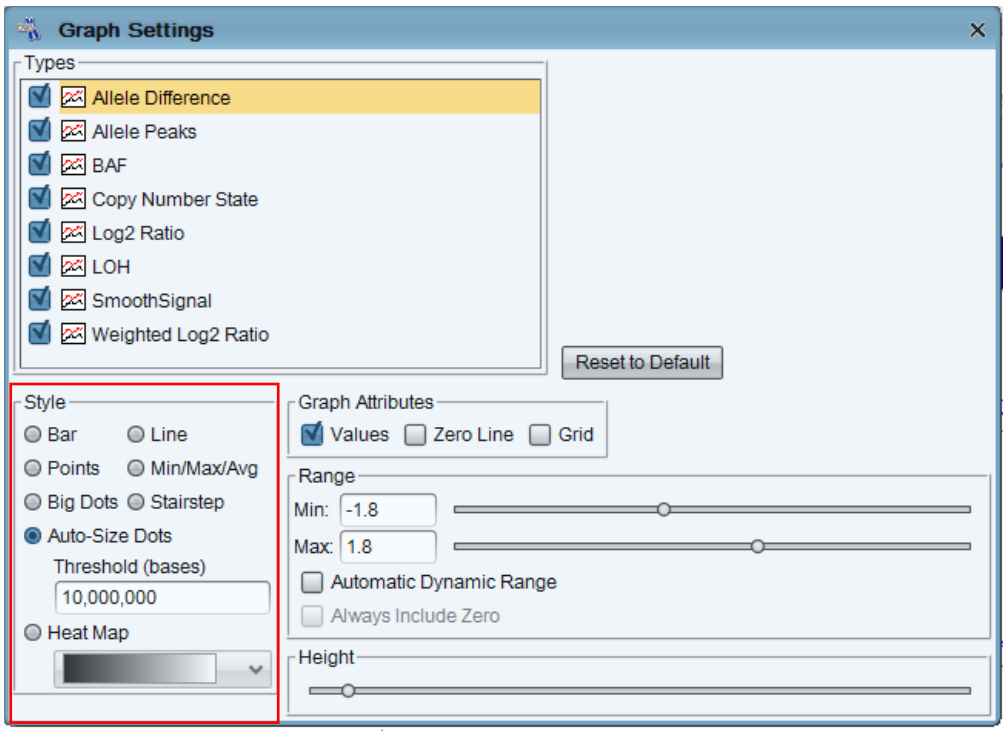


Figure 187 Graph Settings window.

Note: Line, Min/Max/Avg, and Stairstep are not available for CytoScan™ XON Arrays due to the differential coloring based on Level assignment.

- **Bar** – Individual values are shown as vertical bars that are one base wide for position graphs. (Figure 185)

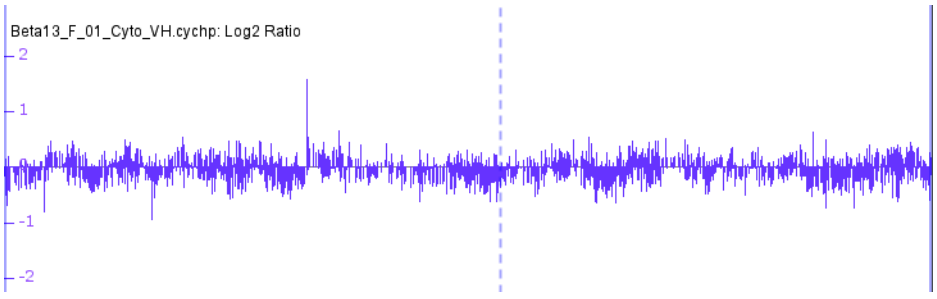


Figure 188 Bar.

- **Line** – Subsequent values are linked with a line. Even if the input file was not sorted, the values are connected in order along the genomic coordinate axis. (Figure 185)

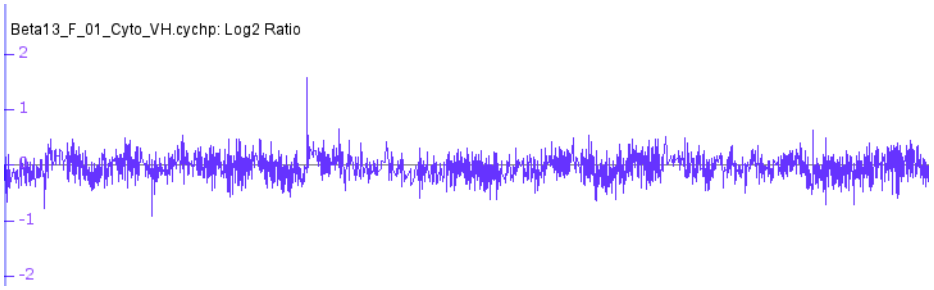


Figure 189 Line.

- **Points** – Shows a single dot for each data value. (Figure 185)

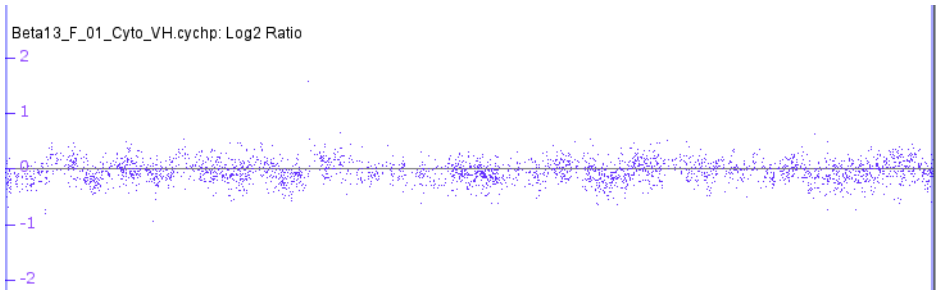


Figure 190 Points.

- **Big dots** – Shows a single big dot for each data value. (Figure 185)

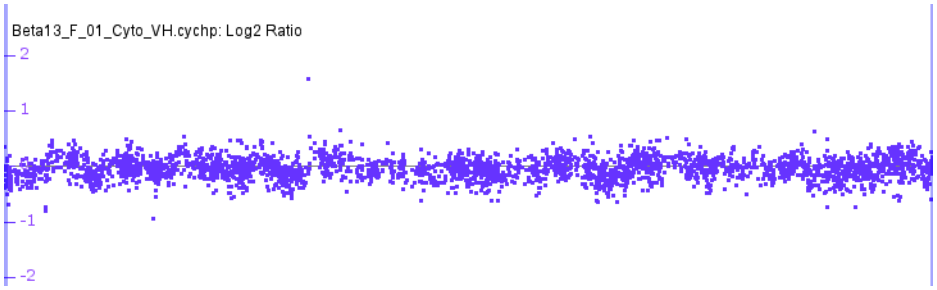


Figure 191 Big dots.

- **Min/Max/Avg** – This style is especially useful for showing very densely populated graphs with data points for large numbers of positions. (Figure 185)

Note: This data style is not available for CytoScan™ XON Arrays due to regions annotated by Levels.

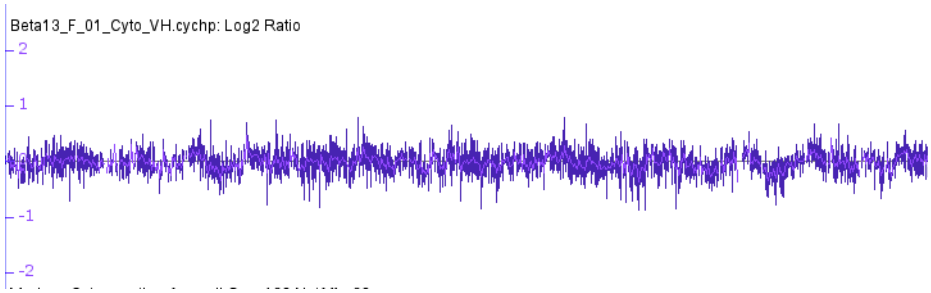


Figure 192 Min/Max Average.

When Detail View is zoomed all the way in, the display is equivalent to the Line style. When zooming out, ChAS starts to summarize values. When the scale of the display reaches the point where individual x-values are associated with multiple score values, ChAS picks the maximum and minimum values and draws a vertical bar between them. In addition, ChAS draws lines through the average of all the data points represented at each x value.

- **Stairstep** – Similar to the bar graph style, except that bar widths along the horizontal axis are stair-stepped. (Figure 185)

Note: This data style is not available for CytoScan™ XON Arrays due to regions annotated by Levels.

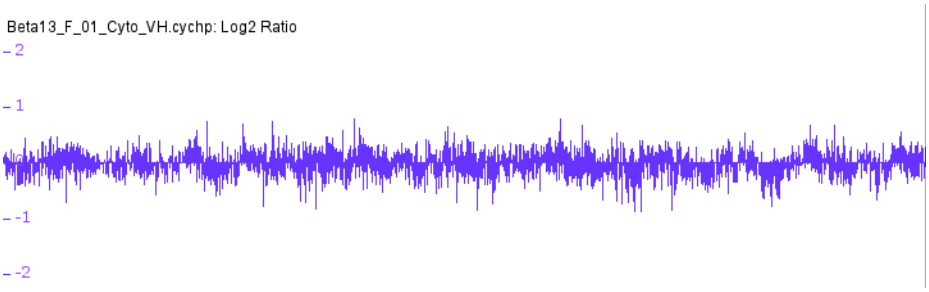


Figure 193 Stairstep.

For example, if position 100 has a value of 50 and position 200 has a value of 75 and there are no values in between, then ChAS will draw a bar of height 50 that starts at position 100 and stops at position 200. Then, at position 200, ChAS will draw a new bar of height 75 that terminates at the next location with a value.

- **Auto-Size Dots** - Transition from Points to Big Dots when zooming in the Detail View. You can select the window size (in base pairs) in which the transition occurs. (Figure 185)

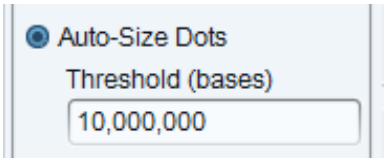


Figure 194 Auto-Size dots.

- **Heat map** – Instead of showing relative intensity via the height of the line at each pixel or coordinate as in most other graph styles, a heat map shows expression levels via color or brightness of the line at each pixel or coordinate (Figure 185). This graph style is useful if you want areas of unusual values to jump out at you. If a graph does not render or is hard to see, adjust the visible bounds of the graph until features are readily visible. Several heat map color schemes are available to choose from.

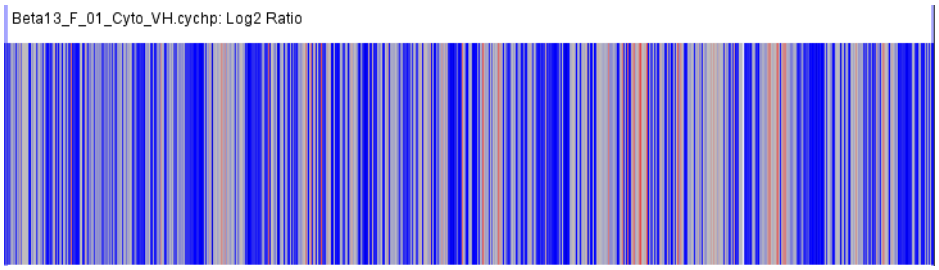


Figure 195 Heat map.

There are now 2 Red/Gray/Blue heat maps.

One is designed to look good for copy number data scaled from 0 to 4 (or 1 to 3), with 2 = normal, and the other is designed to look good for copy number data scaled from 0 to 5 with 2 = normal.

The user must be careful to test that the heat map scaling is appropriate for the data.

Change graph attributes

You can display graphs with:

- Value scale
- Zero Line
- Grid

Change graph attributes

- Select the attributes you want to display in the Detail View. (Figure 185)

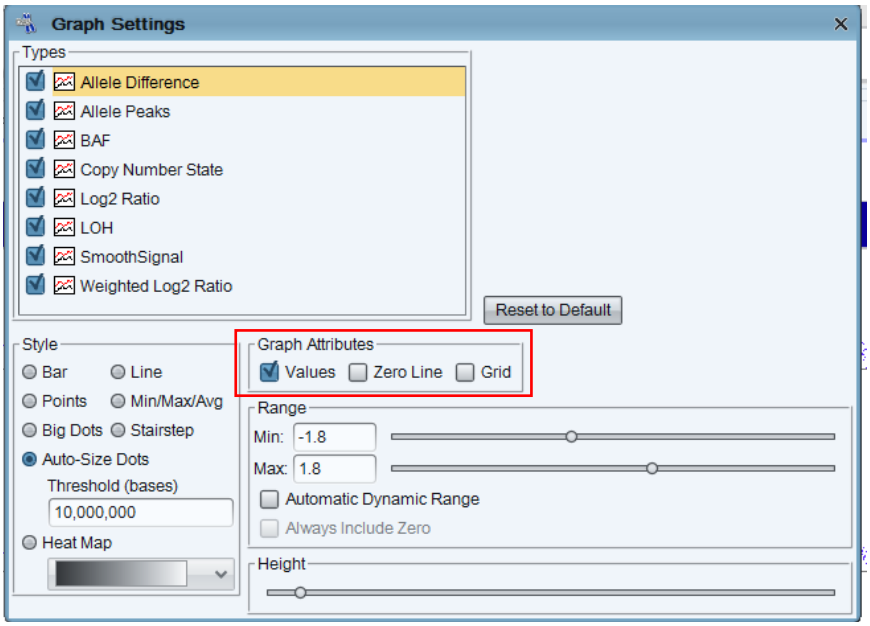


Figure 196 Graph Settings window.

Change scale

Changing the visible bounds involves changing the scale of the graph by setting the maximum and minimum values to be displayed.

To set these visible bounds, use the **Range** section of the **Graph Properties** dialog.



Set specific minimum and maximum values

- Use the sliders, or type in values to the boxes.

  These values are applied to each selected graph. You are free to set maximum and minimum values that cover a range smaller or larger than the actual range of your data.

  – Select the **Automatic Dynamic Range** checkbox to auto-set the Y-axis min/max range.
  – Select the **Always include Zero** checkbox to include a 0 point of reference on the Y axis.

Note: The algorithm detects CN state up to 4 for Genome-Wide Human SNP Array 6.0 CNCHP files and CytoScan™ Array CYCHP files. The TuScan algorithm detects CN State >50 for OncoScan™ FFPE assay OSCHP files. In the Graphs Settings, the Copy Number State range can be set to what is appropriate to the data.

Change the vertical height of a graph

- Use the Height Slider to stretch all the graph type in the vertical direction.



The graph height slider is used to increase or decrease the size of a given graph type. The size is specified in a relative manner. The final graph size will depend on the number of other graphs and annotations being displayed.

Learn more about features

You can use the following tools to learn more about features in the different views:

- "Pop-ups" on page 191
- "Right-click menu options" on page 193
- "Selection Details table" on page 195
- "Link to external websites" on page 201

Pop-ups

You can mouse over a feature in any of the views to display a popup box with information on the feature. The information provided depends on the type of data that the mouse arrow is on.

Pop-ups are available for:

- Cytobands
- Detected segments
- Graph data
- Marker position indicators
- Histograms
- Reference annotations

Note: You should expand the reference annotations before selecting one to avoid selecting multiple annotations. See "Expand and contract annotations" on page 183.

- Displayed Region files, including
  - Overlap Map Regions
  - CytoRegions

The information displayed differs depending upon the type of feature selected, as shown in the following samples.

For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).

You can learn more about the terms used in the pop ups in "Selection Details table" on page 195.

Turn pop-ups on or off

1. From the **View** menu, select **Mouse-over Pop-ups**.

   The information (Figure 197, Figure 198, Figure 200, and Figure 201) can include custom properties created by a user (see "View and edit annotations" on page 278 for more information).

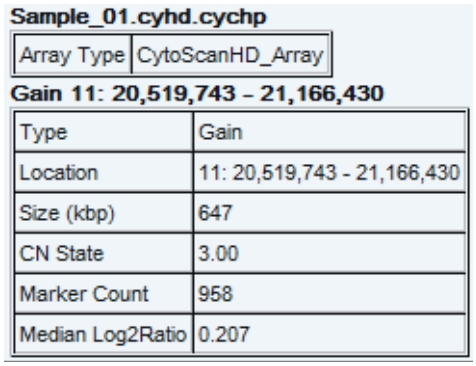
Sample_01.cyhd.cychp

Array Type	CytoScanHD_Array

Copy Number State

Type	Copy Number State
NetAffx Version	33
Array Type	CytoScan HD Array
Nearest Coord	16,501,954
Nearest Value	2

Figure 197 Pop-up for CN State Graph.



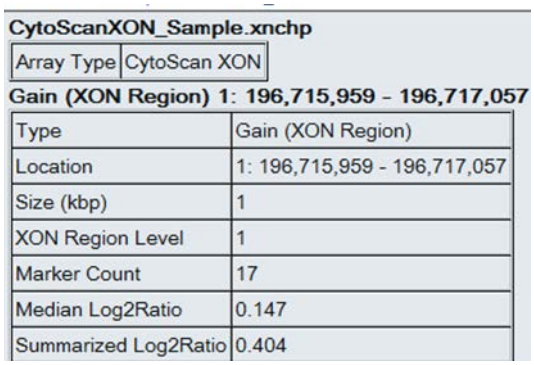
Sample_01.cyhd.cychp

Array Type	CytoScanHD_Array

Gain 11: 20,519,743 – 21,166,430

Type	Gain
Location	11: 20,519,743 - 21,166,430
Size (kbp)	647
CN State	3.00
Marker Count	958
Median Log2Ratio	0.207

Figure 198 Pop-up for a Gain Segment.



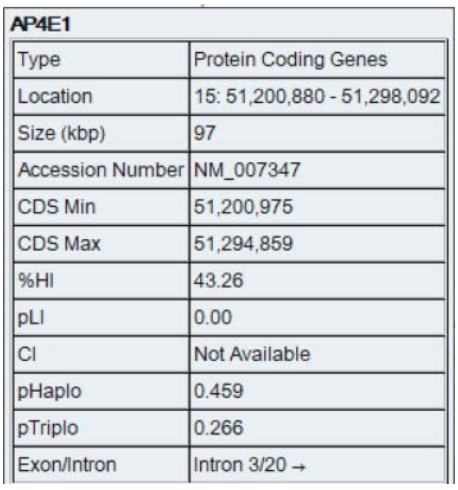
CytoScanXON_Sample.xnchp

Array Type	CytoScan XON

Gain (XON Region) 1: 196,715,959 – 196,717,057

Type	Gain (XON Region)
Location	1: 196,715,959 - 196,717,057
Size (kbp)	1
XON Region Level	1
Marker Count	17
Median Log2Ratio	0.147
Summarized Log2Ratio	0.404

Figure 199 Pop-up for an XON Region Gain Segment.



AP4E1

Type	Protein Coding Genes
Location	15: 51,200,880 - 51,298,092
Size (kbp)	97
Accession Number	NM_007347
CDS Min	51,200,975
CDS Max	51,294,859
%HI	43.26
pLI	0.00
CI	Not Available
pHaplo	0.459
pTriplo	0.266
Exon/Intron	Intron 3/20 →

Figure 200 Pop-up for a Gene.

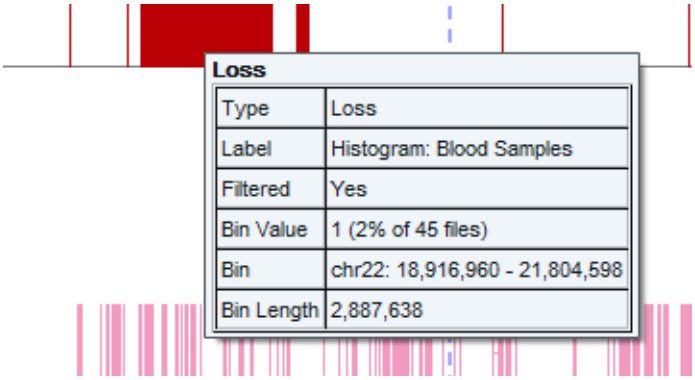


Figure 201 Pop-up for a Histogram.

Right-click menu options

You can right-click any of the following types of features to open a menu with options for learning more about the feature:

- Detected segments (Figure 185)
- Histograms (Figure 185)
- Reference annotations, including cytobands (Figure 185)

Note: You should expand the reference annotations before selecting one to avoid selecting multiple annotations. See "Expand and contract annotations" on page 183.

- Displayed Region files, including (Figure 185):
  - Overlap Regions
  - CytoRegions

Note: Not all options are available for the different feature types. Also, In the Detail View you can select multiple items of different types. The available options will differ, depending upon the number and types you have selected.

Some menu options are common to the different types of features:

- Number of items selected (if more than one item is selected, the options available may differ, depending upon the type and number of items).
- **Zoom to selection:** See "Zoom to a selected item" on page 171.
- **Selection Details:** Opens the Selection Details box with information about the feature. See "Selection Details table" on page 195.
- **Add to a file:** Add the selected segment, annotation, or region to a region file. See "Add regions to a new or existing AED file" on page 273.
- **View/Edit Annotation Properties:** Displays the Annotations Properties window for the selected feature. You may or may not be able to edit the properties. See "View and edit annotations" on page 278.
- **Query ChAS DB:** Displays the segments in the database that match the user defined Overlap and Coverage threshold settings. See "Set up a ChAS DB query" on page 363.

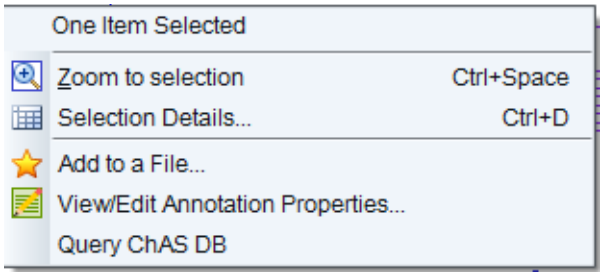


Figure 202 Segment right-click menu.

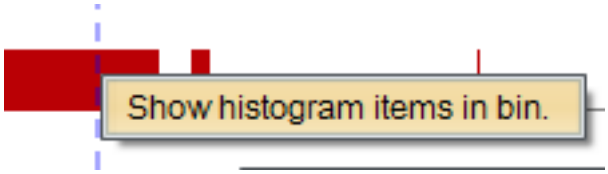


Figure 203 Histogram right-click menu.

Special options for annotations include:

- Link to remote web site for more information. (Figure 185)

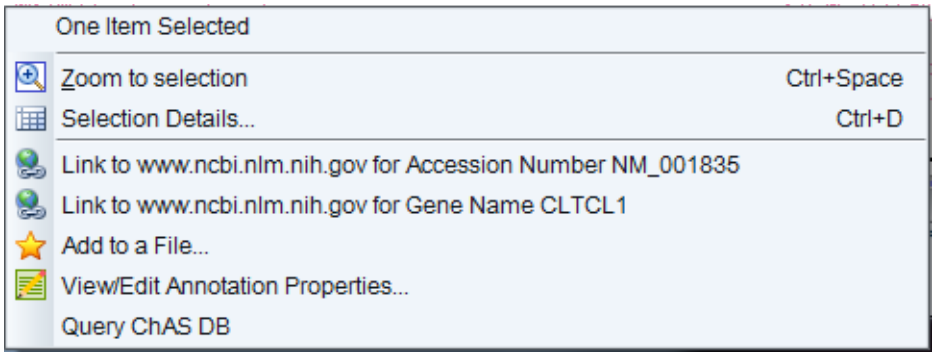


Figure 204 Annotation right-click menu.

Custom functions for regions include (Figure 185):

- **New User Annotation:** Opens the New User Annotation window, which enables you to add user annotation to a region. For more information, see "Create a New User Annotation" on page 285.

- **Increment Counter:** Increments the counter in the annotation properties, allowing you to track the number of times a feature has been seen. For more information about the annotation properties, see "View and edit annotations" on page 278.

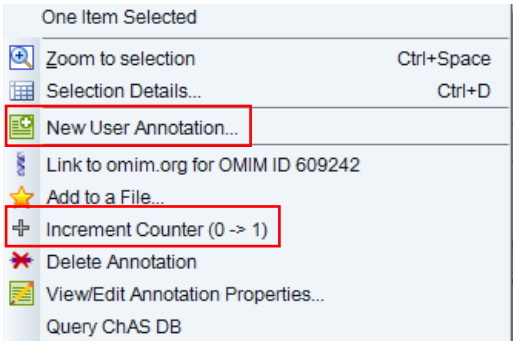


Figure 205 Region right-click menu.

Selection Details table

The Selection Details table (Figure 206) displays information available for items selected in the graphic display views (Karyoview, Chromosome View, and Detail View). It is accessed by right-clicking an item in one of the views and selecting **Selection Details**.

The information is presented in 2 tables:

- Upper table shows one item per row: Provides summation feature and PDF or tab-separated text file export capabilities.
- Lower table shows one item per column: Provides ability to export the table to a tab-separated text file.

To reorder the columns in the upper table, drag a column header left or right. The corresponding row in the lower table is automatically moved to the new location in the table.

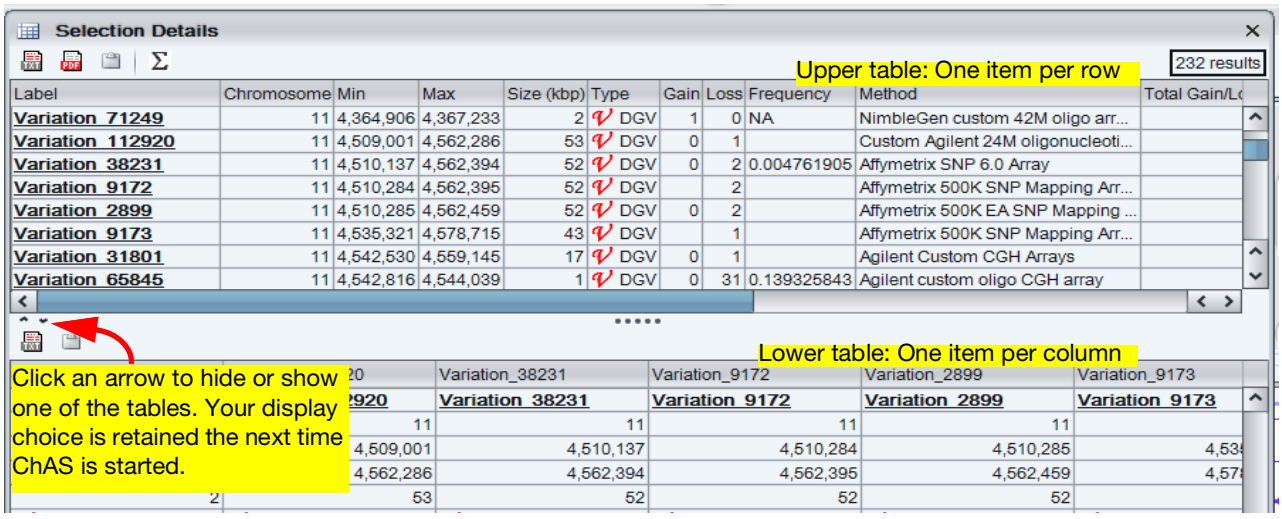


Figure 206 Example: Selection Details table.

The Selection Details table may include the following columns:

Table 28 Selection Details table.

Column	Description
Common	
Label	Identifier for the item.
Chromosome	Chromosome on which the item is located.
Min	Zero-based index position of the first base pair in the sequence.
Max	Zero-based index position of the last base pair in the sequence, plus one. Adding one ensures that the length of any (hypothetical) segment containing a single marker would be one, and ensures that the coordinates match the coordinate system used in BED files. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).
Size (kbp)	Size of the item.
Type	Type of segment (Gain, Loss, Gain Mosaic, LossMosaic, LOH and LOHMosaic) or annotation.
Segments	
File	File the segment was detected in.
CN State	Copy Number State (not displayed for LOH segment types). The expected Copy Number State on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes. See "LOH segments on X and Y chromosomes" on page 46 for more information.
Mean Marker Distance	Length of the segment in base pairs divided by the number of markers in the segment.
Interpretation	User-editable field for free-text interpretation on the segment
Call	User-editable field populated by a user-configurable drop list of Calls.
Inheritance	User-editable field populated by a user-configurable drop list of Inheritance.
Curation By	The current computer Operating System login ID and ChAS user profile name at the time that the Call or Interpretation field was last edited.
Curation Time	The time and date when the Call or Interpretation field was last edited.
Materially Modified Segment	Indication that segment was previously merged, deleted, or had its start or end boundary, type, or state altered by a ChAS user. (ChAS-based processes of Smoothing and Joining are not “Modifications”, nor are making Calls or Interpretations, in this context).
Materially Modified By	The current computer Operating System login ID and ChAS user profile name at the time that the segment was last materially modified.
Materially Modified Time	The time and date when the Segment was last materially modified.
Max % Overlap	The highest percentage by which some item(s) in the Overlap Map overlaps the segment. Segments completely overlapped by an Overlap Map item are 100% overlapped. This number is used for Filtering Segments out by “Overlap”.

Table 28 Selection Details table. (Continued)

Column	Description
Overlap Map Items (% of Segment overlapped)	Item(s) in the Overlap Map which overlap the segment, followed by the percentage by which the segment is overlapped by that Item.
CytoRegions	Names of the CytoRegions with which the segment shares coordinates.
Use in Report	Allows manual selection of Segments for export to a Segments Table PDF, DOCX, or Text rather than all segments in the table.
Marker Count	Number of markers in the segment.
Cytoband Start	Cytoband in which the segment begins.
Cytoband End	Cytoband in which the segment ends.
Genes	List of RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Gene Count	A count of the gene names listed in the Genes column
DGV	List of DGV variations that share coordinates with the segment.
sno/miRNA	List of sno/miRNA features that share coordinates with the segment.
OMIM Genes	List of OMIM™ Genes that share coordinates with the segment.
Disorder Causing OMIM Genes	List of OMIM™ Genes designated with a Phenotype Map Key of 3.
Disorder Causing OMIM Genes Count	A count of the Disorder Causing OMIM Gene names listed in the Disorder Causing OMIM Genes Count.
OMIM Gene Count	A count of the OMIM Gene names listed in the OMIM Genes column.
OMIM Phenotype Loci	List of OMIM Phenotype Loci that share coordinates with the segment.
OMIM Region Phenotype Loci	List of OMIM™ Phenotype associated with a genomic region. The OMIM™ Morbidity information is displayed when using all 3 OMIM tracks. (OMIM™ Genes, OMIM™ Phenotype Loci and OMIM™ Region Phenotype Loci)
MANE	Select transcripts are shown in blue. Transcripts from MANE Plus Clinical are shown in red.
Segmental Duplications	List of Segmental Duplications that share coordinates with the segment.
Smoothed/Joined	Indication that segment was created by smoothing or joining 2 or more segments in the initial segment detection.
Segment Label	A label comprised of the segment's Type, State, and Filename.
Segment Name/ID	File-specific identifier assigned to the detected segment.
Start Marker	The array marker name which marks the beginning of the segment.
End Marker	The array marker name which marks the end of the segment.
Preceding Marker	The array marker just above the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.

Table 28 Selection Details table. (Continued)

Column	Description
Preceding Marker Location	The coordinate location of the array marker just above the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Following Marker	The array marker just below the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Following Marker Location	The coordinate location of the array marker just below the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Mean Log2 Ratio	The mean of all the Log2 Ratio values contained in the segment.
Mean Weighted Log2 Ratio	The mean of all the Weighted Log2 Ratio values contained in the segment.
Microarray Nomenclature	An ISCN-based description of the segment.
Sample UUID	Unique identifier for the CHP file.
Max % Coverage	The highest percentage by which a segment covers some item(s) in the Overlap Map.
Number of Overlap Map Items	Number of Overlap Map items which share genomic coordinates with the segment.
% of Overlaps Map Item covered by Segment	Overlap Map Item and the percentage by which it is covered by the segment.
Full Location	Chromosome Start and Stop in a user-friendly format for use in external databases.
Median log2	The median of all the Log2 Ratio values contained in the segment.
DB Count Both	Number of segments in the database meeting both the user defined thresholds of minimum Percent Overlap Count and Coverage Count.
DB Coverage Count	Number of segments in the database meeting the minimum Percent Coverage Count.
DB Overlap Count	Number of segments in the database meeting the minimum Percent Overlap Count.
XON Region Level	The annotation Level assigned to this region of the genome.
Summarized Log 2 Ratio	The median of the LR, after transformation to adjust for individual marker responsiveness.
Genes	
Chromosome	Chromosome on which the item is located.
Min	Zero-based index position of the first base pair in the sequence.
Max	Zero-based index position of the last base pair in the sequence, plus one. Adding one ensures that the length of any (hypothetical) segment containing a single marker would be one, and ensures that the coordinates match the coordinate system used in BED files. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).

Table 28 Selection Details table. (Continued)

Column	Description
Size (kbp)	Size of the item.
Type	Type of segment (Gain, Loss, Gain Mosaic, LossMosaic, LOH and LOHMosaic) or annotation.
Accession Number	Unique identifier assigned to the sequence in GenBank.
CDS Min	Minimum position of the coding sequence (BED-style coordinates).
CDS Max	Maximum position of the coding sequence (BED-style coordinates).
Strand	The sequence strand of the item.
%Hi	Dosage sensitivity indicator derived from DECIPHER (https://decipher.sanger.ac.uk/). The lower the percentage, the more likely the gene is to be dosage sensitive.
pLI	Probability of loss intolerance. Genes with higher numbers are more likely to be dosage sensitive. Derived from gnomAD (https://gnomad.broadinstitute.org/).
CI	Dosage sensitivity indicator derived from gnomAD (https://gnomad.broadinstitute.org/) for hg38 only. Not yet implemented by gnomAD.
pHaplo	Probability of Haploinsufficiency): The higher the value (0–1), the more likely to be dosage sensitive. Collins et al. A cross-disorder dosage sensitivity map of the human genome. 2022. PMID: 35917817
pTriplo	Probability of Triplosensitivity): The higher the value (0–1), the more likely to be dosage sensitive. Collins et al. A cross-disorder dosage sensitivity map of the human genome. 2022. PMID: 35917817
Ensembl Genes	
CDS Min	Minimum position of the coding sequence (BED-style coordinates).
CDS Max	Maximum position of the coding sequence (BED-style coordinates).
Strand	The sequence strand of the item.
OMIM	
OMIM Gene Title	The title of the gene associated with the OMIM™ entry.
OMIM Gene Symbol List	A list of genes associated with the OMIM™ entry.
OMIM Disorder	Disorder associated with the OMIM™ entry.
OMIM Phenotype Key	Indicates how this phenotype was placed on the map.
OMIM Gene Symbol	Symbol of the gene based on gene title.
OMIM Phenotype Loci	
OMIM Phenotype ID	Unique identifier to an OMIM™ phenotype.
OMIM Phenotype Map Key	Indicates how this phenotype was placed on the map.

Table 28 Selection Details table. (Continued)

Column	Description
OMIM Phenotype Locus Description	Describes the phenotype or disorder associated at the OMIM™ Phenotype Loci.
OMIM Region Phenotype Loci	
OMIM ID	Unique identifier to an OMIM™ phenotype.
Cytoband	Location of the region.
Min	Start location of Genome.
Max	End location of Genome.
Gene Symbol	Symbol of the gene (based on gene title).
Gene Title	The title of the region associated with the OMIM™ entry.
Comment	Additional information for the entry from OMIM.
Gene-Phenotype Loci Data	Information of Gene-Phenotype relationships for the region.
Sentimental Duplications	
Score	Score based on the raw BLAST alignment score. The score for segmental duplications is set to zero in NetAffx™ annotation 31 and higher.
FracMatch	The fraction of matching bases.
FracMatchIndel	The fraction of matching bases with Indels.
Strand	The sequence strand of the item.
MANE	
Type	Type of MANE transcript (MANE Select, MANE Plus Clinical)
Location	Start and End location of Genome.
Size	Size of the transcript.

Note: Thermo Fisher Scientific does not generate or verify the information for genes, FISH clones, Segmental Duplications, sno/miRNAs, DGV annotations, OMIM™ data, or MANE. Segmental Duplication and sno/miRNA annotations do not have any unique terms; but sno/miRNA annotations use the "type" field to indicate subtypes like "cdBOX" and "HAcaBOX".

Some information may not be displayed, depending upon the feature type. The information can include custom properties created by a user (see "View and edit annotations" on page 278).

You can export data from the table using the standard table export tools (see "Export table data" on page 393).

You can perform multi-column sorts. See "Sort by columns" on page 306.

Link to external websites

You can view a selected area within the Detail View at one of the following public sites via https:

- **UCSC**
- **Ensembl**
- **Toronto DGV**
- **ClinVAR**
- **ClinGen**
- **DECIPHER**
- **Load TaqMan Assays Track**

View a selected area at a public site

1. In the Detail View, zoom and scroll to the area of interest. (Figure 206)

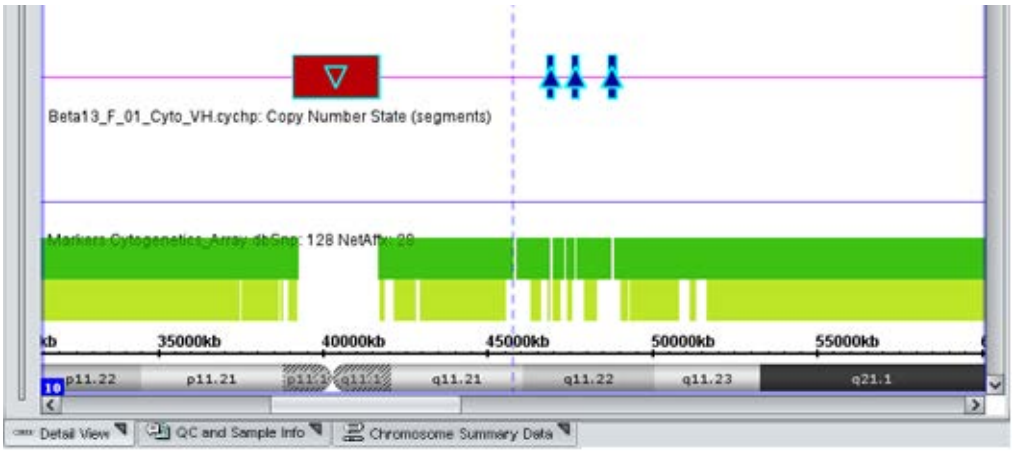


Figure 207 Selected area in Detail View.

2. From the View menu, select **View Region at [site name]** or click the appropriate site's toolbar button.

   A browser opens, displaying the selected area of the chromosome.

Link to TaqMan™ copy number and genotyping assays

View and order TaqMan™ assays for CN

TaqMan™ assays can easily be accessed from within ChAS. These assays can be used for confirmation of copy number aberrations. TaqMan™ assays can only be ordered based on hg38 genome coordinates.

Do the following for the region(s) you would like to view and order TaqMan™ assays for Copy Number:

1. Locate the region containing the aberration in the Detail View.
2. Select the TaqMan™ shortcut in the toolbar (Figure 208) to view which TaqMan™ assays are available within the genomic coordinates populated in the text box at the top center of the browser.

   The TaqMan™ assays load in a track in the Detail view.

Note: This track is only available for the genomic coordinates for the current query. To view TaqMan™ assays in another region of the genome, repeat steps 1 and 2.

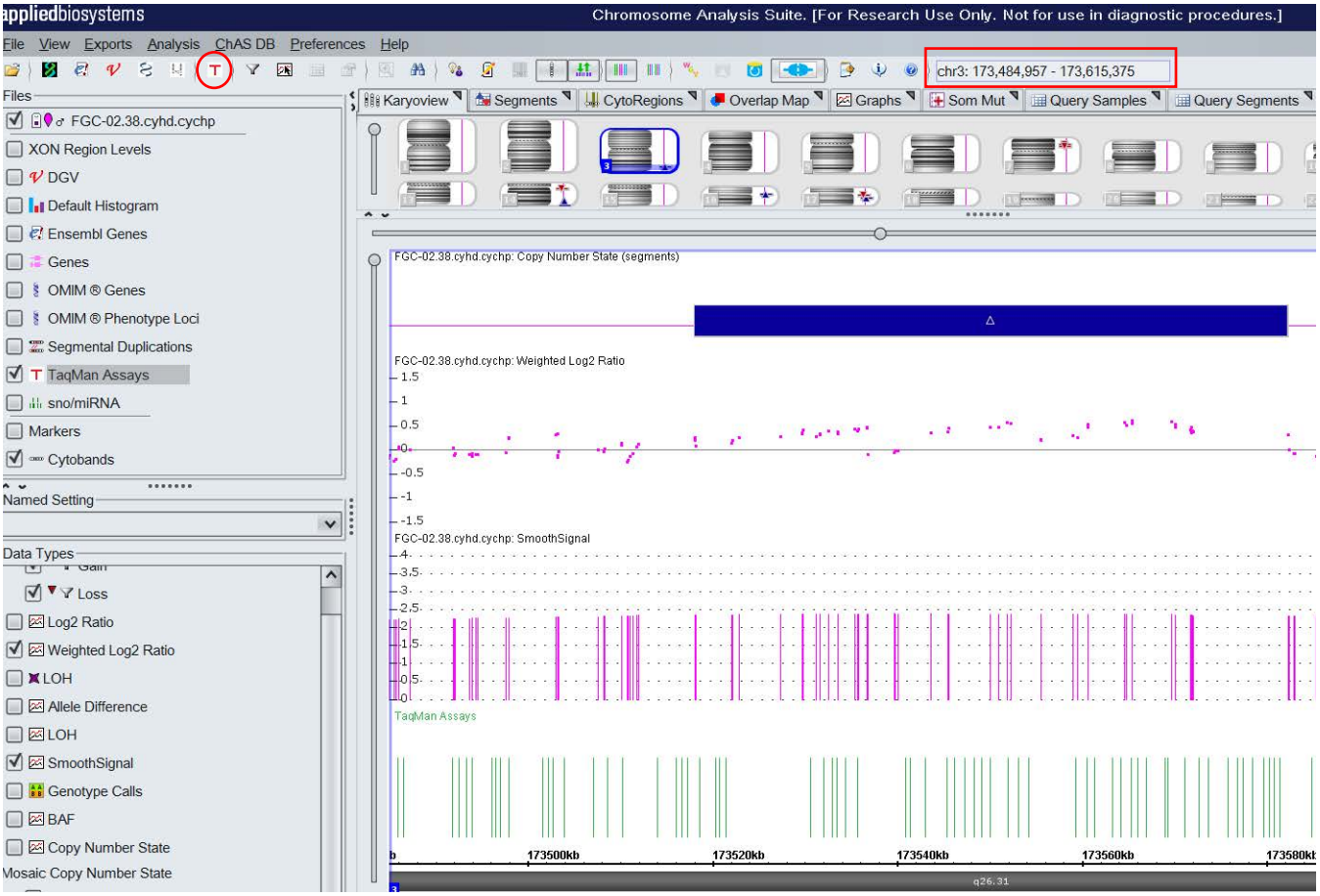


Figure 208 TaqMan™ Shortcut icon and the genomic coordinate.

3. Right-click the TaqMan™ assays you want to order, then click **Order TaqMan assay** to link out to the website, as shown in Figure 209.

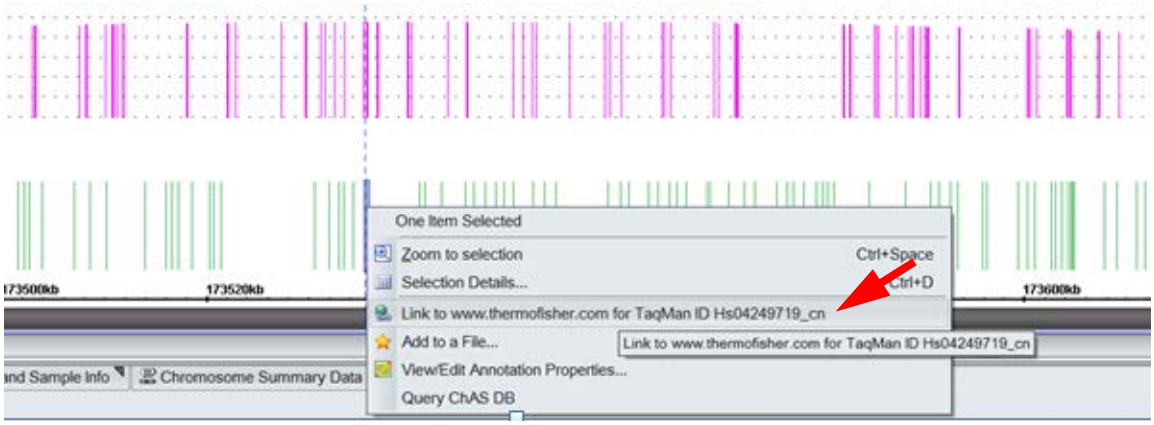


Figure 209 Link to selected TaqMan ID.

View and order TaqMan™ assays for genotyping

TaqMan™ assays for genotyping can be ordered from VCF files that contain dbSNP IDs.

Note: The VCF file must contain an rsID for the SNP to directly access the TaqMan™ website for that SNP. Also, TaqMan™ assays can only be ordered based on hg38 genome coordinates.

1. Load a VCF file clicking **File → Open**.
2. Right-click an SNP for which you would like to view and order a TaqMan™ assay.

   A menu appears. (Figure 208)

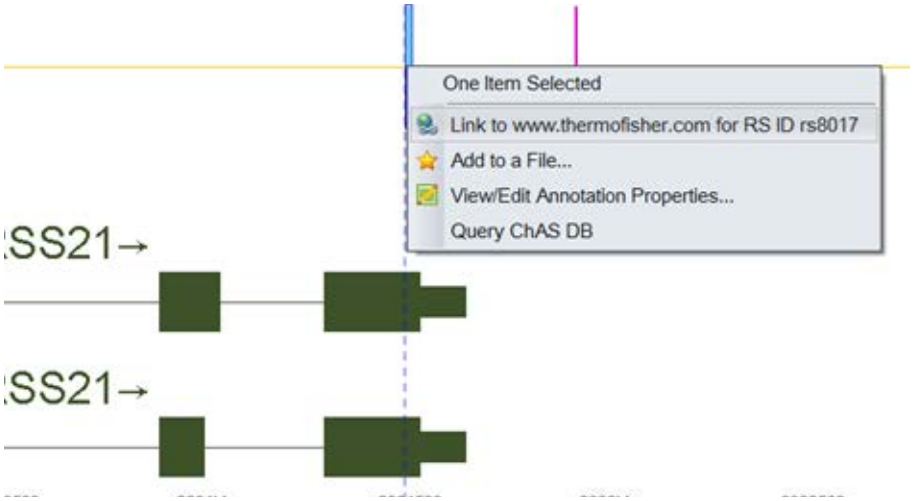


Figure 210 Right-click menu for TaqMan™ ordering.

3. Click the provided "Link to" link.

   You are directed to the TaqMan™ website for details about the assay.

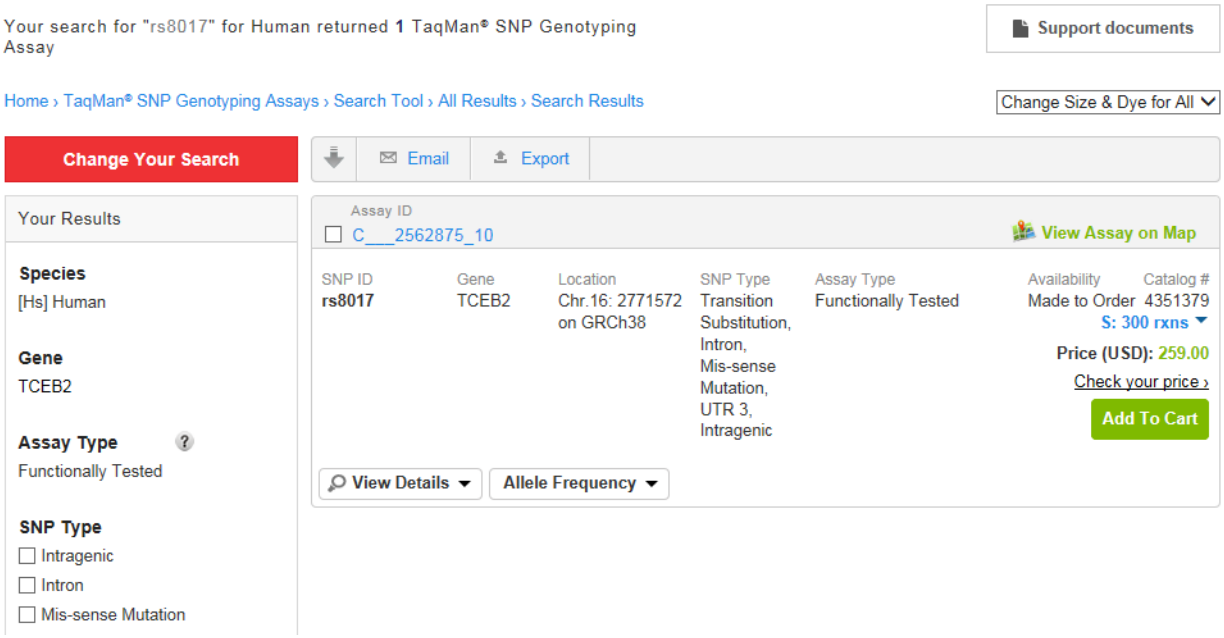


Figure 211 Assay details example.

9 Filter segments

ChAS enables you to filter the detected segments using different segment parameters, concealing segments that do not meet requirements for significance for:

- Marker Count
- Length
- XON Level Assignment (XNCHP only)
- Percent Mosaicism

You can apply these filters to different segment types, using different parameters for each type. The filtering is done on the fly, with changes to the parameters reflected in the different views as they are made.

A segment must pass all filter requirements for the segment type to be displayed.

You can apply different filter values for areas inside CytoRegions and areas outside the CytoRegions (genomewide). See "Use filters with CytoRegions" on page 259.

The Overlap Map filter is described in "Use the overlap map and filter" on page 262.

Filter settings are saved when a Named Setting is created and can be reapplied. See "User profiles and named settings" on page 408.

IMPORTANT! The Filters set in the browser are NOT linked to the filters for the MSV. The same filter settings should be set in both the ChAS Browser and the MSV separately. The MSV does have a flag to indicate when filter settings do not match. For more information, see the *Reproductive Health Research Analysis Software User Guide* (Pub. No. MAN0027973).

Apply segment parameter filters

Open the Segment Filters window

1. Select **View** → **Segment Filters** on the menu bar.
2. Click the **Segment Filters** toolbar icon.
3. Right-click a segment type in the **Data Types** list, then select **Filters…** from the right-click menu. (Figure 212)

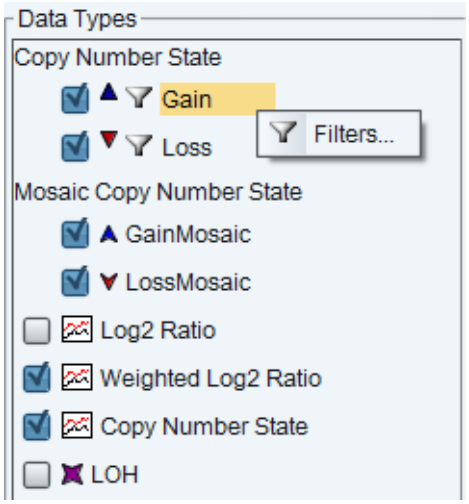


Figure 212 Right-click menu.

Note: If you use the right-click menu option, only the filter settings for the selected segment type are displayed.

The **Segments Filters** window opens. (Figure 213)

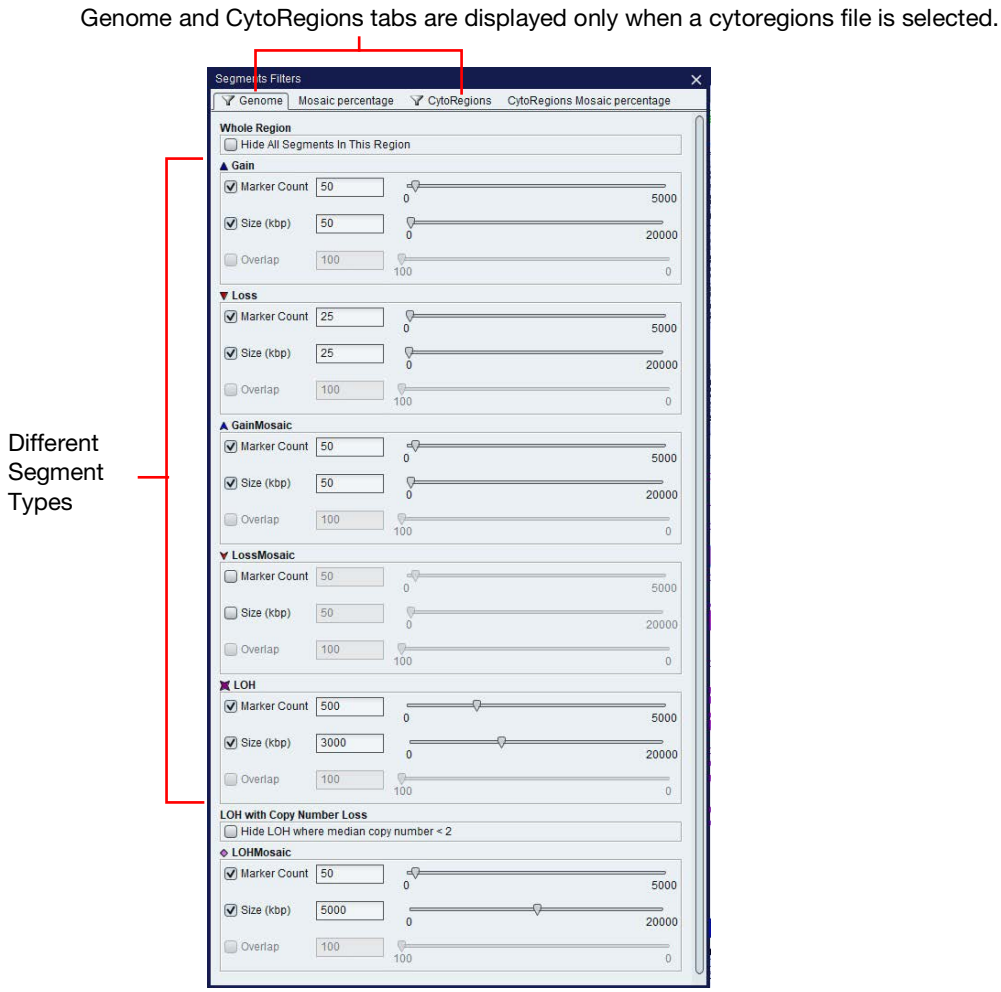


Figure 213 Segment Filters window.

The following parameter options for filtering apply to both the Genome tab and the CytoRegions Tab.

Note: The Overlap Map filtering parameter is set using the same window. The Overlap Map function is described in "Use the overlap map and filter" on page 262.

- For XON, select level checkbox(es) to reveal any XON region segment calls in regions assigned to the level(s) you selected.

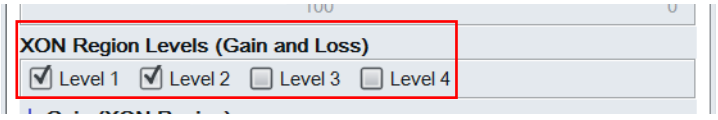


Figure 214 Level selections.

Table 29 Segment Filter options.

Option	Function
Hide All Segments in this Region	Hides all the segments. This is particularly useful when using a CytoRegions file for CytoScan™ XON arrays. Select the Hide All Segments in this Region checkbox on the Genome Tab so that *only* segments overlapping CytoRegions are shown. **Note:** This option is only available with a CytoRegions file is assigned.
Hide LOH where median CN < 2	Selecting this option will hide LOH segments that are assigned a median copy number less than 2. LOH with median copy number of 2 or higher will still be displayed.
Marker Count	The number of markers the segment encompasses from start to finish. A segment must have at least as many markers as you specify to be displayed. Each marker represents a probe which represents a sequence along the genome at a particular spot. Markers are probe sequences of DNA, each sized from 12–50 base pairs long, depending on the type of array data. The 12–50 bp sequence is unique to that one spot on the genome it represents.
Size	Based on the start and end markers of a segment. Because each segment represents a single place in the genome, you can measure from start to end, in DNA base pairs, and by filtering, demand a segment be at least that long to be visualized.
XON Segment Level	Based on the Level Assignment to the region in the genome. • Level 1: Medical Research exome and cancer • Level 2: ClinVar genes not covered in Level 1 • Level 3: Other OMIM™ genes • Level 4: Opportunistic regions from Refseq/UCSC/Enseble/LOVD. The XON Segment Filters can be used to narrow down the number of XON segments to review based on their annotation level assignment. Those regions assigned as Level 1 contain genes/ regions that have been identified as part of the Medical Research Exome along with regions associated with cancer. By selecting only Level 1 in the filter settings, only XON segment calls in regions assigned as Level 1 are displayed. XON segments for all other Levels are hidden from view as well as hidden in the Segments Table. To expose XON segment calls in other regions simply check the box for those Levels in the Filters Windows. For more details on CytoScan XON analysis workflow recommendations, Appendix G, "Recommended CytoScan™ XON Array workflows" on page 466.
Percent Mosaicism	Hides the mosaic segments based on the defined threshold and parameters. Multiple filters (rules) can be assigned allowing gains/losses and autosomes/sex chromosomes to be filtered independently.

Use segment parameter filters

1. Select the checkbox next to the parameters you want to use as filters. (Figure 215)

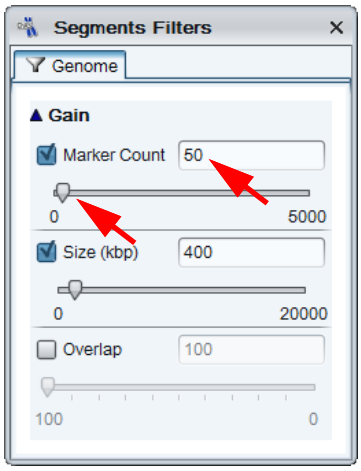


Figure 215 Gain Segment filter settings.

2. Use the slider to set the value for the parameter or enter a value in the provided text field. (Figure 215)

Note: As you move the slider from left to right, more segments are removed.

Your filtered results are displayed instantly in all tables and graphs. For information about using the Overlap setting, see "Use the overlap map and filter" on page 262. For information about using different filtering settings in CytoRegions, see "Use filters with CytoRegions" on page 259.

Use percent mosaicism filters

The percent mosaicism filters tab allows you to apply multiple rules on filtering copy number mosaic segments.

Segments that are mosaic gains can be filtered for a different percentage than mosaic losses. Mosaic segments on autosomes can be filtered differentially from the sex chromosomes as well. Copy Number mosaic segments that intersect with a cytoregion can be differentially filtered relative to the filters set for the rest of the genome.

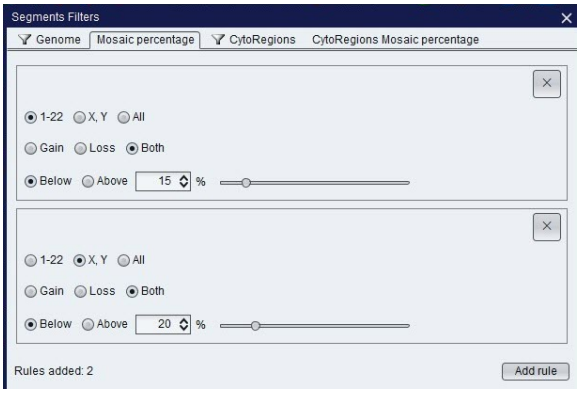


Figure 216 Mosaic percentage filter.

In Figure 216, the autosomes (1–22) will filter both mosaic gain and loss segments at 15% and lower. Both mosaic gain and loss segments on the sex chromosomes (X and Y) will be filtered at 20% and lower.

Bypass segment filters

A specific segment can be assigned to ignore the current filter settings. The segment will still displays even though its marker or size thresholds are not met. To use this feature:

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click the segment you want to bypass the segments filters with.

   A menu appears.

2. Click **View/Edit Annotation Properties**.

   The Annotation Properties window appears. (Figure 217)

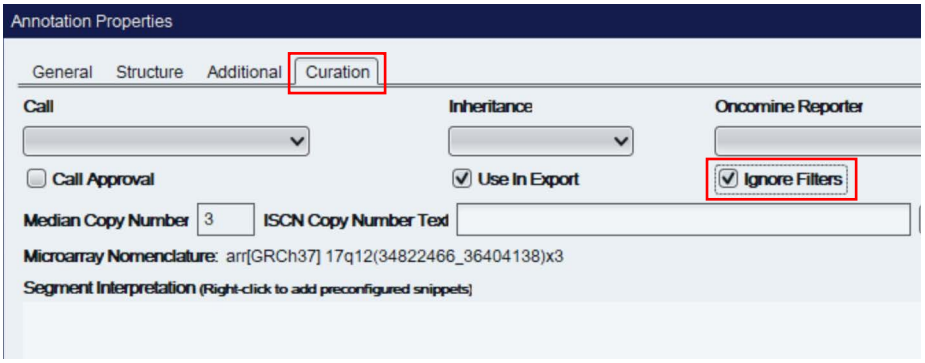


Figure 217 Annotation Properties window.

3. Click the **Curation** tab.
4. Select the **Ignore Filters** checkbox.
5. Click **OK**.

   The segment you wanted to bypass your filter settings with is now displayed.

10 Segment modification

Edit segment data overview

- ChAS enables you to edit data segments; merge, delete, draw *de novo*, adjust boundaries, and change the Copy Number State of CHP data segments, or undo your changes.
- The instant a file is modified by editing of one or more segments, having a segment interpreted, or called, ChAS auto-generates a "sidecar" or CHP Change Archive (CHPCAR) file which saves these modifications. ChAS then uses this CHPCAR file for further user-defined edits and modifications, while your original (native) xxCHP file (CYCHP, CNCHP, or OSCHP) is safeguarded (remains untouched).
- The smoothing, joining and XON merging settings for each CHP file are locked at the time of CHPCAR file creation, while unmodified CHP files without CHPCAR files will still respond to array type-specific changes in smoothing and joining settings.
- In order for the changes stored in the CHPCAR file to show up in the CHP file data displayed in the ChAS Browser, the CHP file and CHPCAR file must be stored in the same directory/folder (the xxCHP file is still the only file that is required to be "loaded" into the browser, the CHPCAR file's edits, calls and interpretations will load when the CHP file is loaded).
- CHPCAR files are named using the entire CHP file's name, and contain the extension: ***.chpcar**
- Changing the name of either file and not the other to match will disrupt the ability for the files to be recognized as associated with each other in the ChAS Browser.
- CHP files which have associated CHPCAR files detected (and in use), display as a special "CHP"  icon in the **File → Open** dialog window, and in the **Files** tree of the Browser.
- Move the CHP and CHPCAR files together when moving or archiving data.
- Two people using CHAS on different systems should NOT attempt to edit a CHP file at the same time.
- Modification made to segments in the ChAS Browser will also be updated in the MSV.
- Files downloaded from ChAS DB can only use the delete segment option for editing segments.

Note: Segment modification does not apply to ReproSeq™ Aneuploidy data.

Use edit mode

IMPORTANT! Ensure that the Edit Mode feature is turned on BEFORE you start editing segments. Segments can not be published to ChAS DB while in Edit Mode. Edit Mode must be turned off to enable the Publish function.

Edit Mode is accessible in 3 places:

1. The Browser's icon row (top).



2. The Detail View's icon row (top right)



3. Click **View → Edit Mode**

   By default, Edit Mode is OFF. Click  (located on the Browser's top icon row or above the Detail View) to turn Edit Mode ON.

   - Click  to turn Edit Mode OFF and remove all visual indications of your segment changes.
     - When Edit Mode is **ON**, deleted segments are visible, and edited segments appear distinct from non-edited segments.
     - When Edit Mode is ON, a track on a dotted axis line will appear showing the original calls made by the software for comparison with the manual modifications on the segment track.
     - When Edit Mode is **OFF**, deleted segments are invisible, and edited segments look identical to non-edited segments.

IMPORTANT! Turn Edit Mode OFF, before exporting a report of your data. Also, Edit Mode must be OFF, before publishing to the database.

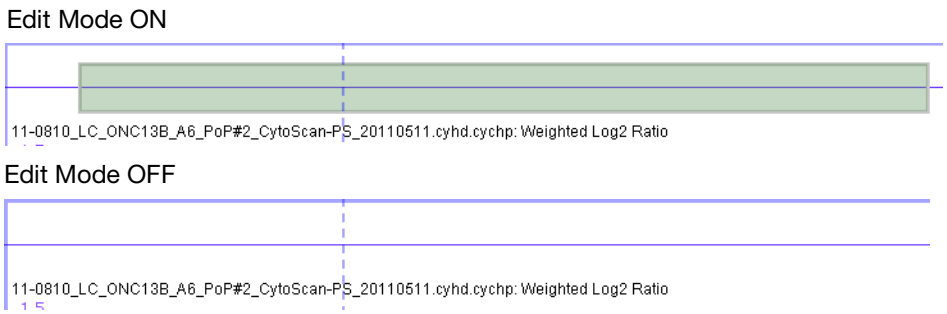


Figure 218 Edit Mode ON/OFF Deleted Segment example.

Types of segment editing

- "Track original calls" on page 213.
- "Merge all segments types" on page 214.
- "Delete all segments types" on page 220.
- "Edit the start/end coordinates of all segment types" on page 222.
- *"De novo segment drawing" on page 224*.
- "Change all copy number segment types" on page 227.
- "Promote copy number mosaic segments" on page 228.
- "Edit the Microarray Nomenclature (ISCN 2013) and Microarray Nomenclature fields" on page 232.

Note: Before you start any segment editing, ensure that the Detail View tab is selected, as shown in Figure 219.

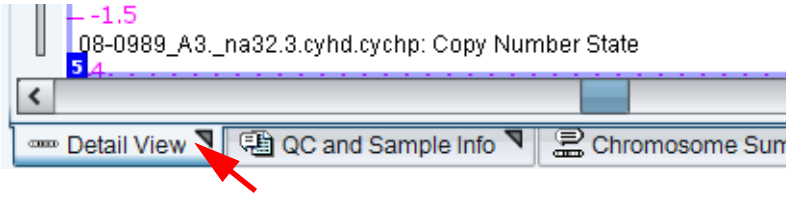


Figure 219 Detail tab (bottom left).

Track original calls

Original calls can be tracked to view original segment calls made by the software.

- This track is only visible when Edit Mode is ON.
- This track is only for visualization in the Detail View and is not populated elsewhere in the software.
- The Original Calls track will disappear when the Edit Mode is OFF.

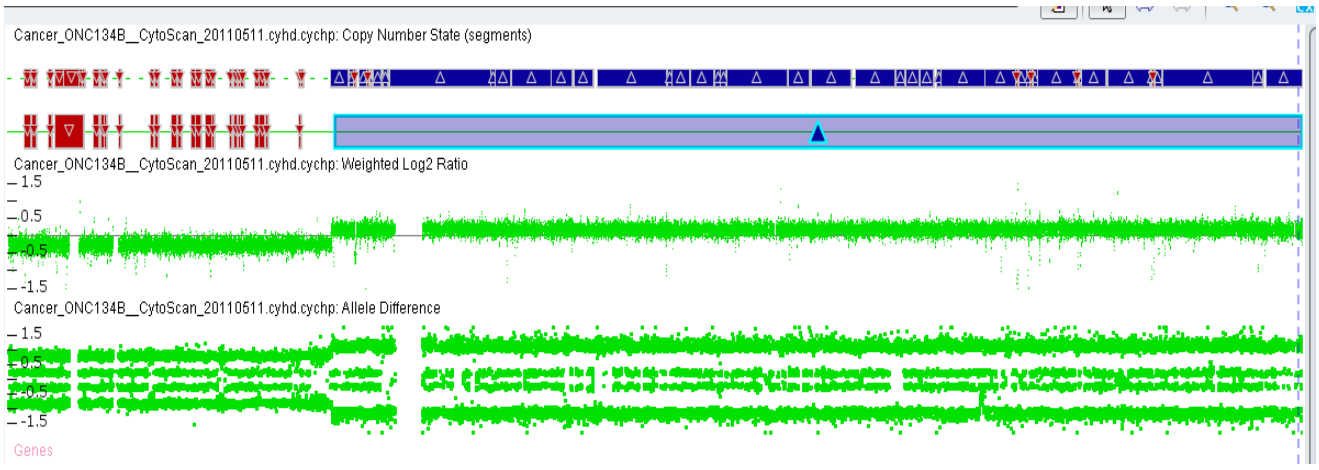


Figure 220 Original Call Track in Edit Mode example.

Merge all segments types

There are 2 ways to merge segments:

- "Merge segment groups" on page 214.
- "Segment to segment merge" on page 216.

Merge segment groups

Note: Merging segment groups together, cancels out any previously assigned Calls. However, undoing the group of merged segments (page 219) reinstates their original Calls.

1. Click **File → Open**.

   Your Sample File data folder window appears.

2. Click to select the file you want to edit, then click **Open**.

   The file appears in the ChAS Browser's Detail view.

3. Click, hold, then move the mouse to frame the segments you want to merge together. (Figure 221)

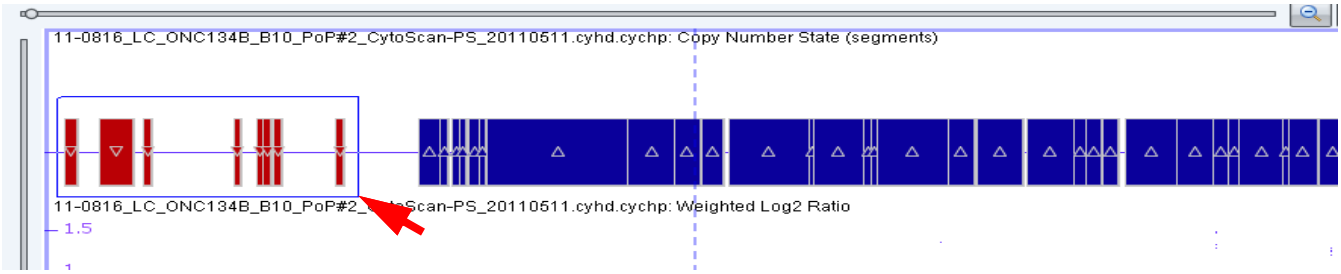


Figure 221 Framing your segments.

4. Release the mouse button.

   Your selected segments, including their Loss symbols are now highlighted in **blue**, as shown in the following example. (Figure 222)

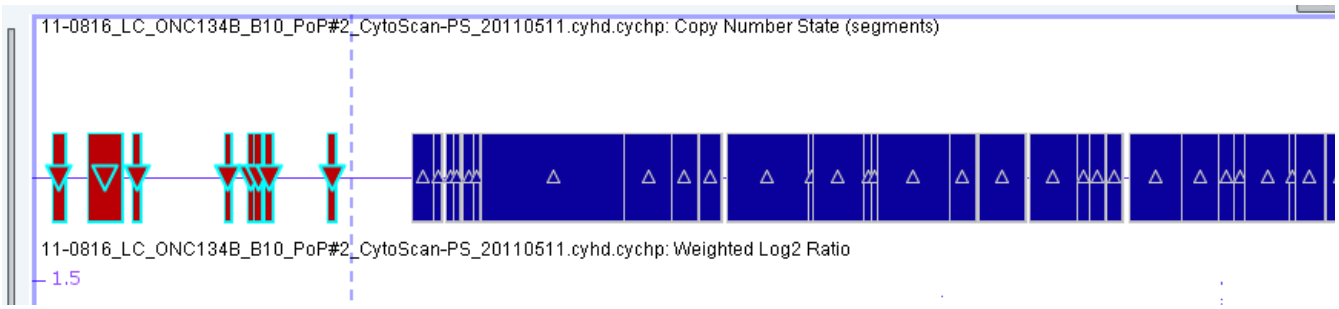


Figure 222 Framed segments now highlighted.

5. Right-click a highlighted segment.

   The following menu appears. (Figure 223)

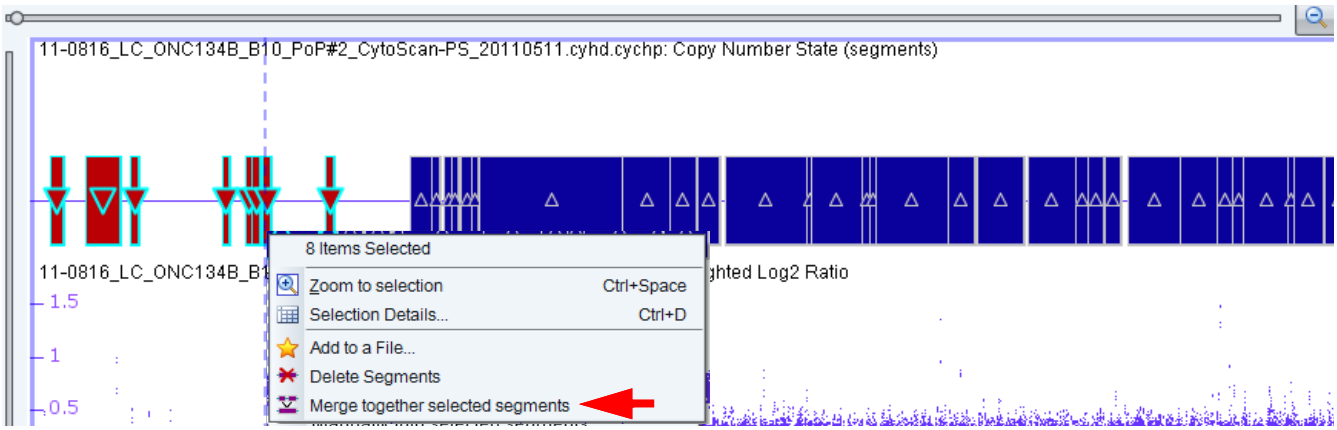


Figure 223 Right-click menu.

6. Click **Merge together selected segments**.

   A message regarding your planned merge may appear. (Figure 223)



Figure 224 Merge All? message.

7. Click **OK** to acknowledge it.

   The Pick a state window appears. (Figure 223)

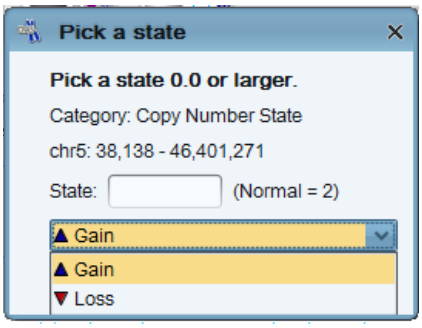


Figure 225 Pick a state window.

8. In the **State** field, enter a Copy Number, then use the dropdown list to select a Type (Gain/Loss) for the new segment. (Figure 223)

   Note: For CytoScan™ XON arrays (XNCHP files), it is not required to enter a copy number state value.

9. Click **OK**.

In cases where the segment to be merged into a group contains a **Call or Interpretation**, the following message appears: (Figure 223)

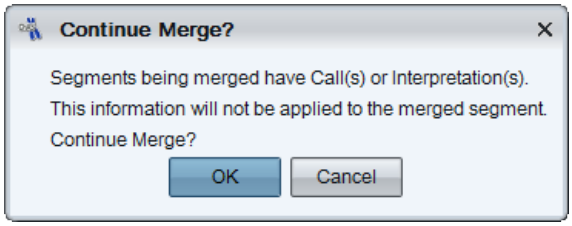


Figure 226 Merge Warning message.

10. Acknowledge the message, then click **OK**.

    Your selected segments are now merged together and appear as shown. (Figure 234)

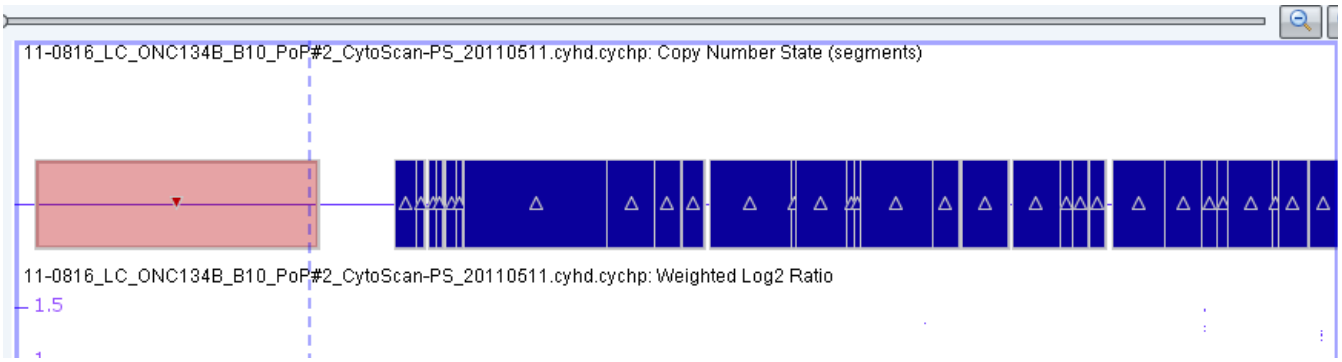


Figure 227 Merged segments.

Segment to segment merge

IMPORTANT! When merging segments during the editing of the start or end of one particular segment, only the segment whose start or end you are editing has the option of having its Call or Interpretation saved (or not).

Segments which are being engulfed by the edit start/end procedure being performed will not have the option of having their Call or Interpretations placed on the resulting segment. However, un-doing the edit resulting in this type of merging (page 219) will reinstate previous Call and Interpretation information for all the original segments involved.

1. Click **File → Open**.

   Your Sample File data folder window appears.

2. Click to select the file you want to edit, then click **Open**.

   The file appears in the ChAS Browser's Detail view.

3. Click to select the segment you want to merge with another. (Figure 228)



Figure 228 Selected segment to merge with another.

4. Click the **Start Editing** button.
5. A shaded area over your selected segment appears. (Figure 229)

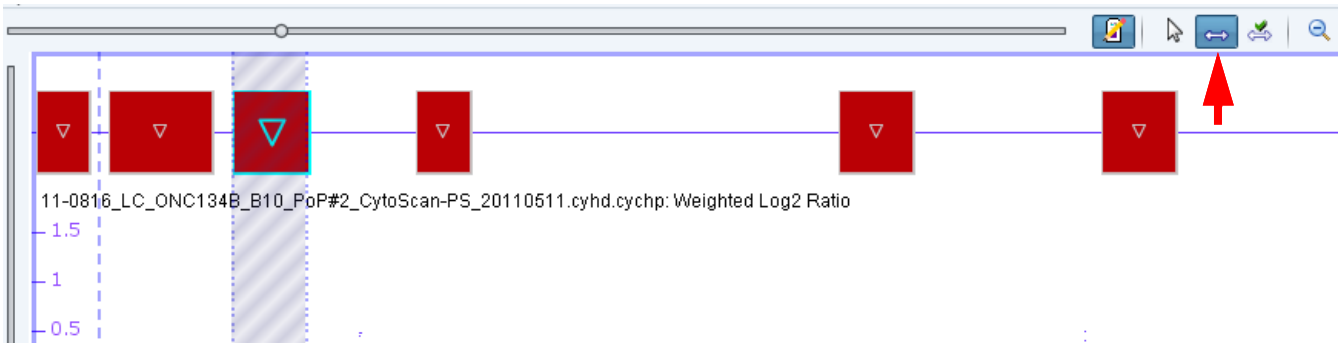


Figure 229 Click **Start Editing** button.

6. Click, then drag the shaded area over the segment you want to merge it with. Ensure that you overlap your target segment just slightly, as shown. (Figure 230)

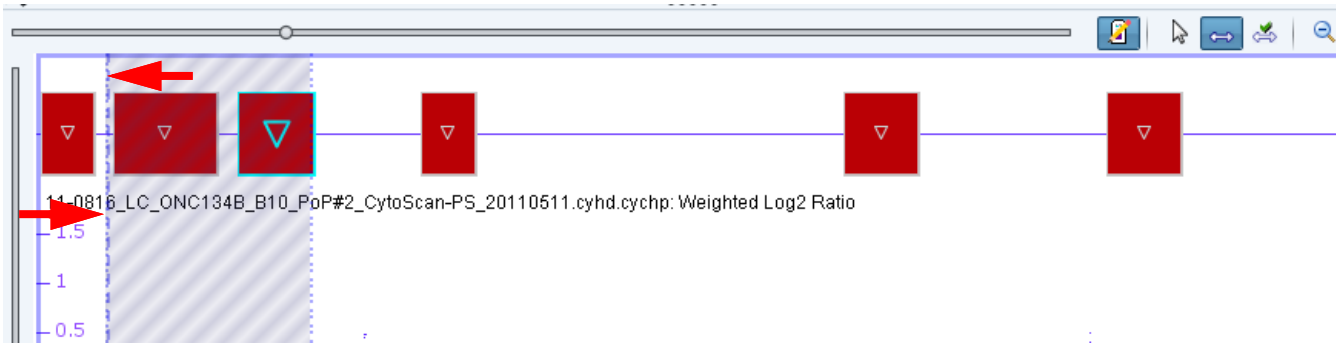


Figure 230 Click **Start Editing** button.

7. Click the **Start/End** button.

   The following message appears: Figure 231

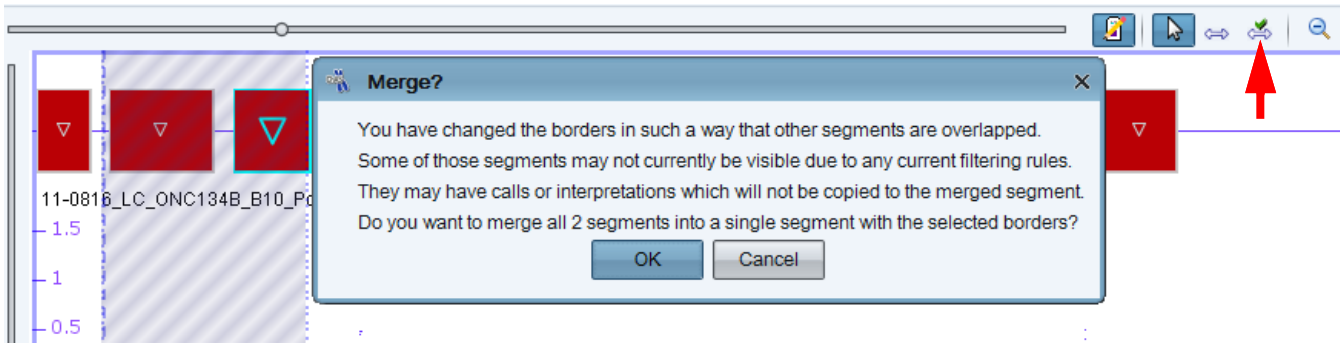


Figure 231 Click **Start Editing** button.

8. Click **OK** to acknowledge the message.

   If the segment whose Start or End you are editing contains **Call or Interpretation** information, the following message appears: Figure 232

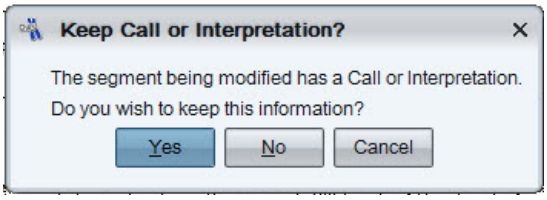


Figure 232 Keep Call or Interpretation message.

9. Click the appropriate button.

   Your 2 segments are now merged together. While Edit Mode is **ON**, the segment appears in a distinct color. (Figure 233). When Edit Mode is **OFF**, the edited segments look identical to non-edited segments.

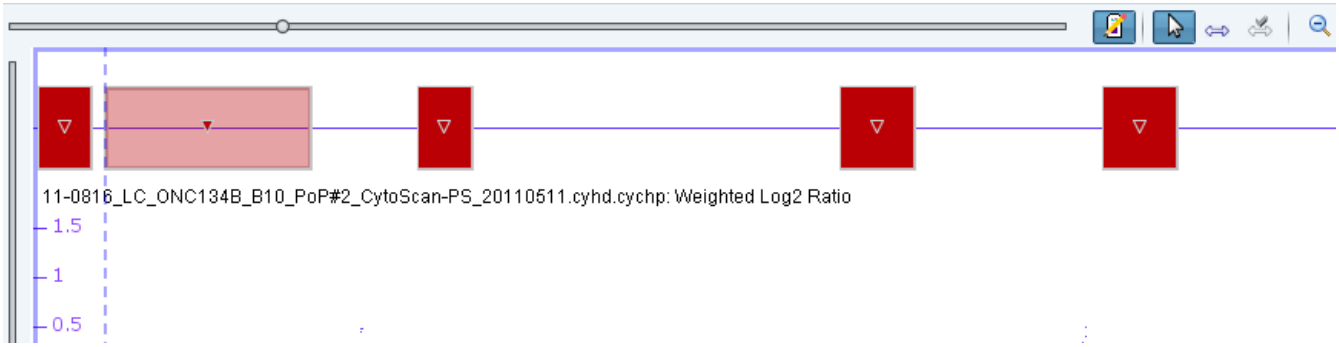


Figure 233 Click **Start Editing** button.

Unmerge

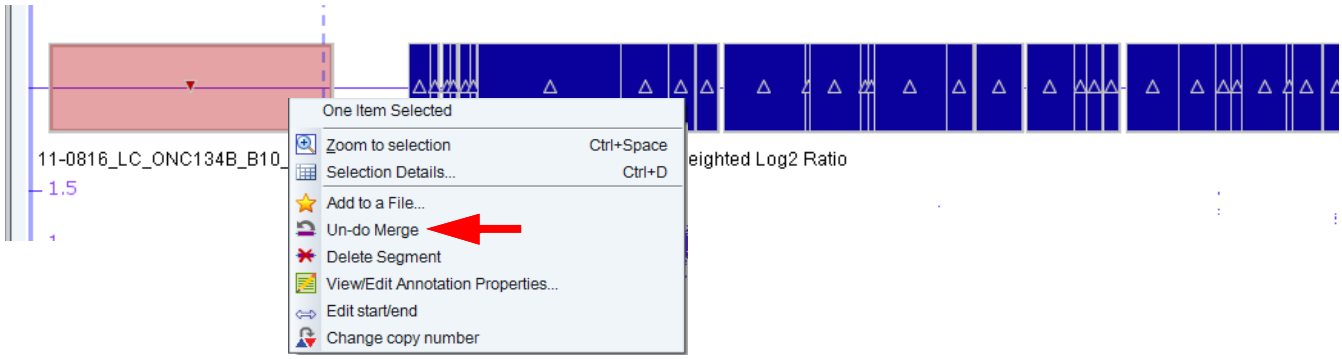


Figure 234 Merged segments.

Un-do merged segments

1. Right-click the newly merged segments (Figure 234), then click **Un-do Merge**.

   The following message appears. (Figure 234)

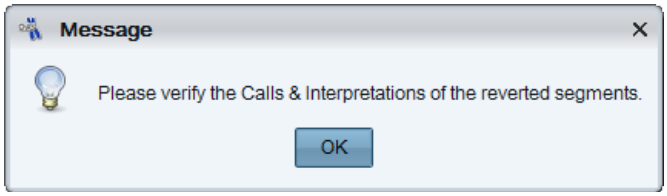


Figure 235 Confirm deletion.

2. Click **OK** to acknowledge the message.

   Your previously merged segments return to their original states, including any Calls or Interpretations made on the original segments (prior to their merging).

Delete all segments types

1. Click **File → Open**.

   Your Sample File data folder window appears.

2. Click to select the file you want to edit, then click **Open**.

   The file appears in the ChAS Browser.

3. Click, hold, then move the mouse to frame the segments you want to delete. (Figure 236)

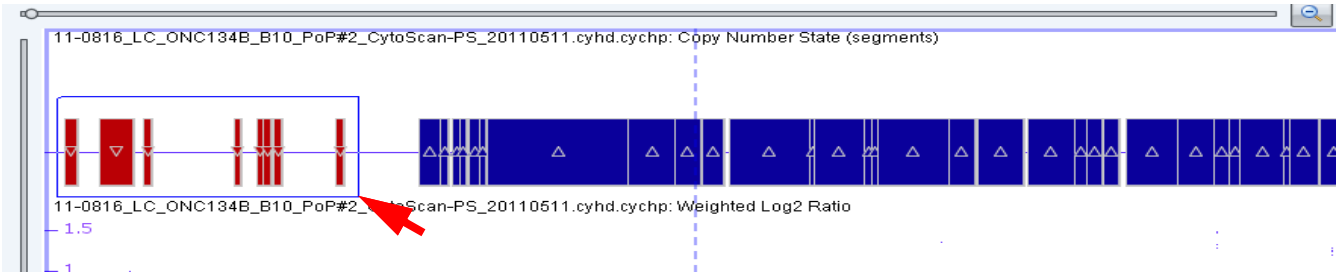


Figure 236 Framing your segments for deletion.

4. Release the mouse button.

   Your selected segments, including their Loss symbols are now highlighted, as shown in the following example. (Figure 237)

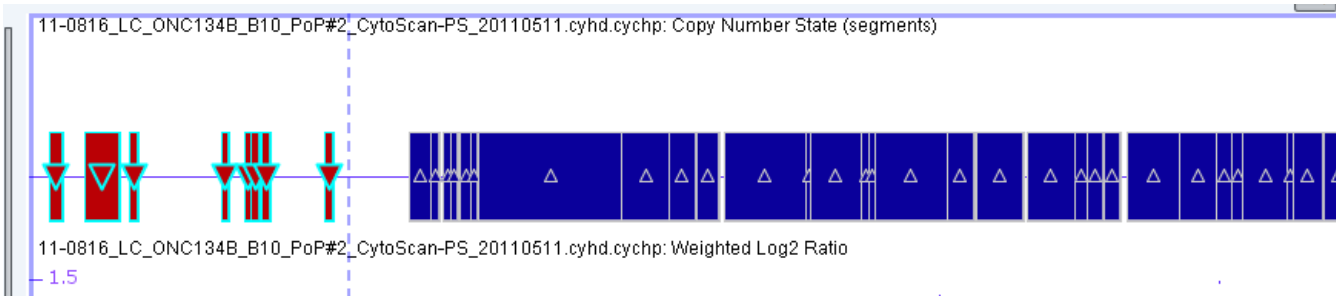


Figure 237 Framed segments now highlighted.

5. Right-click a highlighted segment.

   The following menu appears. (Figure 238)

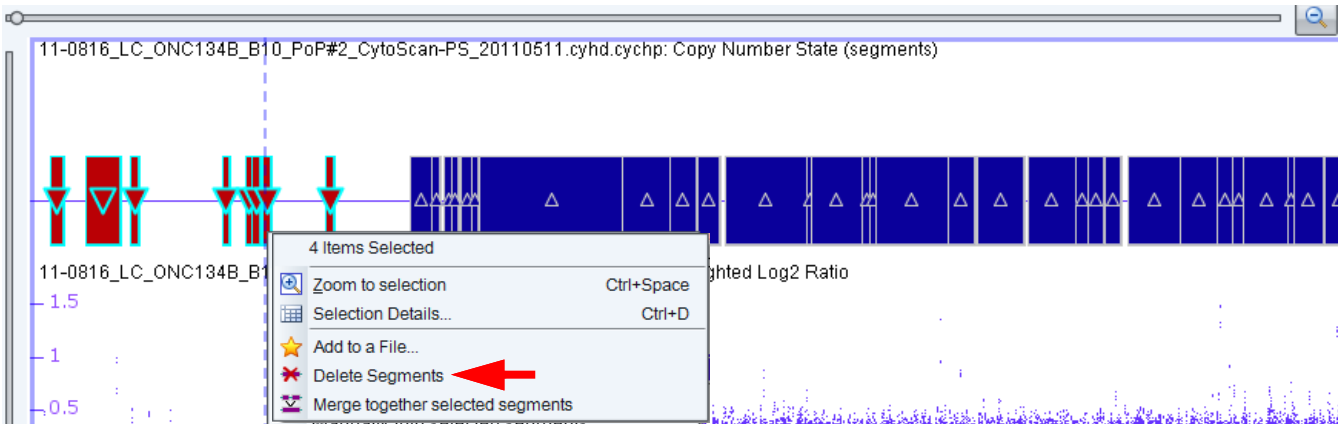


Figure 238 Right-click menu.

6. Click **Delete Segments**.

   A message regarding your planned deletion appears. (Figure 238)

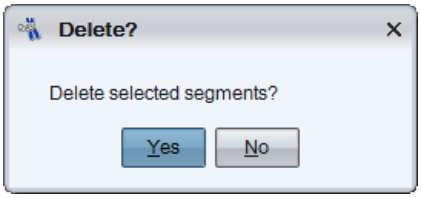


Figure 239 Confirm deletion.

7. Click **Yes** to acknowledge it.

   Your selected segments are deleted and graphically represented as "ghosts" while Edit mode is **ON**. (Figure 240). When Edit Mode is OFF, deleted segments are invisible and NOT displayed in any view or table.

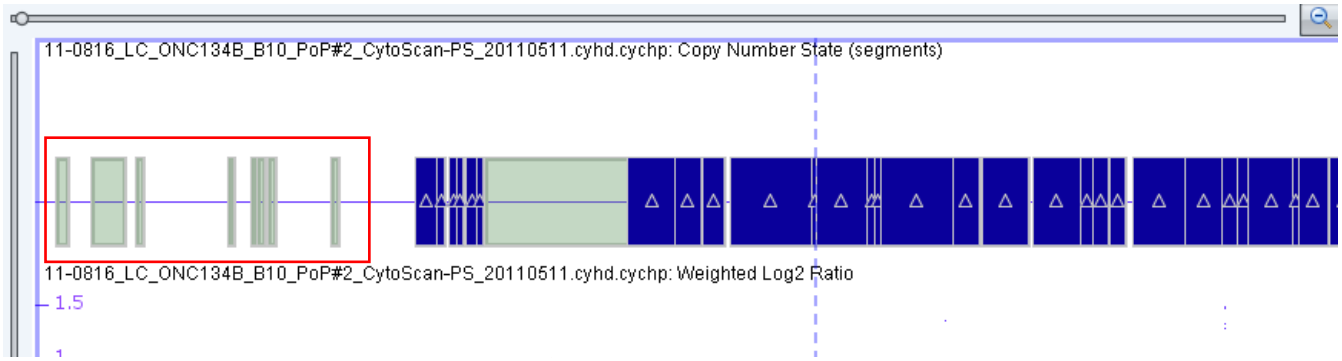


Figure 240 Deleted segments.

Note: Even though your selected segments are deleted, ChAS preserves their data for future reference. See "Right-click menu options" on page 193 for information about how to view and edit segment properties and view segment details.

Un-delete a deleted segment(s)

1. Right-click the deleted segment(s), then click **Un-do Delete**.

   The following message appears. (Figure 240)

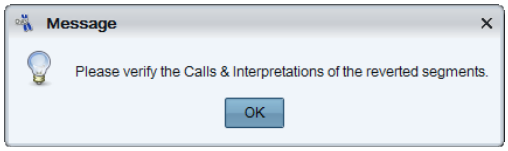


Figure 241 Confirm deletion.

2. Acknowledge the message, then click **OK**.

   Your previously deleted segment(s) return to their original states, including any Calls or Interpretations made on the original segments (prior to their deletion).

Edit the start/end coordinates of all segment types

IMPORTANT! Only the boundaries of 1 segment, can be adjusted/modified at one time.

Note: Segment start or end boundaries can be moved left and/or right. The Adjusting/Modifying Segment Boundaries example that follows, shows how to move a segment end boundary farther to the right.

1. Using the zoom tools and scroll bars (if needed), identify the segment whose start, end, or both you want to modify.
2. Click the segment to highlight it. (Figure 240)

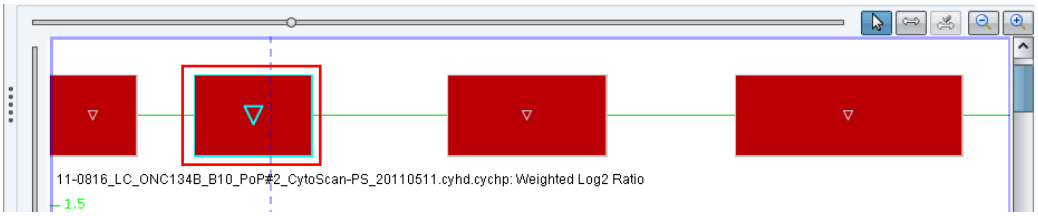


Figure 242 Selected segment.

3. Click the **Start editing...** button.

   The following appears: (Figure 243 on page 222)
4. Place the mouse cursor on the very-right edge of the current segment boundary. (Figure 240)

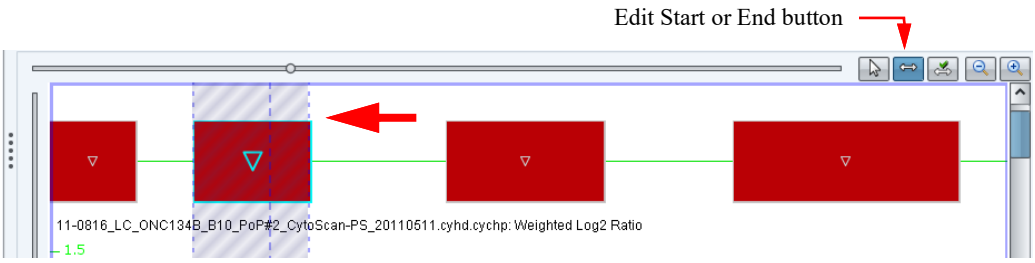


Figure 243 Selected segment - Start editing.

5. Click, then drag the segment's right-edge boundary to the right, then stop at an appropriate point. (Figure 240) You will *only* be allowed to set the Start or End position to match the position of a marker probeset.

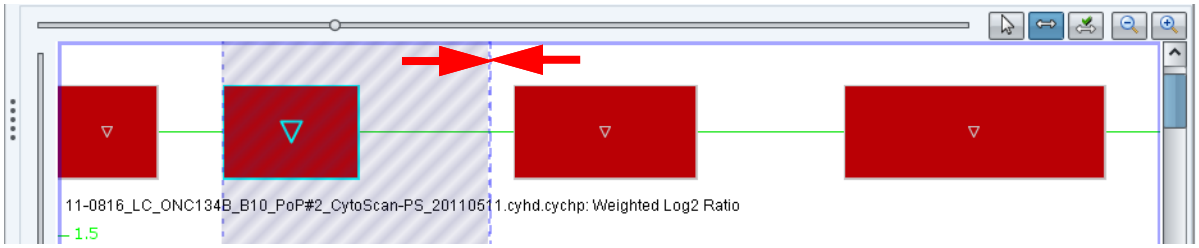


Figure 244 Selected segment - Start editing.

6. Click the **Set start/end**... button. (Figure 240)

   The newly adjusted/modified segment boundary appears as shown. (Figure 240)

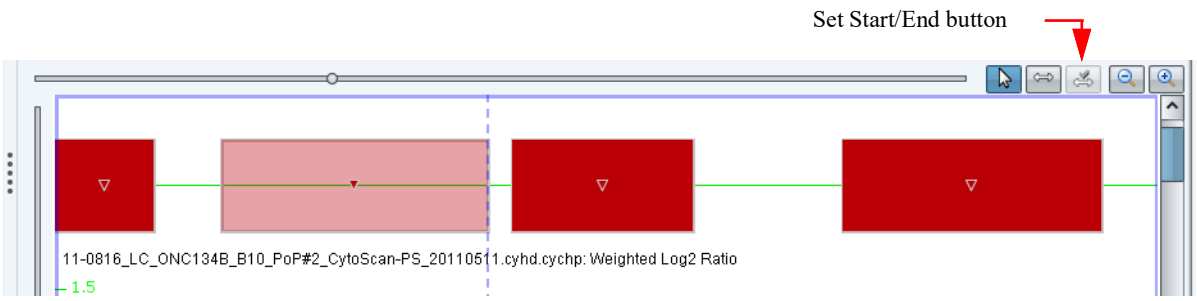


Figure 245 Selected segment - Start editing.

7. ChAS auto-calculates various properties of the newly edited segment. Mouse over the segment to view its new details. (Figure 240)

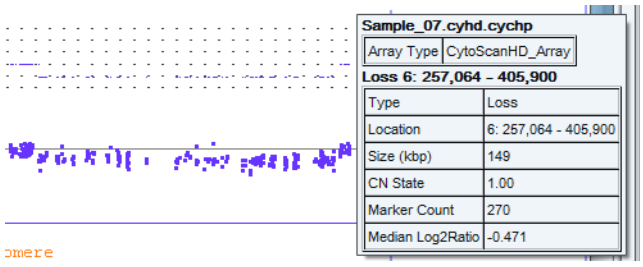


Figure 246 Selected segment - Start editing.

Un-do the edited start/end coordinates of a segment

1. Right-click the previously adjusted/modified segment, then click **Un-do Edit**.

   The segment returns to its previous state, including, previous boundaries, calls, and interpretations.

De novo segment drawing

1. Within an existing segment track, locate an empty space where you want to draw in a brand new segment.
2. Right-click inside this empty space.

   A **Create a Copy Number State segment around XX,XXX,XXX** ribbon appears (Figure 240)



Figure 247 Selected segment - Change Copy Number - Pick a state window.

3. Click the ribbon, as shown in Figure 247.

   The **Pick a state** window appears. (Figure 240)

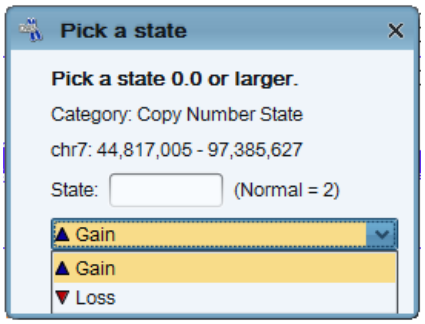


Figure 248 Drawing a new segment -- Pick a state window.

4. Enter a **Copy Number** and **Type** for the new segment.
5. Click **OK**.

Your newly drawn segment appears. (Figure 240)

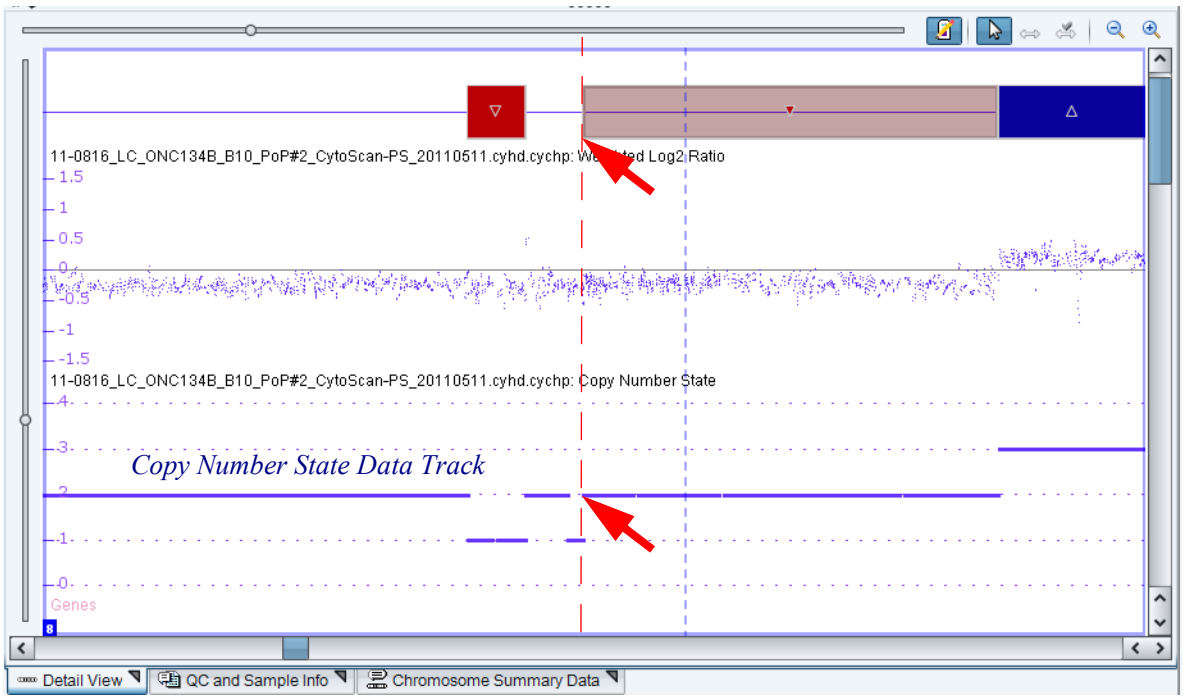


Figure 249 Newly drawn segment limited in size by the Copy Number State track.

IMPORTANT! Newly drawn segments will stop when they encounter another segment, whether or not that segment is currently drawn or whether it is currently filtered out; see Figure 249 for an example in the CN State data track which is used to draw Copy Number Segments of Gain and Loss.

New segments will also stop when they encounter the last appropriate marker on the chromosome. Because of this, new segments drawn will vary in their initial size.

6. Move the mouse cursor over the newly drawn segment to reveal its properties. (Figure 240)

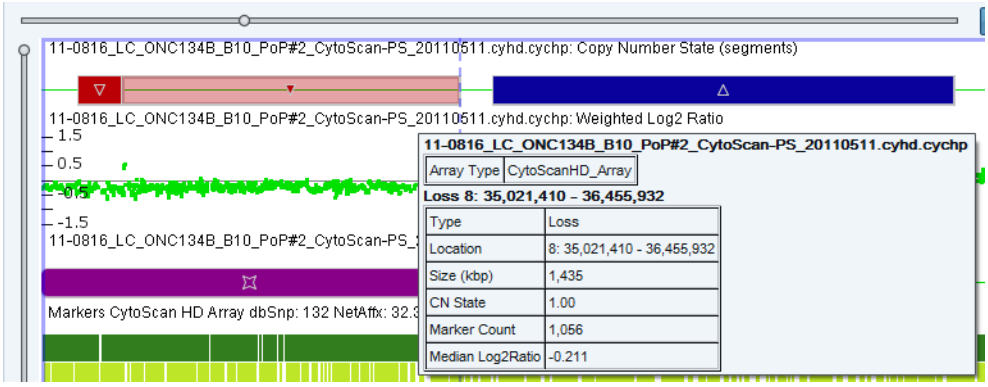


Figure 250 Newly drawn segment - Mouse over to see its properties.

Un-do a segment *de novo* drawing

1. Right-click the newly drawn segment.

   The following menu appears. (Figure 240)

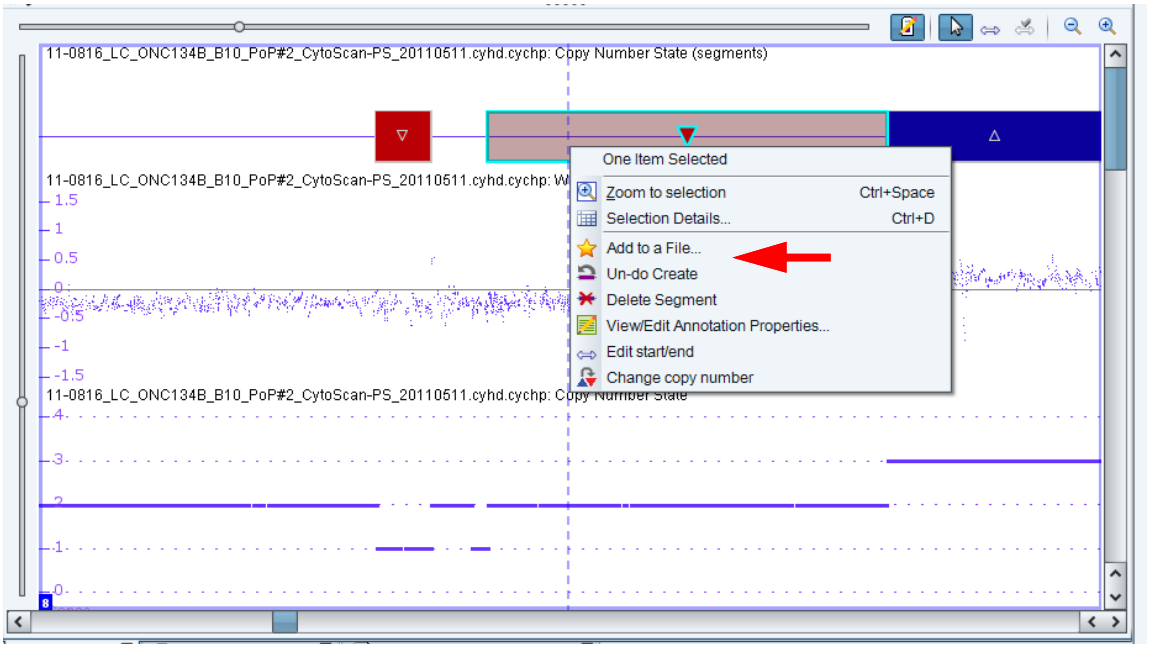


Figure 251 Newly drawn segment - Un-do drawn segment.

2. Click **Un-do Create**.

   The previously drawn segment is removed, along with any Calls or Interpretations it had been given. (Figure 240)

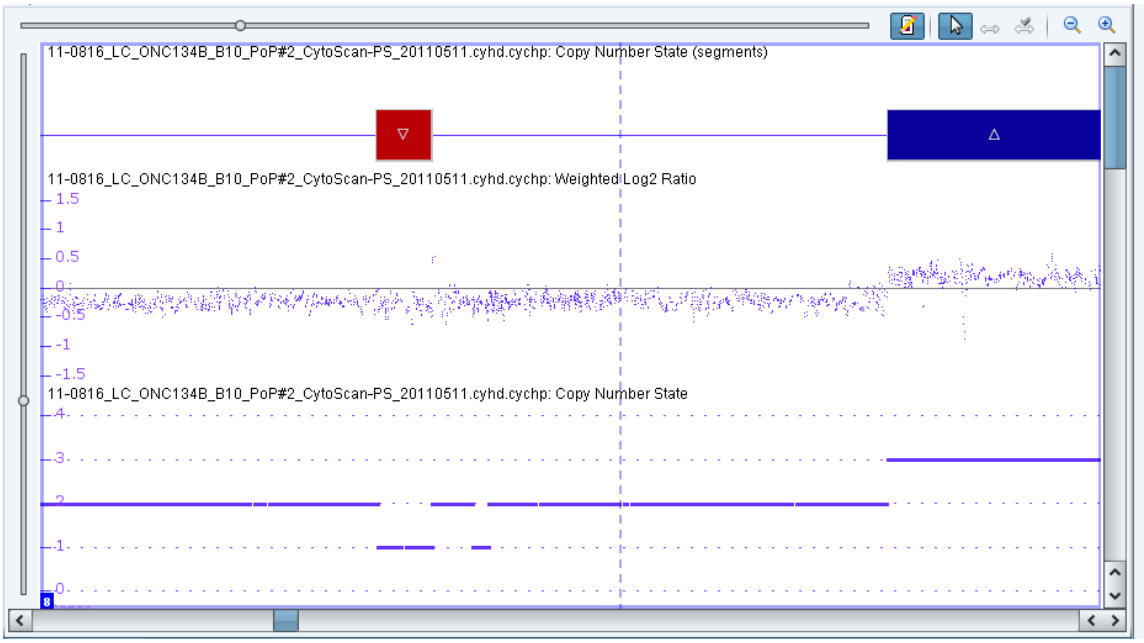


Figure 252 Newly drawn segment - Drawn segment removed.

Change all copy number segment types

Note: For CytoScan™ XON Arrays only, you are not required to assign a copy number to the segment.

1. Right-click the segment that contains the copy number you want to change
2. Click **Change copy number**. (Figure 240)

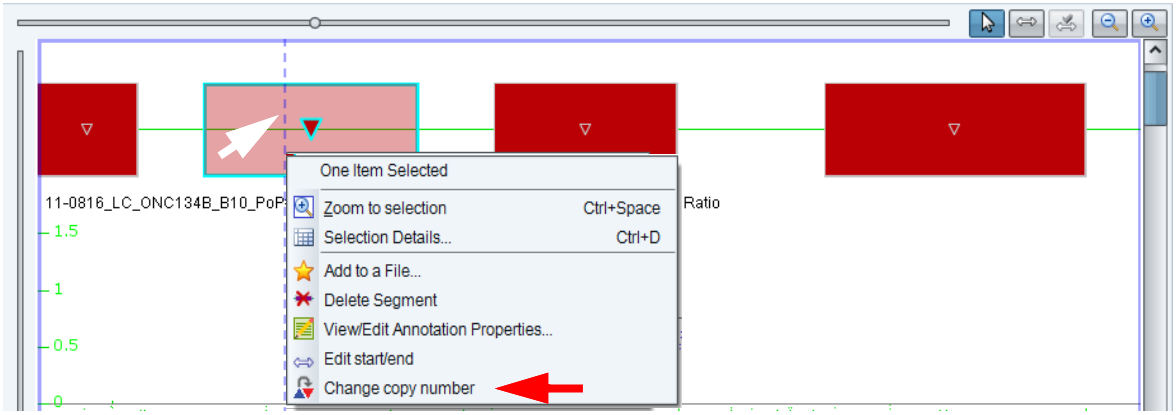


Figure 253 Selected segment - Change copy number.

The **Pick a state** window appears. (Figure 240)



Figure 254 Selected segment - Change Copy Number - Pick a state window.

3. Enter a new copy number **State** value and **Type** (Gain/Loss).

Note: For CytoScan™ XON Arrays (XNCHP files), it is not required to enter a copy number state value.

4. Click **OK**.

The segment now reflects your revised copy number **State** and **Type**.

Un-do a copy number change

1. Right-click the segment you performed a Copy Number State change on, then click **Un-do Edit**.
2. The following message appears. (Figure 255)

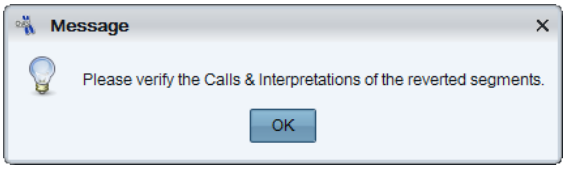


Figure 255 Confirm deletion.

3. Click **OK**. to acknowledge the message.

   The segment's original Copy State Number is reinstated.

Promote copy number mosaic segments

IMPORTANT! Segments in the Copy Number Mosaic Segment Track are NOT uploaded to the database. Mosaic LOH segments CAN be directly published to the ChAS database and DO NOT need to be promoted to the LOH track prior to publishing.

To capture the information for a copy number mosaic segment in the database, that segment must be "promoted" to the Copy Number State track. This is done to reduce redundancy in those regions in which segments were called by both the copy number algorithm and the mosaic detection algorithm. (Figure 256)

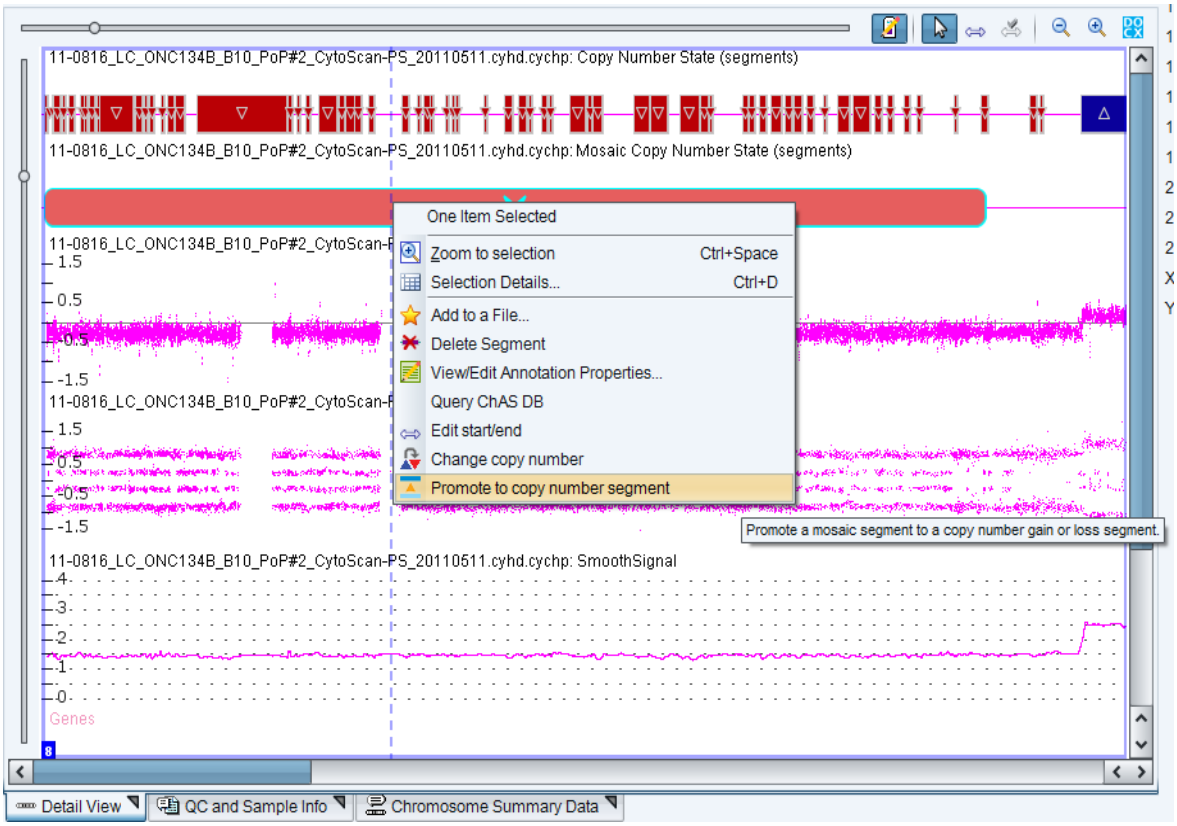


Figure 256 Promoting Mosaic Segments - Example.

Promoted mosaic segments maintain their non-integer copy number state, marker count, median log2 ratio, genome coordinates and size when they are promoted to the copy number state track. (Figure 257)

The mosaic gain segments will have the same blue/red used for integer copy number Gains/Losses, however, they maintain their non-integer copy number state to indicate they are a mosaic.

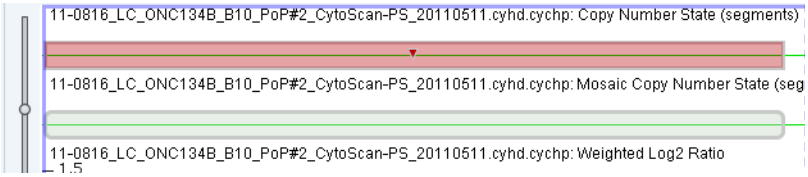


Figure 257 Promoted Mosaic Segment to Copy Number State Track.

Modified segments in the segments table

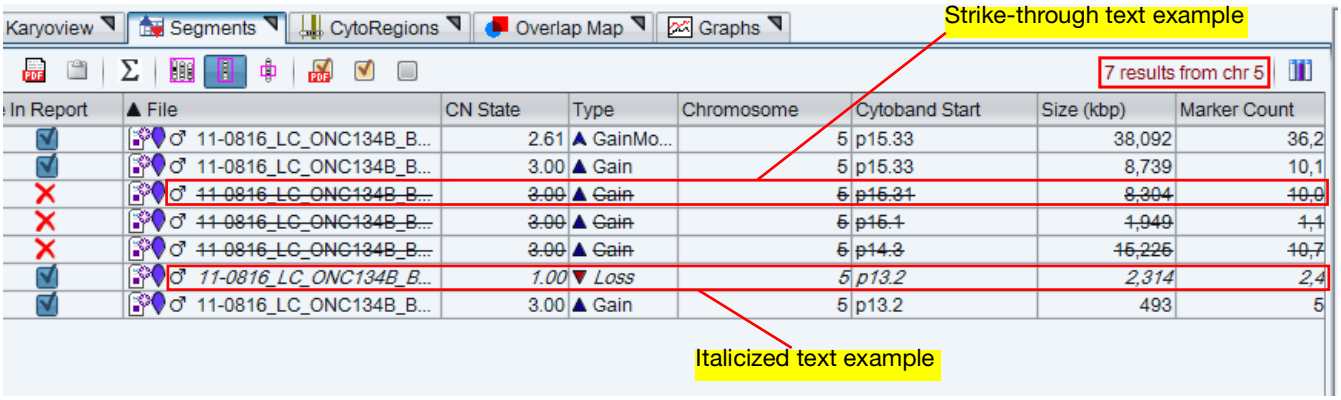
IMPORTANT! When exporting a Segments table to text (TXT), note that deleted segments are part of the export when Edit Mode is On, and will NOT be part of the export when Edit Mode is Off.

In PDF reports, deleted segments are never shown in graphical views, nor are they listed in the Segments Table, as *Edit Mode is required to be OFF to generate a PDF report.*

Edit mode on

When the Edit Mode is ON  , the Modified segments appear differently within the Segments table, as shown in Figure 258.

- Deleted Segments are represented with a **red X** and a **strike-through** line. Deleted segments do NOT show up in PDF reports because PDF reports cannot be created while Edit Mode is **ON**.
- Materially Modified Segments (including segments that have been merged, boundaries edited, or had their Copy Number States changed) are represented with ***italicized*** text.



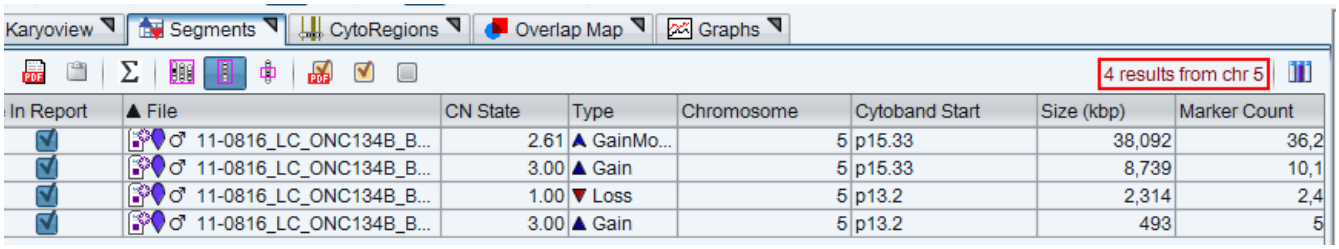
In Report	File	CN State	Type	Chromosome	Cytoband Start	Size (kbp)	Marker Count
☑	♂ 11-0816_LC_ONC134B_B...	2.61	▲ GainMo...	5	p15.33	38,092	36,2
☑	♂ 11-0816_LC_ONC134B_B...	3.00	▲ Gain	5	p15.33	8,739	10,1
✗	~~♂ 11-0816_LC_ONC134B_B...~~	~~3.00~~	~~▲ Gain~~	~~5~~	~~p15.31~~	~~8,304~~	~~10,0~~
✗	~~♂ 11-0816_LC_ONC134B_B...~~	~~3.00~~	~~▲ Gain~~	~~5~~	~~p15.1~~	~~1,949~~	~~1,1~~
✗	~~♂ 11-0816_LC_ONC134B_B...~~	~~3.00~~	~~▲ Gain~~	~~5~~	~~p14.3~~	~~15,225~~	~~10,7~~
☑	*♂ 11-0816_LC_ONC134B_B...*	*1.00*	*▼ Loss*	*5*	*p13.2*	*2,314*	*2,4*
☑	♂ 11-0816_LC_ONC134B_B...	3.00	▲ Gain	5	p13.2	493	5

Figure 258 Segments table with Edit mode ON.

Edit mode off

Turning the Edit Mode  OFF, displays the Segments table as follows: (Figure 259)

Note: The 3 deleted segment rows which were in strike-through text are removed from the table when Edit Mode is OFF. In addition to when Edit Mode is OFF, the rows which indicated Modified segments are no longer italicized.



In Report	File	CN State	Type	Chromosome	Cytoband Start	Size (kbp)	Marker Count
☑	♂ 11-0816_LC_ONC134B_B...	2.61	▲ GainMo...	5	p15.33	38,092	36,2
☑	♂ 11-0816_LC_ONC134B_B...	3.00	▲ Gain	5	p15.33	8,739	10,1
☑	♂ 11-0816_LC_ONC134B_B...	1.00	▼ Loss	5	p13.2	2,314	2,4
☑	♂ 11-0816_LC_ONC134B_B...	3.00	▲ Gain	5	p13.2	493	5

Figure 259 Segments table with Edit mode OFF.

Remove all edits made to a sample

1. Right-click the File name in the Files list, then select **Discard Changes**. (Figure 259)

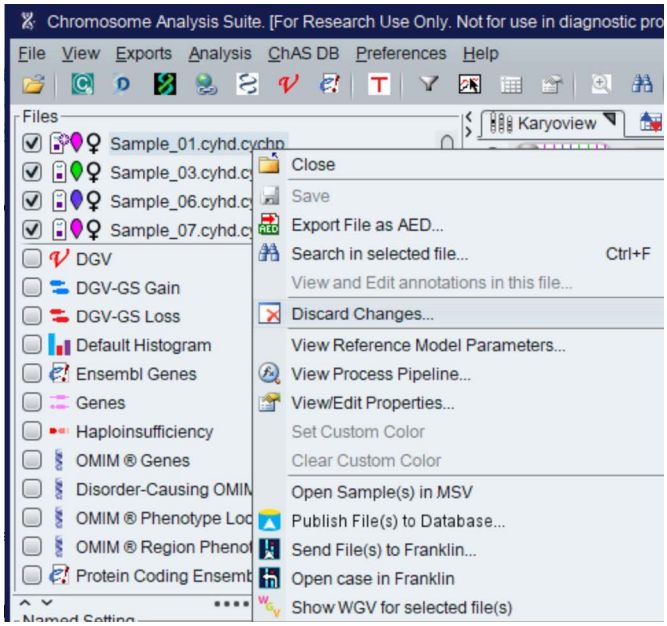


Figure 260 Discard Changes.

The Discard Changes window appears and displays the following options:

- Purge All Edits: Reverts all edits to segments and removes all calls and interpretations.

Note: The log of the edits that have been performed on the sample can still be viewed.

- **Clear Edit Log**: Purges all edits and clear the log of any edits performed on the sample.
- **Discard all changes**: Purges all edits, clears the edit log, and deletes the CHCAR file.
- Check the appropriate box, then click **Yes**. (Figure 259)

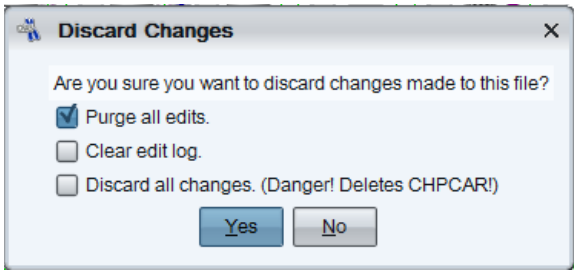


Figure 261 Discard Changes Confirmation window.

Edit the Microarray Nomenclature (ISCN 2013) and Microarray Nomenclature fields

The View/Edit Annotation Properties Curation tab enables you to update the copy number nomenclature in the Microarray Nomenclature (ISCN 2013) and Microarray Nomenclature fields.

Example: For Mosaic segments, the default name will show a range of copy number such as x2–3. If you have the percent mosaicism information, this part of the Microarray Nomenclature can be updated to reflect the percent mosaicism by typing x3[0.6]. The field is updated as follows:

Default name: arr[GRCh37] 5p15.33p13.2(113576_38205477)x2-3

Updated name: arr[GRCh37] 5p15.33p13.2(113576_38205477)x3[0.6]

1. Right-click the Microarray Nomenclature cell for the segment you would like to update, then click **View/ Edit Annotation Properties**, as shown in Figure 262.

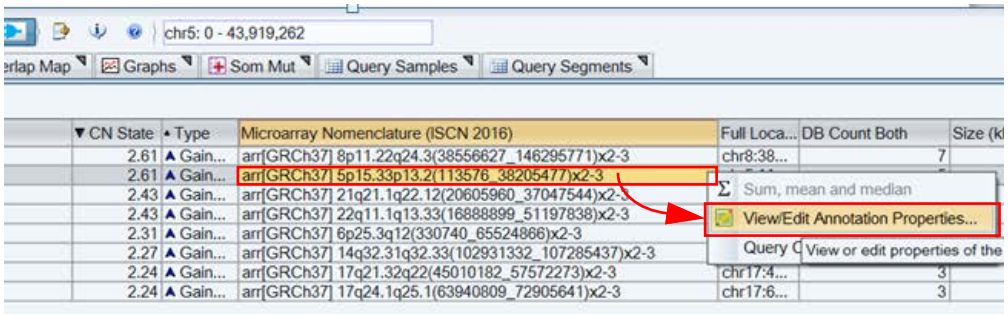


Figure 262 Discard Changes.

The Annotation Properties window appears. (Figure 259)

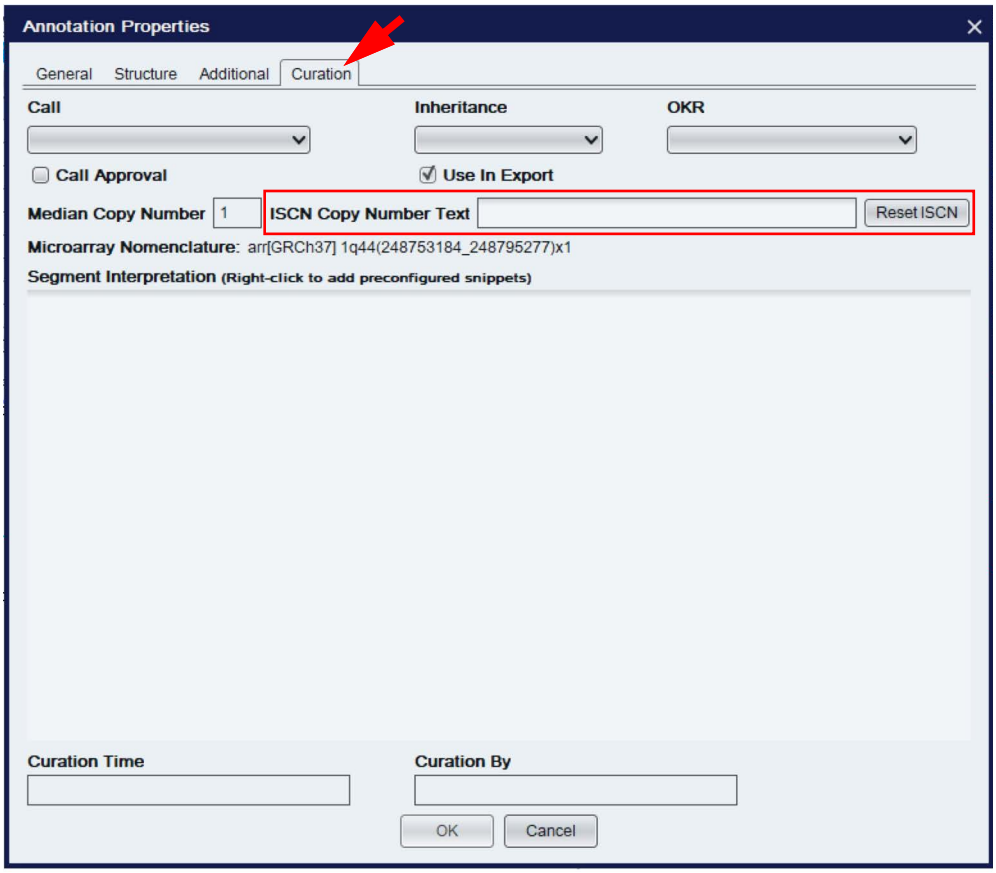


Figure 263 Discard Changes.

2. Click the **Curation** tab.
3. At the **ISCN Copy Number Text** field (Figure 259), type in the copy number nomenclature text you want, then click **OK**.

Note: This field can be edited for LOH segments to designate copy number in the Microarray Nomenclature field for LOH segments as well.

4. Optional: To reset the text to the original default format, click the **Reset ISCN** button.

Note: To automatically add the Inheritance field to the Microarray Nomenclature ISCN 2016 field, see "Add or remove inheritance calls" on page 245.

11 Sample and segment annotations

Sample annotations

Sample file level annotations such as Sample-type, Phenotype, and Sample Interpretation can be added to each sample.

Add, remove, and change the order of sample type text

1. Click **Preferences → Edit User Configurations** or click  on the upper toolbar.
   The User Configuration window appears.
2. Click the **Vocabularies** tab, then click the **Sample Type** tab. (Figure 264)

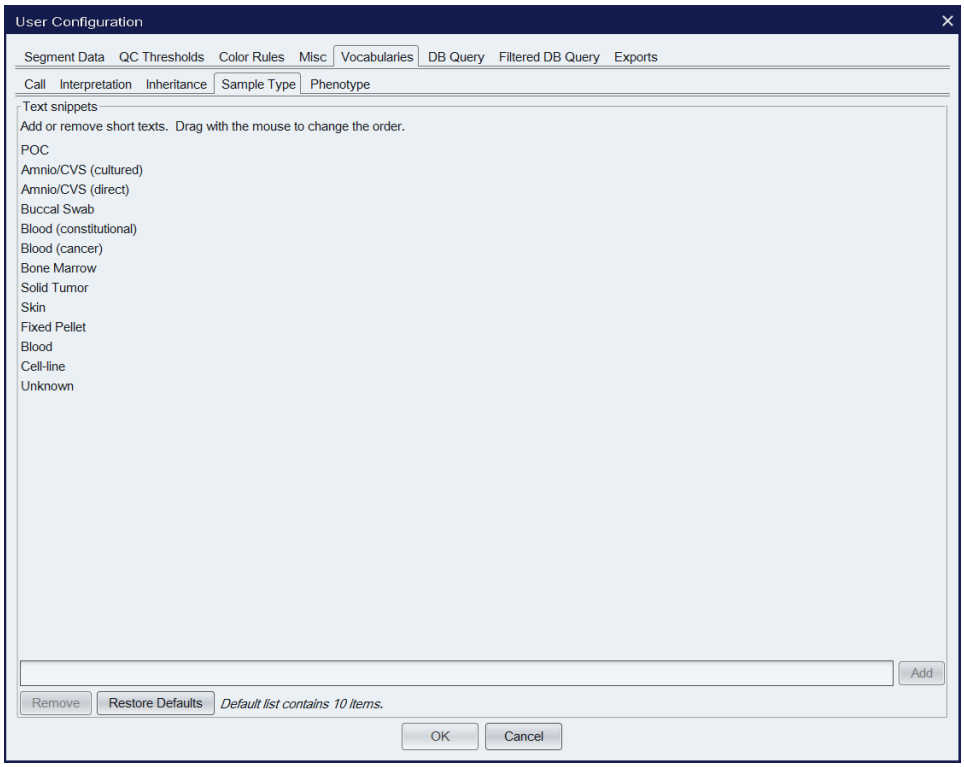


Figure 264 Sample Type window tab.

Add a Sample Type

1. Click inside the **Add Sample Type** field, then enter your new Sample Type.
2. Click **Add**.

Delete a Sample Type

1. Click to highlight the Sample Type you want to delete.
2. Click **Remove**.

Re-arrange the order currently displayed Sample Types

1. Click to highlight the Sample Type you want to move.
2. Drag and drop it to its new location (order).
3. If needed, repeat steps 1–2 to re-arrange additional Sample Types.

Restore the factory default Sample Types

1. Click **Restore Defaults**.
   The factory default Sample Types are restored.

Add, remove, and change the order of phenotype text

1. Click **Preferences → Edit User Configurations** or click  on the upper toolbar.
   The User Configuration window appears.
2. Click the **Vocabularies** tab, then click the **Phenotype** tab.
   The following window tab appears: (Figure 265)

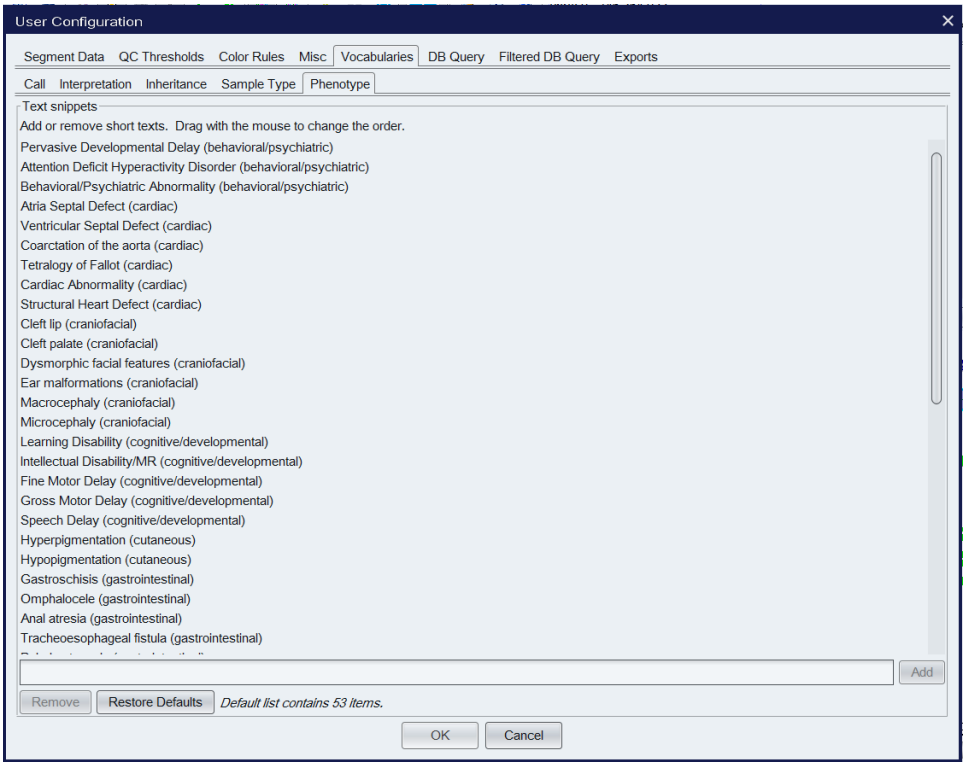


Figure 265 Phenotype window tab.

Add a short Phenotype text

1. Click inside the Add Phenotype Type field, then enter your new Phenotype.
2. Click **Add**.

Delete a short Phenotype text

1. Click to highlight the Phenotype you want to delete.
2. Click **Remove**.

Re-arrange the currently displayed Phenotypes order

1. Click to highlight the Phenotype you want to move.
2. Drag and drop it to its new location (order).
3. If needed, repeat steps 1–2 to re-arrange additional Phenotypes.

Restore the factory default Phenotypes

1. Click **Restore Defaults**.
   The factory default Phenotypes are now restored.

Add annotations at the sample (xxCHP) file level

1. Right-click a File name you want to add a Sample level annotation to.
   A menu appears.
2. Click **View/Edit Properties**.
   The File Properties window appears. (Figure 266)

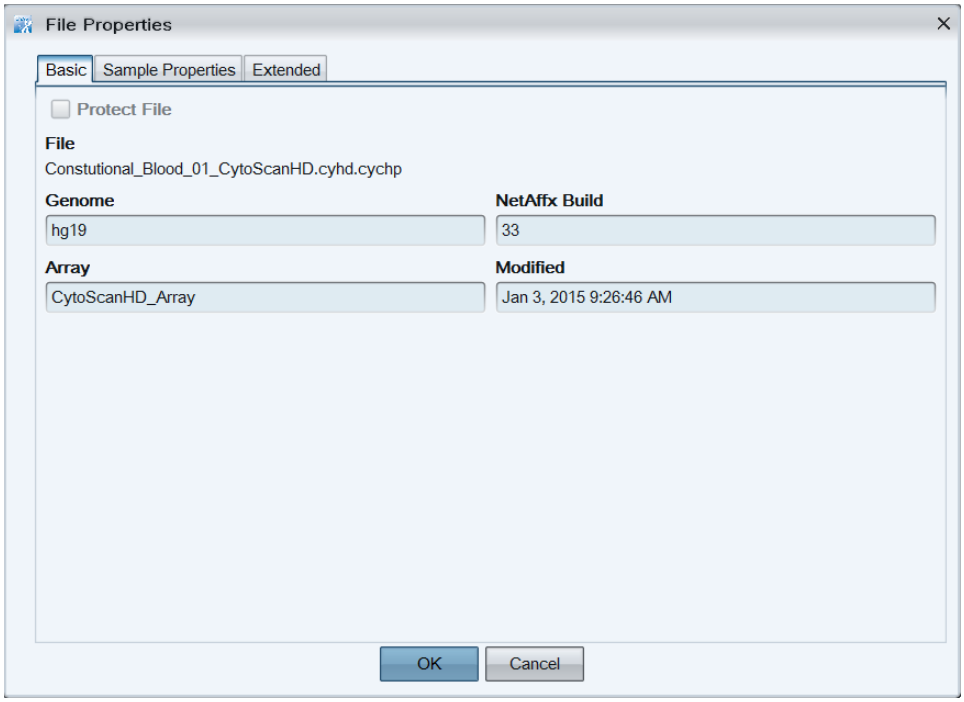


Figure 266 File Properties window.

3. Click the **Sample Properties** tab. (Figure 267)

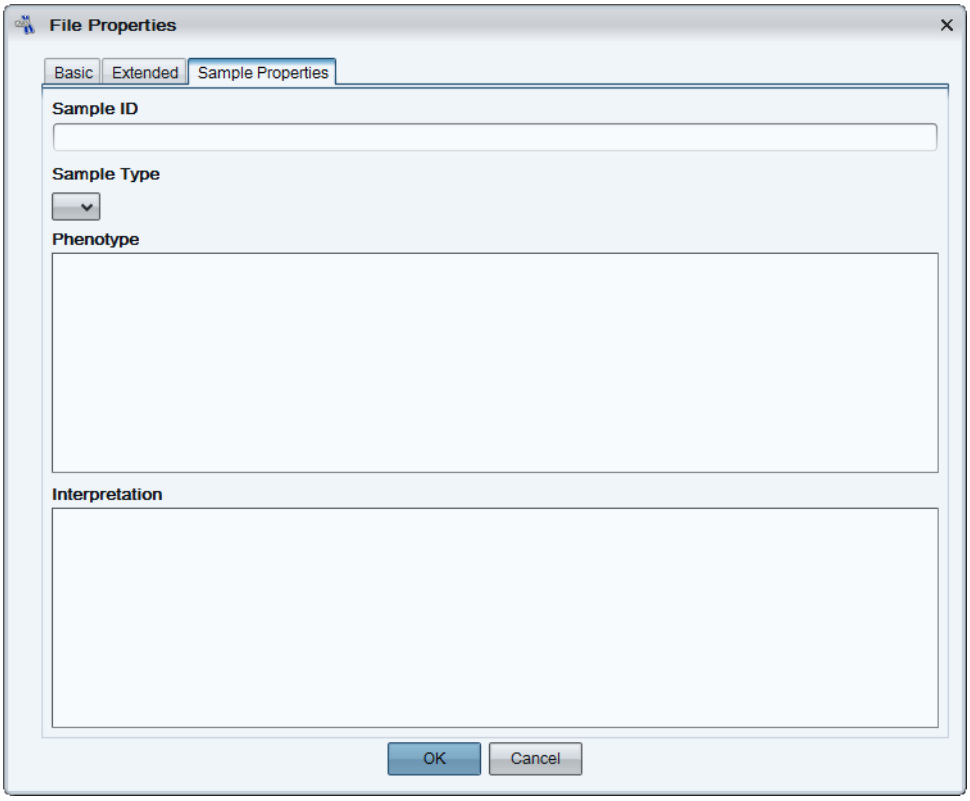


Figure 267 Sample Properties window.

4. Use this window to Add/Enter **Sample ID**(s), choose a **Sample Type**(s), enter **Phenotype**(s), and sample **Interpretation**(s).

Note: The Sample ID defaults to the File Name, but you can edit/change the Sample ID name if you want.

5. Click **OK**.

Segment annotations

Segment level annotations such as Call, Interpretation, and Inheritance can be added to segment data.

Set up the calls feature

Note: If you are using a user profile from a previous version of ChAS, your default set of Calls will NOT appear in the Calls dropdown list. To restore them, click Edit User Configurations → Vocabularies → Calls, then click the **Restore Defaults** button, as shown in Figure 268 on page 238.

Add and remove calls

1. Click **Preferences → Edit User Configurations** or click  on the upper toolbar.

   The User Configuration window appears.

2. Click the **Vocabularies** tab.

   The **Call** tab appears. (Figure 268)

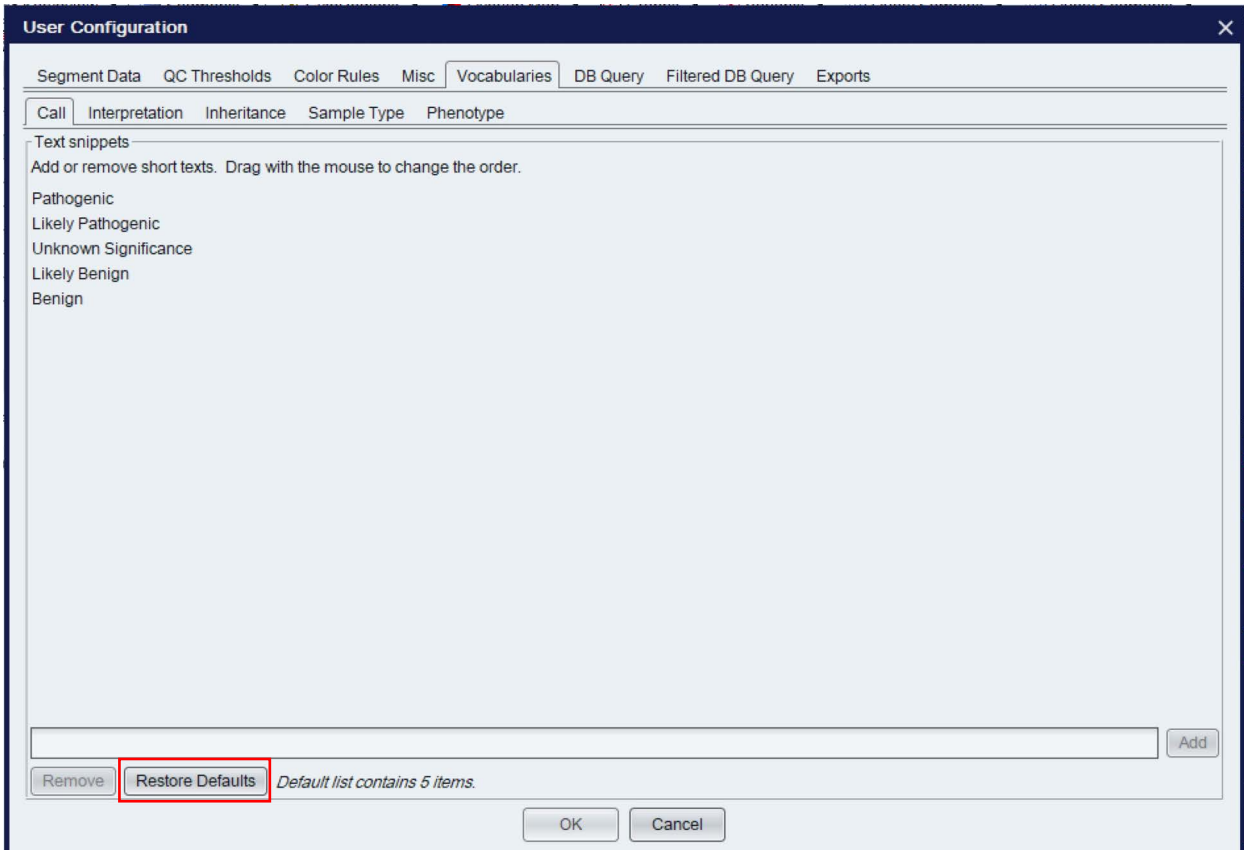


Figure 268 User Configuration window - Vocabularies tab window.

Add, delete, and re-arrange calls

The Calls window enables you to add, delete, and Re-arrange current Calls.

Add calls to the Call dropdown list

1. Click inside the **Add Call** field, then enter your new Call.
2. Click **Add**.

   Your newly added Call now appears in the Call dropdown list.

Delete calls from the Call dropdown list

1. Click to highlight the Call you want to delete.
2. Click **Remove Call**.

   Your newly deleted Call is removed from the **Call** dropdown list.

Re-arrange the order of calls in the Call dropdown list

1. Click to highlight the Call you want to move.
2. Drag and drop it to its new location (order).

   Your Call is now in its revised position/order within the Call dropdown list.
3. If needed, repeat steps 1–2 to re-arrange additional Calls.

Restore the factory default Calls

1. Click **Restore Defaults**.

   The factory default Call(s) are now populated in the Call dropdown list.

Use the calls feature

Method 1: Use the calls feature at the Segments table

1. Click the Browser's **Segments** tab.
2. Scroll the Segment table to the right until you see the **Call** column.
3. Locate the appropriate row.
4. Click inside the field.

   A blue dropdown list appears.
5. Click the dropdown list to reveal the list of available Calls, as shown in Figure 269.

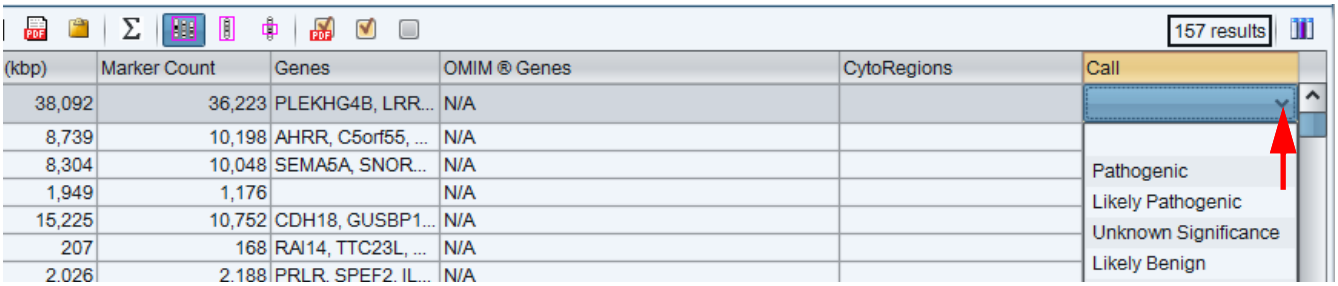


Figure 269 Calls dropdown list bar.

6. Click to select the appropriate Call.
7. Click outside the field or press **Enter** on the keyboard.
8. Your Call is entered. Note that the **Curation By** column is populated with the user's Windows™ login ID (left) and ChAS User Profile ID (right). (Figure 270)

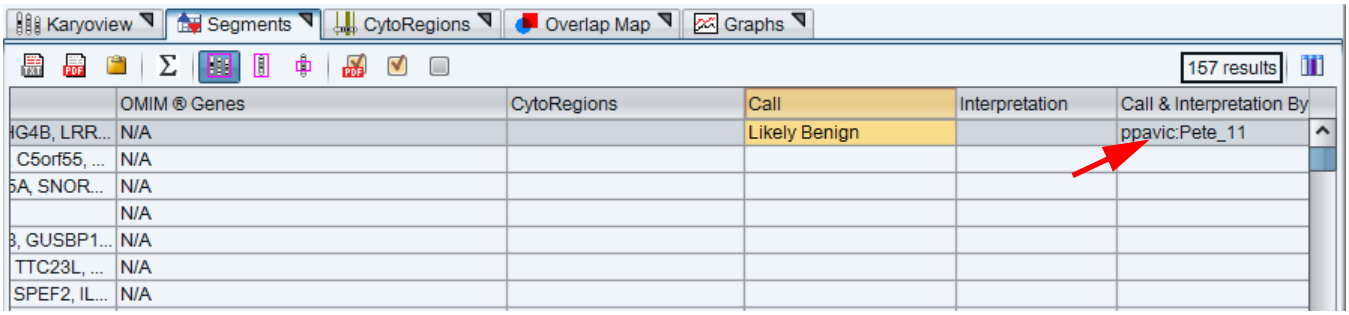


Figure 270 Call is entered and Curation By field is populated.

9. A Call can be assigned to multiple segments at the same time. To do this:
   a. Shift+click or Ctrl+click the Calls fields for the segments you want to assign to the same call to.
   b. Right-click the highlighted area, then click **Set Value**.
   c. Select the **Call** from the dropdown list, then click **OK** to assign that call to all the selected segments.

Method 2: Use the calls feature at the View/Edit Annotation Properties window

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click the segment you want to add an interpretation to, then click the menu selection **View/Edit Annotation Properties**.

   The Annotation Properties window appears.
2. Click the **Curation** tab.

   The Segment Interpretation window pane appears. (Figure 271)

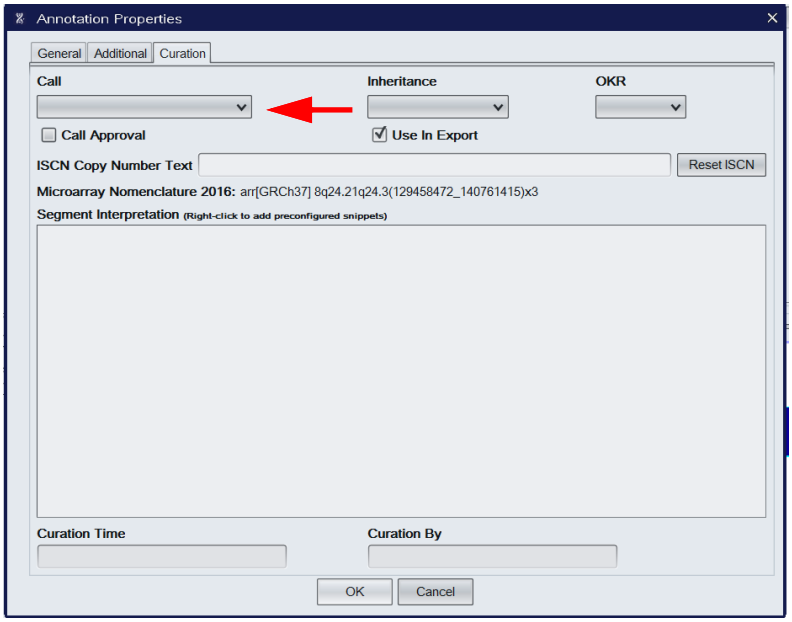


Figure 271 Annotation Properties window - Segment Interpretation.

3. Click the **Call** dropdown list, then select your appropriate interpretation Call. (Figure 272)

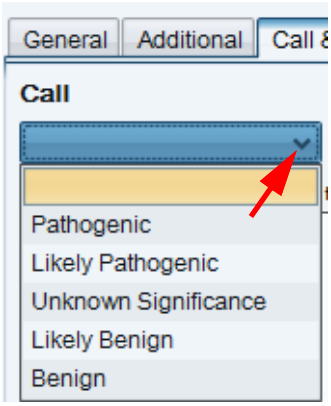


Figure 272 Call dropdown list.

Add or remove interpretation snippets

The Snippets feature can be used in conjunction with the free-typing Interpretation field (below the **Call** dropdown list). It allows for a convenient "shortcut" when common words or phrases are used often in an interpretation.

1. Click **Preferences** → **Edit User Configurations** or click  on the upper toolbar.

   The User Configuration window appears.

2. Click the **Vocabularies** tab, then click the **Interpretation** tab.

   The **Interpretations** tab appears. (Figure 273)

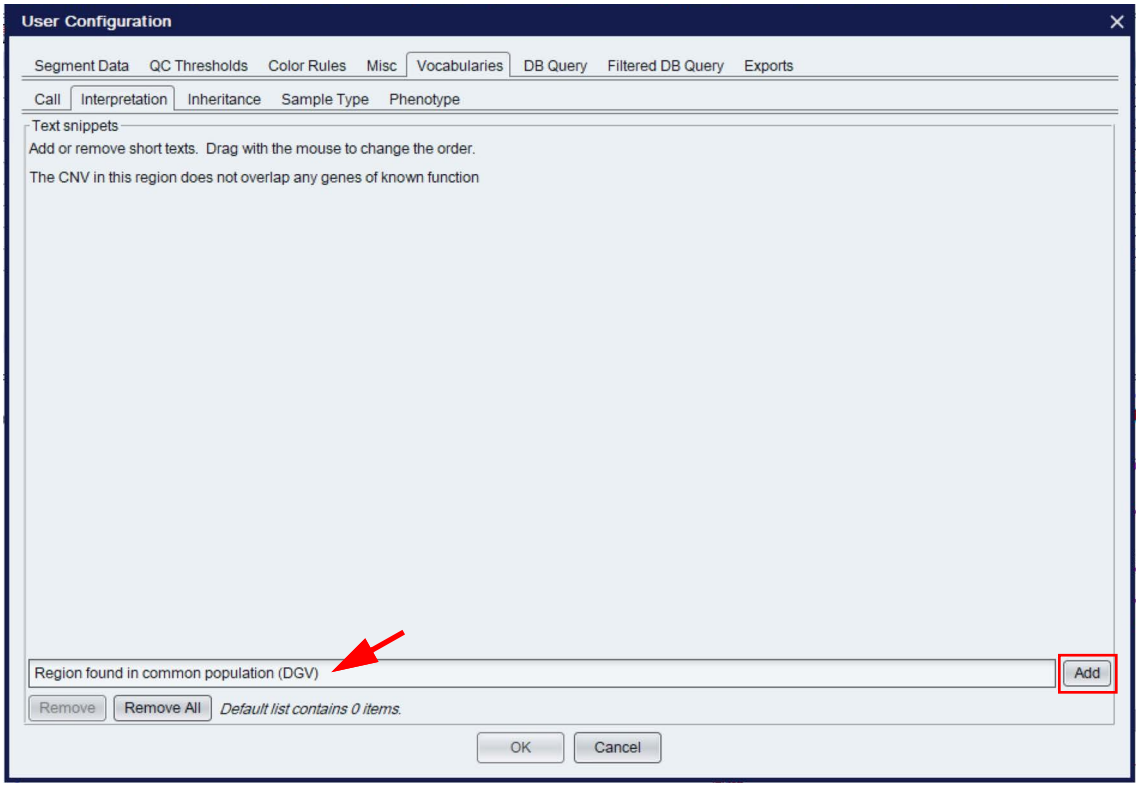


Figure 273 User Configuration window - Interpretation tab.

3. At the Interpretation window, click inside the field shown (Figure 273), then enter the snippet you want to use with your interpretation(s).
4. Click **Add**.

   The snippet now appears and is saved in the Interpretations List pane.
5. Repeat steps 3 and 4 to add additional snippets.
6. Click **OK**.

Remove a saved snippet

1. Click to highlight the snippet you want to remove.
2. Click **Remove**.

Remove multiple saved snippets

1. Shift+click or Ctrl+click to highlight each snippet you want to remove.
2. Click **Remove All**.

Use the interpretation snippets feature

Method 1: Use the interpretation snippets feature at the segments table

1. Click the browser's **Segments** tab.
2. Scroll the Segment table to the right until you see the **Interpretation** column.
3. Locate the appropriate row.
4. Click inside the field.
5. Right-click the flashing cursor. If the field has existing text, place the flashing cursor to the point where you want to add a snippet, then right-click.

   A floating dropdown list bar appears. (Figure 274)

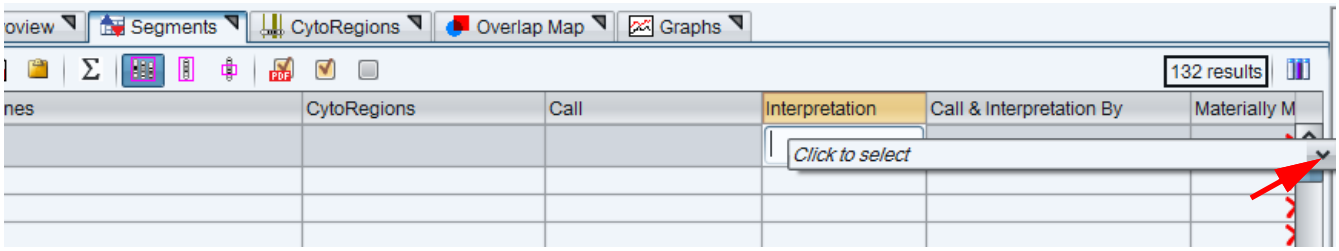


Figure 274 Floating Snippets dropdown list bar.

6. Click the snippet bar's dropdown list to display the available snippets, then click the appropriate snippet. (Figure 274)

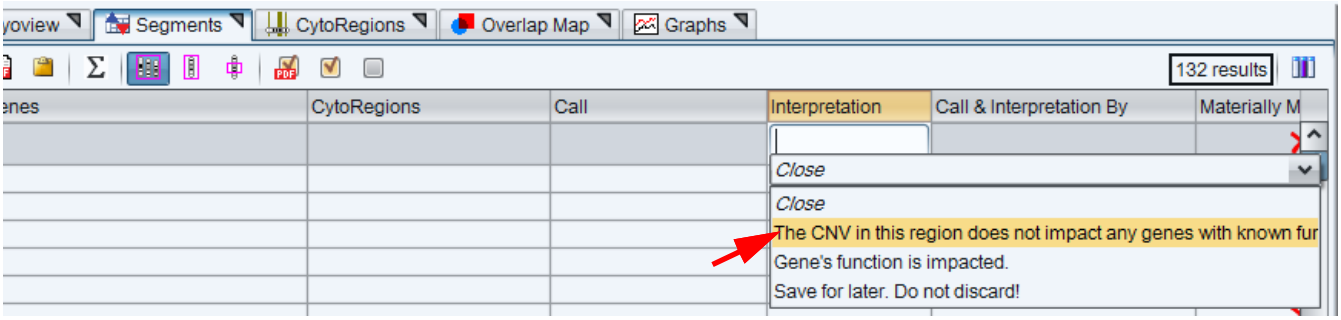


Figure 275 Snippets dropdown list.

The snippet appears in the appropriate Segment Interpretation row, as shown in Figure 276.

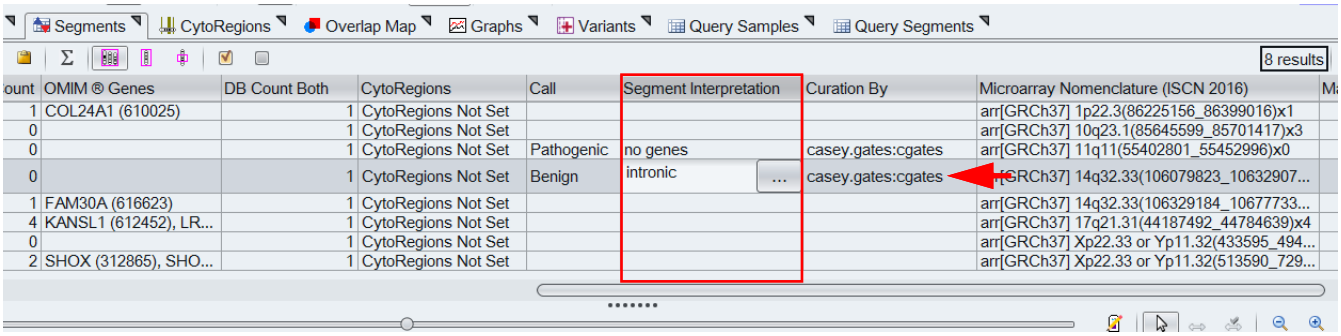


Figure 276 Snippet appears in field.

7. Click outside the field.

   Your snippet is entered.

   Note: The **Curation By** column is populated with the user's Windows™ login ID (left) and ChAS User Profile ID (right), as shown in Figure 276.

Method 2: Use the interpretation snippets feature at the View/Edit annotation properties window

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click the segment you want to add an interpretation to, then click the menu selection **View/Edit Annotation Properties**.

   The Annotation Properties window appears.

2. Click the **Curation** tab.
3. Type in your interpretation. If at any point you want to insert your preset snippet(s), right-click inside the interpretation window.

   A dropdown bar graphic appears. (Figure 277)



Figure 277 Dropdown list bar.

4. Click to select the appropriate snippet or click **Close** to exit. (Figure 278)

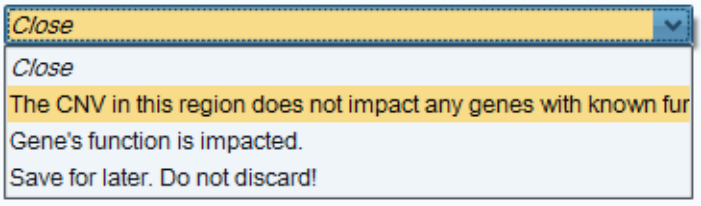


Figure 278 Snippet dropdown list.

Your snippet (preset word, sentence, or phrase) now appears "in line" with your typed text. (Figure 279)

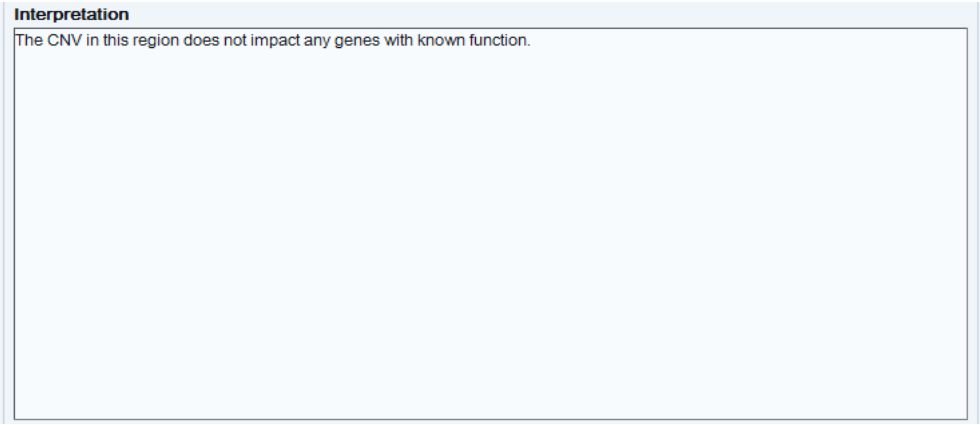


Figure 279 Snippet in the Interpretation field pane.

Add or remove inheritance calls

1. Click **Preferences → Edit User Configurations** or click  on the upper toolbar.

   The User Configuration window appears.

2. Click the **Vocabularies** tab, then click then **Inheritance** tab.

   The Inheritance window tab appears. (Figure 280) The Inheritance window enables you to Add, Delete, and Re-arrange current Inheritance calls.

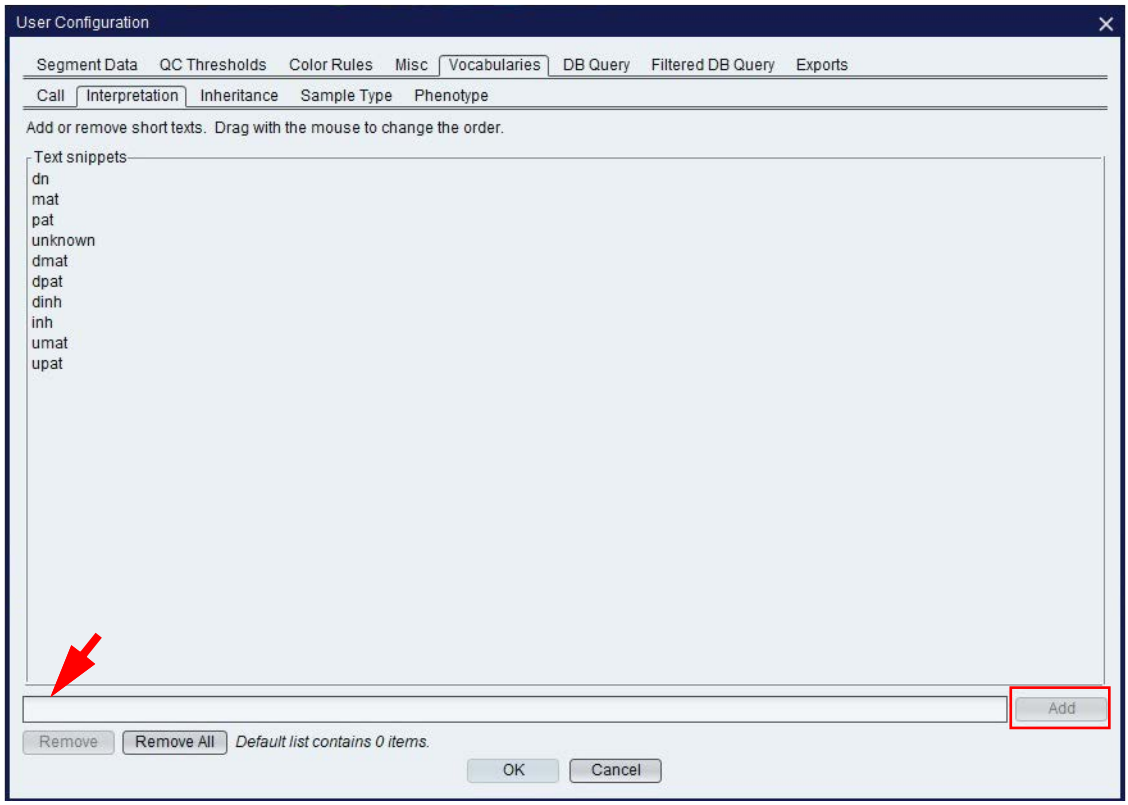


Figure 280 User Configuration window - Inheritance window tab.

3. Select the **Include Inheritance in Microarray Nomenclature** checkbox to have Inheritance column entries automatically appended to the Microarray Nomenclature field.

   Example: If the **dn** is selected in the Inheritance column then the Microarray Nomenclature field would be updated to read: **arr[GRCh37] 22q13.31(44582928_44851090)x3 dn**

Add an Inheritance call to the Inheritance dropdown list

1. Click inside the Add Inheritance Call text field (Figure 280), then enter your new call.
2. Click **Add**.

Delete a call from the Inheritance dropdown list

1. Click to highlight the call you want to delete.
2. Click **Remove**.

Re-arrange the order of calls displayed in the Inheritance dropdown list

1. Click to highlight the Inheritance you want to move.
2. Drag and drop it to its new location (order).

   Your call is now in its revised position/order within the Inheritance dropdown-list.
3. If needed, repeat steps 1–2 to re-arrange additional Inheritance.

Use the inheritance feature

Method 1: Use the inheritance feature from the Segments tab

1. Click the Browser's **Segments** tab.
2. Scroll the Segment table to the right until you see the **Inheritance** column.
3. Click inside the field.

   A blue dropdown list appears.
4. Click the dropdown list to reveal the list of available Inheritance Calls. (Figure 281)

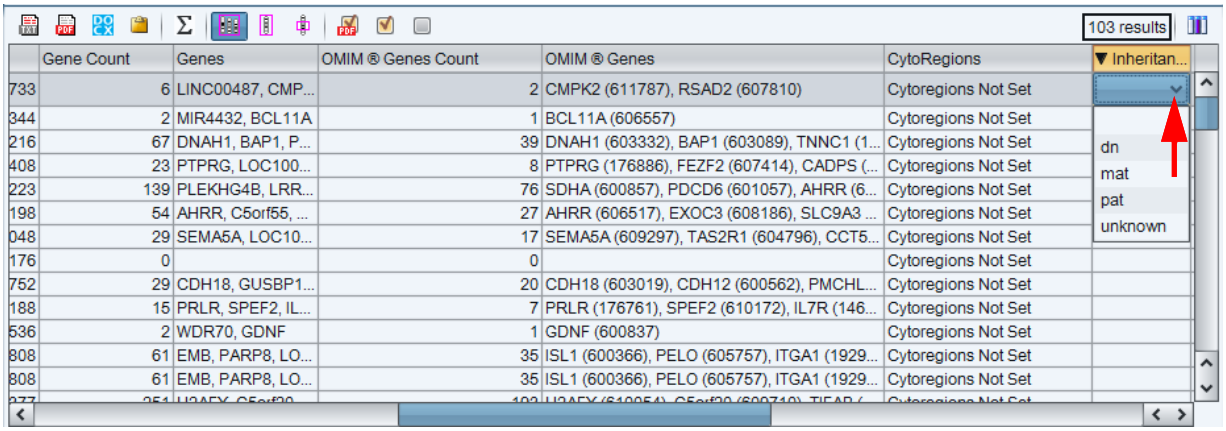


Figure 281 Calls dropdown list.

5. Click to select the appropriate Inheritance.
6. Click outside the field or press **Enter** on the keyboard.

   Your Inheritance is entered.

Method 2: Use the inheritance feature from the View/Edit annotation properties window

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click the segment you want to add an interpretation to, then click the menu selection **View/Edit Annotation Properties**.

   The Annotation Properties window appears.
2. Click the **Curation** tab.
3. Select the Inheritance call from the dropdown list.

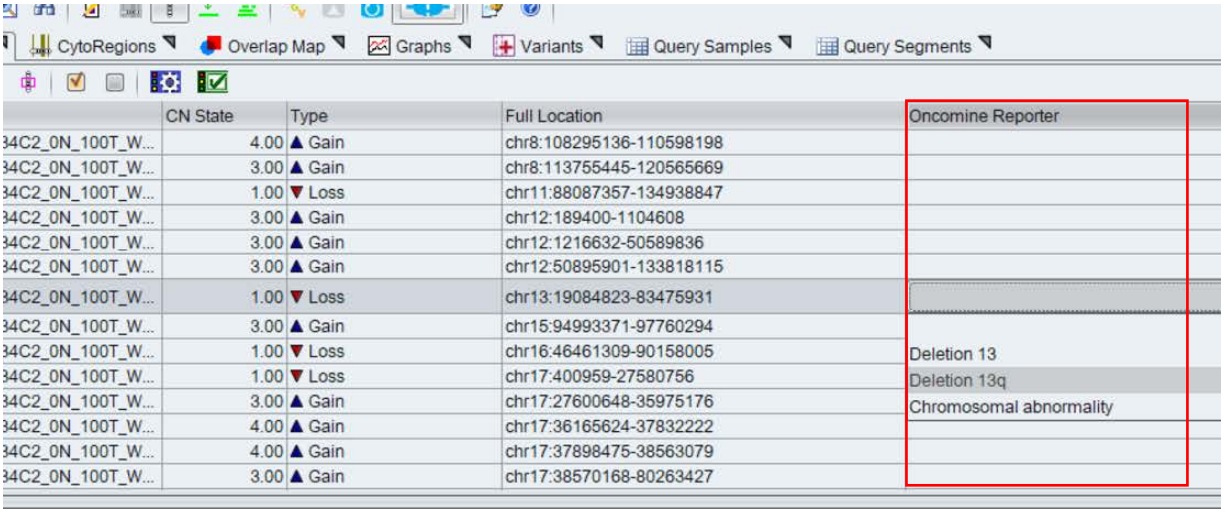
Add Oncomine™ Reporter annotations

You may add annotations based on approved Oncomine™ Reporter search terms. After your segments have been annotated with Oncomine™ Reporter term(s), the Segments Table can be exported as a TXT file and directly uploaded to Oncomine™ Reporter for literature searches based on the assigned annotation.

The annotation options for use with Oncomine™ Reporter are a controlled vocabulary.

Note: The options available in the dropdown list for the Oncomine™ Reporter column are compatible nomenclature with Oncomine™ Reporter application.

1. Select to view the **Oncomine Reporter** column in the Segments Table. See "Select columns to display or hide" on page 307.
2. Click in the **Oncomine Reporter** column for a given segment to assign the appropriate annotation for that segment. Segments on a given chromosome will only see relevant Oncomine Reporter terms for that chromosome. For example, if there is a gain of Chromosome 8q, you will only see Oncomine™ Reporter terms for Chromosome 8. All other terms for other chromosomes are hidden for that segment.



	CN State	Type	Full Location	Oncomine Reporter
34C2_0N_100T_W...	4.00	▲ Gain	chr8:108295136-110598198	
34C2_0N_100T_W...	3.00	▲ Gain	chr8:113755445-120565669	
34C2_0N_100T_W...	1.00	▼ Loss	chr11:88087357-134938847	
34C2_0N_100T_W...	3.00	▲ Gain	chr12:189400-1104608	
34C2_0N_100T_W...	3.00	▲ Gain	chr12:1216632-50589836	
34C2_0N_100T_W...	3.00	▲ Gain	chr12:50895901-133818115	
34C2_0N_100T_W...	1.00	▼ Loss	chr13:19084823-83475931	
34C2_0N_100T_W...	3.00	▲ Gain	chr15:94993371-97760294	
34C2_0N_100T_W...	1.00	▼ Loss	chr16:46461309-90158005	Deletion 13
34C2_0N_100T_W...	1.00	▼ Loss	chr17:400959-27580756	Deletion 13q
34C2_0N_100T_W...	3.00	▲ Gain	chr17:27600648-35975176	Chromosomal abnormality
34C2_0N_100T_W...	4.00	▲ Gain	chr17:36165624-37832222	
34C2_0N_100T_W...	4.00	▲ Gain	chr17:37898475-38563079	
34C2_0N_100T_W...	3.00	▲ Gain	chr17:38570168-80263427	

Figure 282 Oncomine Reporter column example.

For information on Oncomine™ Reporter Table State, see "Saved table states" on page 310.

Track and review the log file

1. Right-click the file, then click **View Process Pipeline**. (Figure 283)

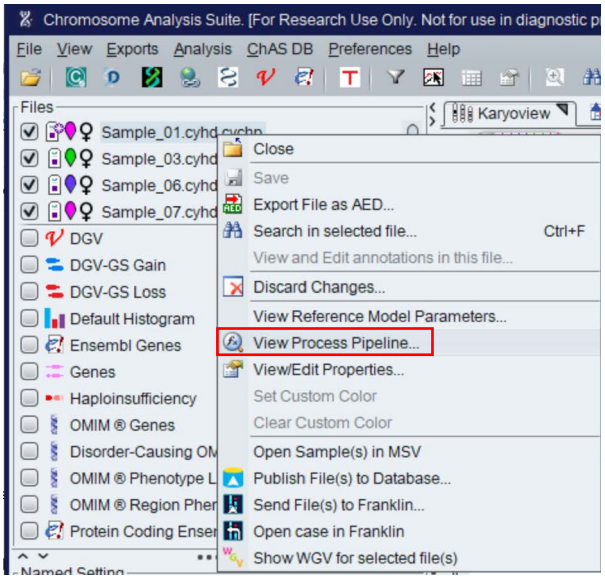


Figure 283 Right-click menu.

The **Process Pipeline** window appears. (Figure 284)

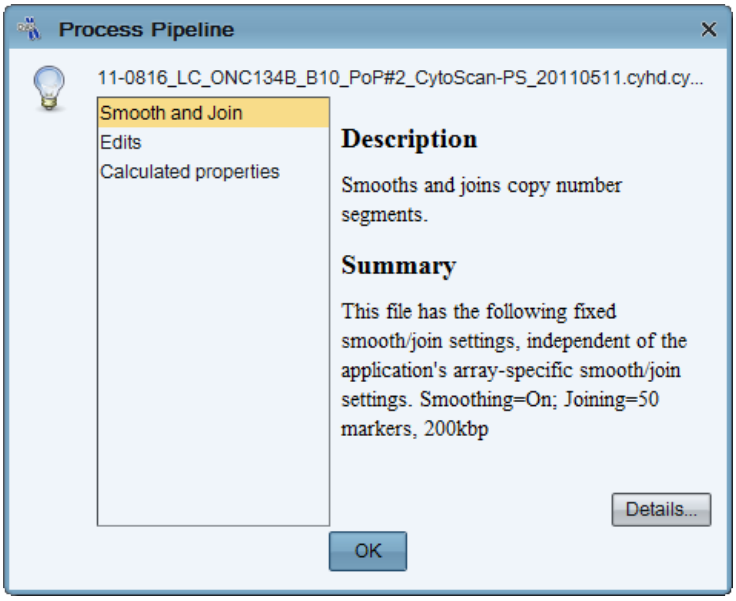


Figure 284 Process Pipeline - Smooth and Join.

2. Click **Smooth and Join** for its description and summary. (Figure 284)
3. Click **Edits** for its description and summary of the file's past user edits. (Figure 285)

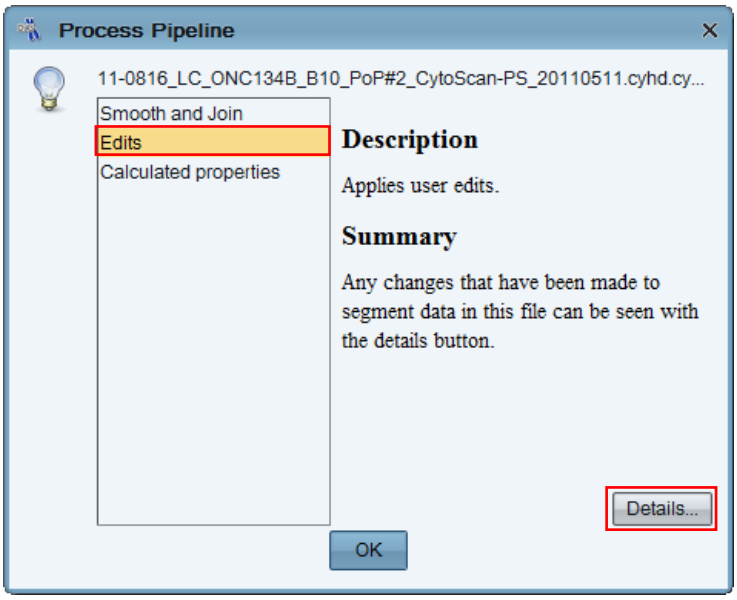


Figure 285 Process Pipeline -Edits.

4. Click **Details.**

   A Edit Details window appears featuring an Edit and Log tab. (Figure 286)

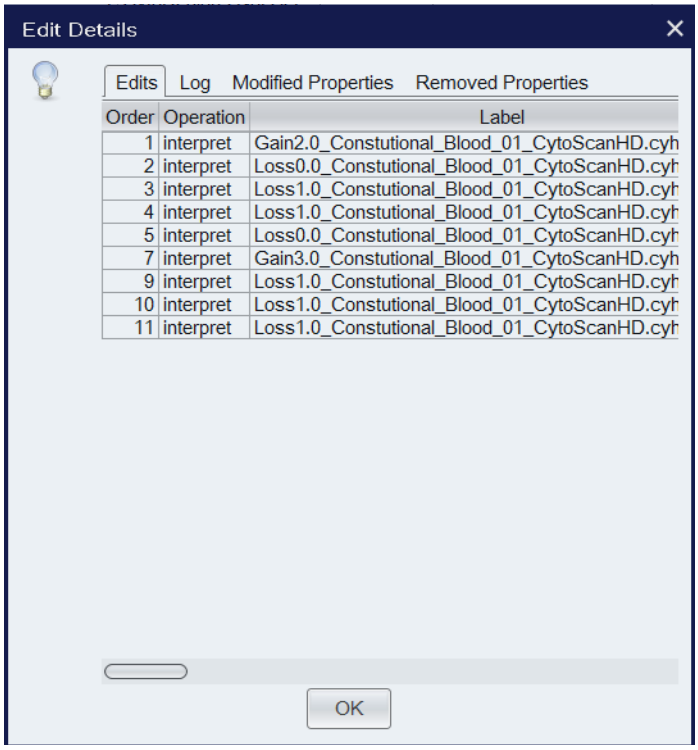
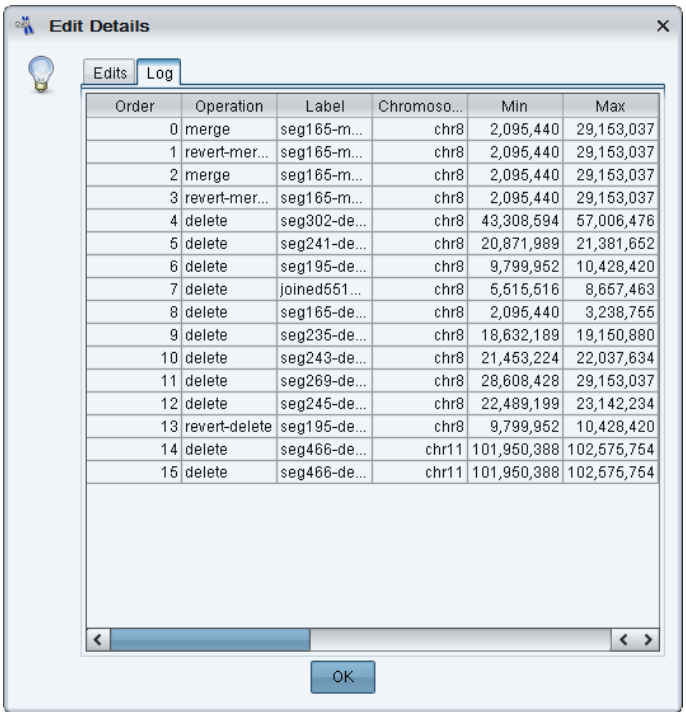


Figure 286 Process Pipeline - Edit tab.

The **Edit** tab lists only recent edits that were NOT reverted. Use the horizontal scroll bar to reveal additional edit information. For a complete historic account of all edits made (including reversions of some edits), click the **Log** tab.

5. Click **OK** to return to the **Process Pipeline** window or click the **Log** tab.

   The **Log** tab displays an extensive summary of the file's edited history to date. Use the horizontal scroll bar to reveal additional log information. (Figure 287)



Order	Operation	Label	Chromoso...	Min	Max
0	merge	seg165-m...	chr8	2,095,440	29,153,037
1	revert-mer...	seg165-m...	chr8	2,095,440	29,153,037
2	merge	seg165-m...	chr8	2,095,440	29,153,037
3	revert-mer...	seg165-m...	chr8	2,095,440	29,153,037
4	delete	seg302-de...	chr8	43,308,594	57,006,476
5	delete	seg241-de...	chr8	20,871,989	21,381,652
6	delete	seg195-de...	chr8	9,799,952	10,428,420
7	delete	joined551...	chr8	5,515,516	8,657,463
8	delete	seg165-de...	chr8	2,095,440	3,238,755
9	delete	seg235-de...	chr8	18,632,189	19,150,880
10	delete	seg243-de...	chr8	21,453,224	22,037,634
11	delete	seg269-de...	chr8	28,608,428	29,153,037
12	delete	seg245-de...	chr8	22,489,199	23,142,234
13	revert-delete	seg195-de...	chr8	9,799,952	10,428,420
14	delete	seg466-de...	chr11	101,950,388	102,575,754
15	delete	seg466-de...	chr11	101,950,388	102,575,754

Figure 287 Log tab.

6. Click **OK** to return to the Process Pipeline window. (Figure 288)

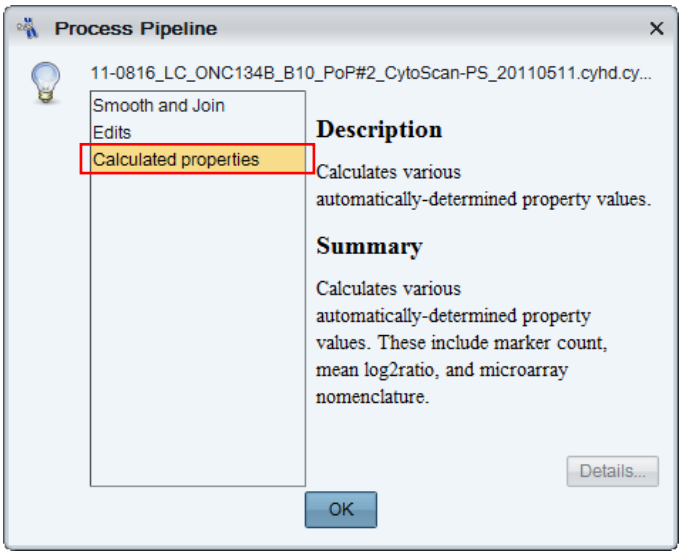


Figure 288 Process Pipeline - Calculated properties.

7. Click **Calculated Properties** for its description and summary.
8. Click **OK** to return to the ChAS Browser.

12 Use CytoRegions

CytoRegions overview

The CytoRegions feature enables you to define parts of the genome that are of special interest.

Note: The CytoRegions feature is designed for use with up to a few thousand regions. Larger numbers of regions can be used, but will impact performance. A reference annotation file, such as Genes, is not recommended for use as a CytoRegions file due to the large number of reference annotations.

To use CytoRegions, you need to select a file(s) with position information for regions of the genome as the CytoRegions file.

- Select Region information files in AED or BED format.
- Use existing Region file(s), or create a new one in AED format in ChAS, then add regions to it by selecting segments, annotations, or regions in other loaded files.
- Add annotations to regions to help you track the information. See "Create and edit AED files" on page 269.

After selecting a CytoRegions file, you can:

- Use the Restricted Mode to display only Segments and graph data that appear in those regions. While in this mode, annotations are not hidden by CytoRegions or by the application of Restricted Mode.
- Use differential filtering options for these regions and for the rest of the genome.
- Protect a CytoRegions file. See "Protect an AED file" on page 288.

Select a CytoRegions information file

Select the CytoRegions file from the available region information files. See "Load files" on page 113. The software automatically checks the hg version of an AED or BED file before loading. See Figure 289 for an example BED file. The file will not be loaded if the hg version does not match what is loaded in the ChAS Browser. If a hg version is not found for the AED or BED file, a warning message appears.

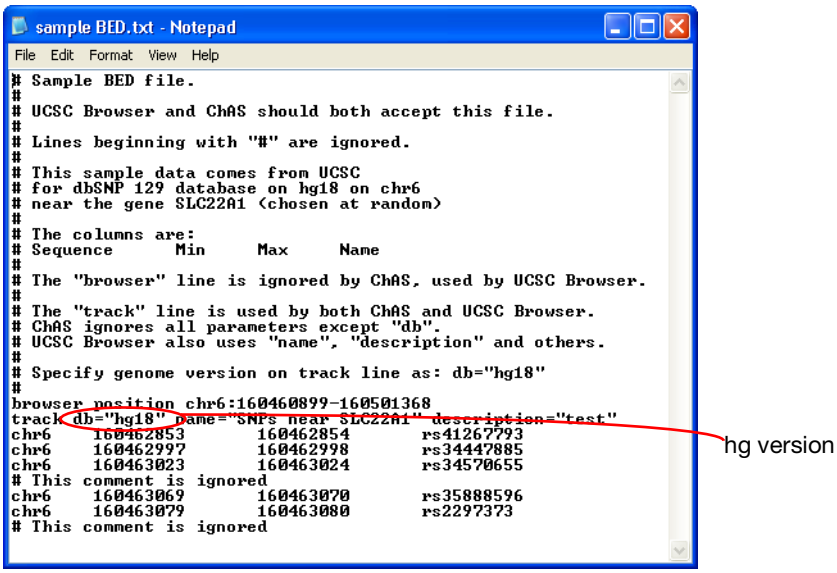


Figure 289 Example BED file.

Do either of the following to select a CytoRegions file:

- In the files list, right-click a file and select **Include as CytoRegion** on the shortcut menu, as shown in Figure 290.
- Multiple files can be selected to be included in the CytoRegions. To do this, right- click each AED/BED file to be included, then select **Include in CytoRegions**.

Note: All files (included in CytoRegions) are treated as they are one file.

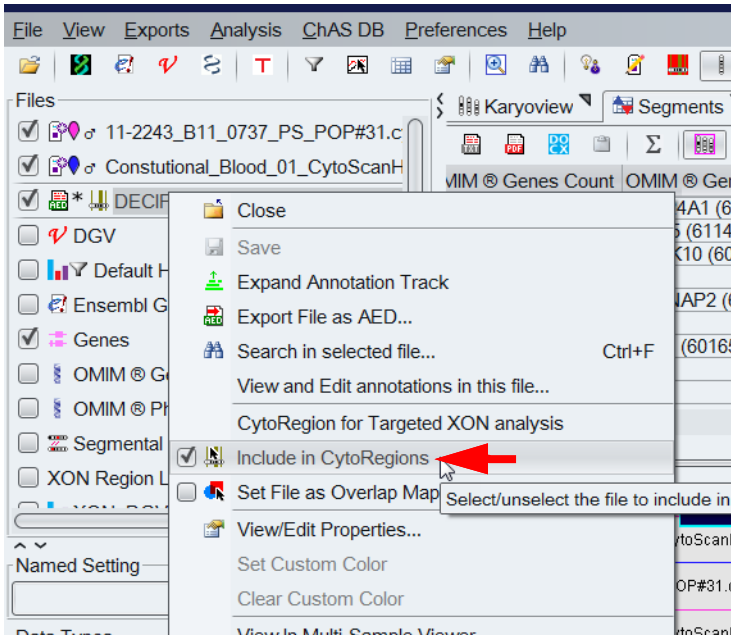


Figure 290 Select a CytoRegions file from the file list.

Or

- From the menu bar, click **View** → **Select as CytoRegions file(s)**.

  Alternatively, in the CytoRegions Tab of the Upper display, click the **Select to include in CytoRegions** icon.

  The Select CytoRegions File(s) window opens. (Figure 291)

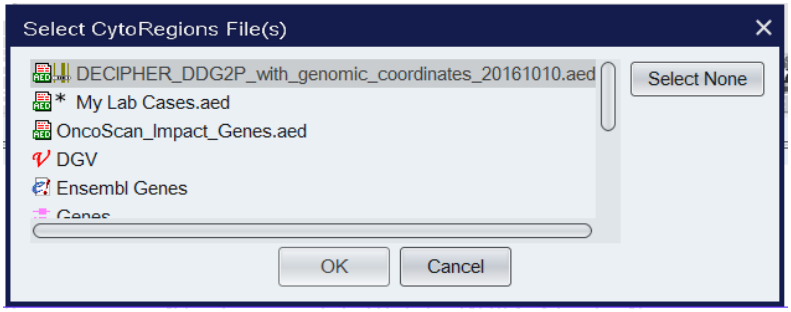


Figure 291 Select CytoRegions File(s) window.

- Select a regions information file(s) to use for CytoRegions, then click **OK**.

Note: You may select more than one Region file (AED/BED) to include as CytoRegions. ChAS handles multiple selected files as single (larger) CytoRegion file.

The **Create New** feature is described in "Create an AED file of annotations" on page 269.

Note: If a region file was selected for the CytoRegion file before shutting off the software, that file is automatically loaded as the CytoRegion file after the software is opened the next time with the same user profile. To clear a CytoRegions file map from ChAS, click **Select None** (Figure 291). Alternatively, right-click the file in the Files list, then deselect **Include in CytoRegions** on the shortcut menu to toggle the check mark off.

View CytoRegions

After selecting a CytoRegions file, it can be displayed in either a graphic view or table.

- "CytoRegions in the graphic views" on page 254
- "CytoRegions table" on page 256

Note: CytoRegions that share genomic coordinates with a detected segment are listed in the "CytoRegions" column of the Segments table. See "Segments table" on page 315.

CytoRegions in the graphic views

Regions specified in the CytoRegions file are displayed as dark gray stripes in the Karyoview and Chromosome Display (Figure 292) and Detail View (Figure 293).

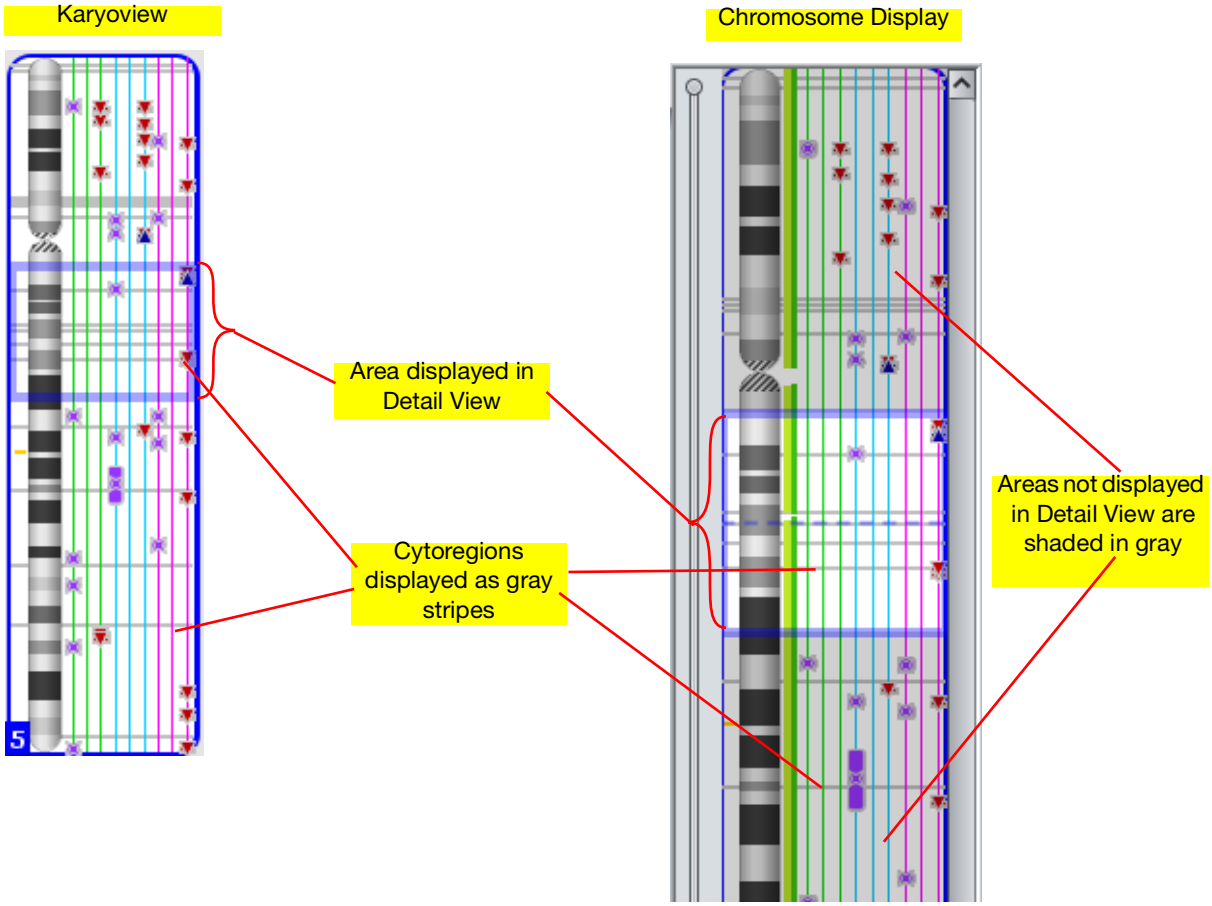


Figure 292 Karyoview and Selected Chromosome View with CytoRegions Displayed.

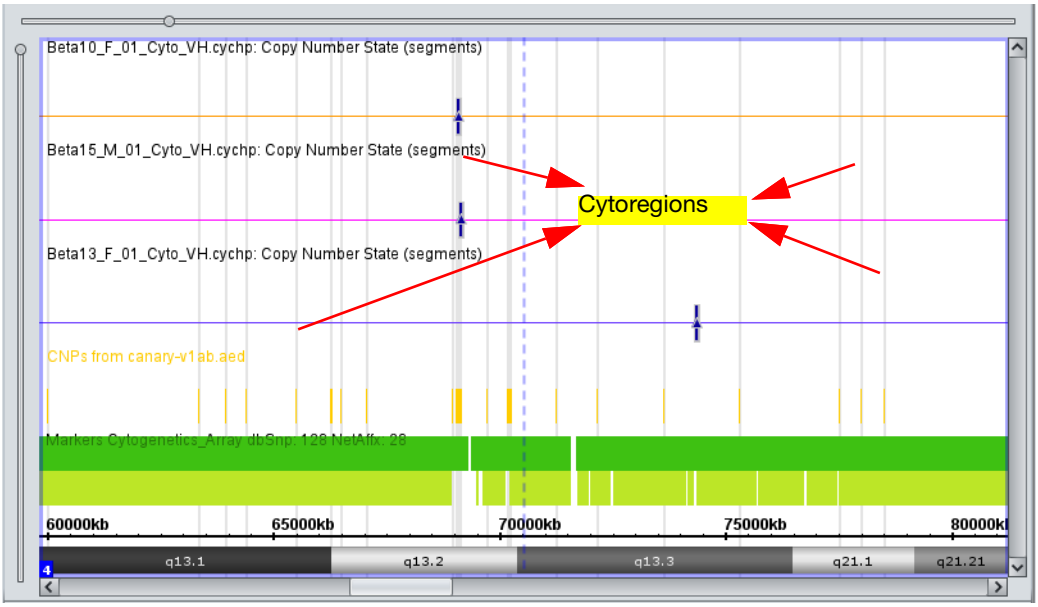


Figure 293 Detail View with CytoRegions displayed.

You can turn off highlights by deselecting the View menu's **Hide CytoRegion Background Highlights** checkbox. (Figure 294) This action keeps the CytoRegions in the Table View, however they are no longer highlighted in the Graph Views.

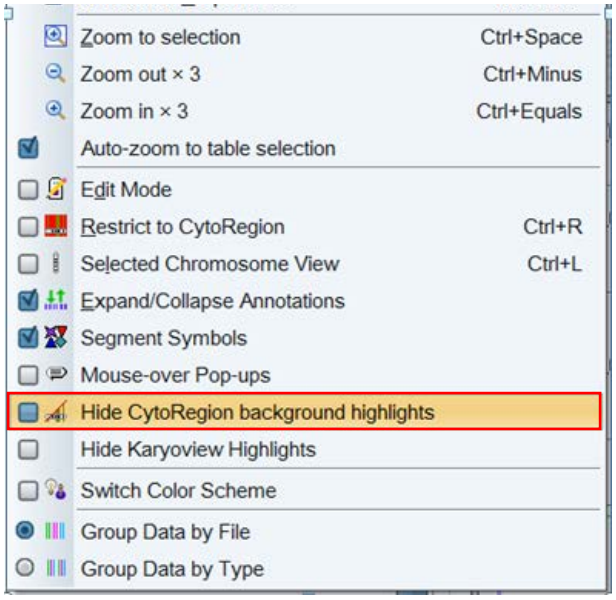


Figure 294 View menu - Hide CytoRegion checkbox.

CytoRegions table

The CytoRegions table (Figure 295) shows the intersections between regions in the designated CytoRegions file and the segments in the Segments table. Every region in the CytoRegions file are listed on at least one line of the CytoRegions table, even if it does not intersect any segments. For those regions which intersect one or more segments, there is 1 table row for each intersection. Depending on the columns which have been used to sort the CytoRegions table, these rows may or may not be near each other. A segment that intersects more than one region in the CytoRegions file appears multiple times in the CytoRegions table, one row for each intersection.

In the CytoRegions table, the "CN State" value corresponds to the state of copy-number and mosaicism segments that intersect the CytoRegion. There is no CN State value for other types of segments that do not correspond to a copy-number call.

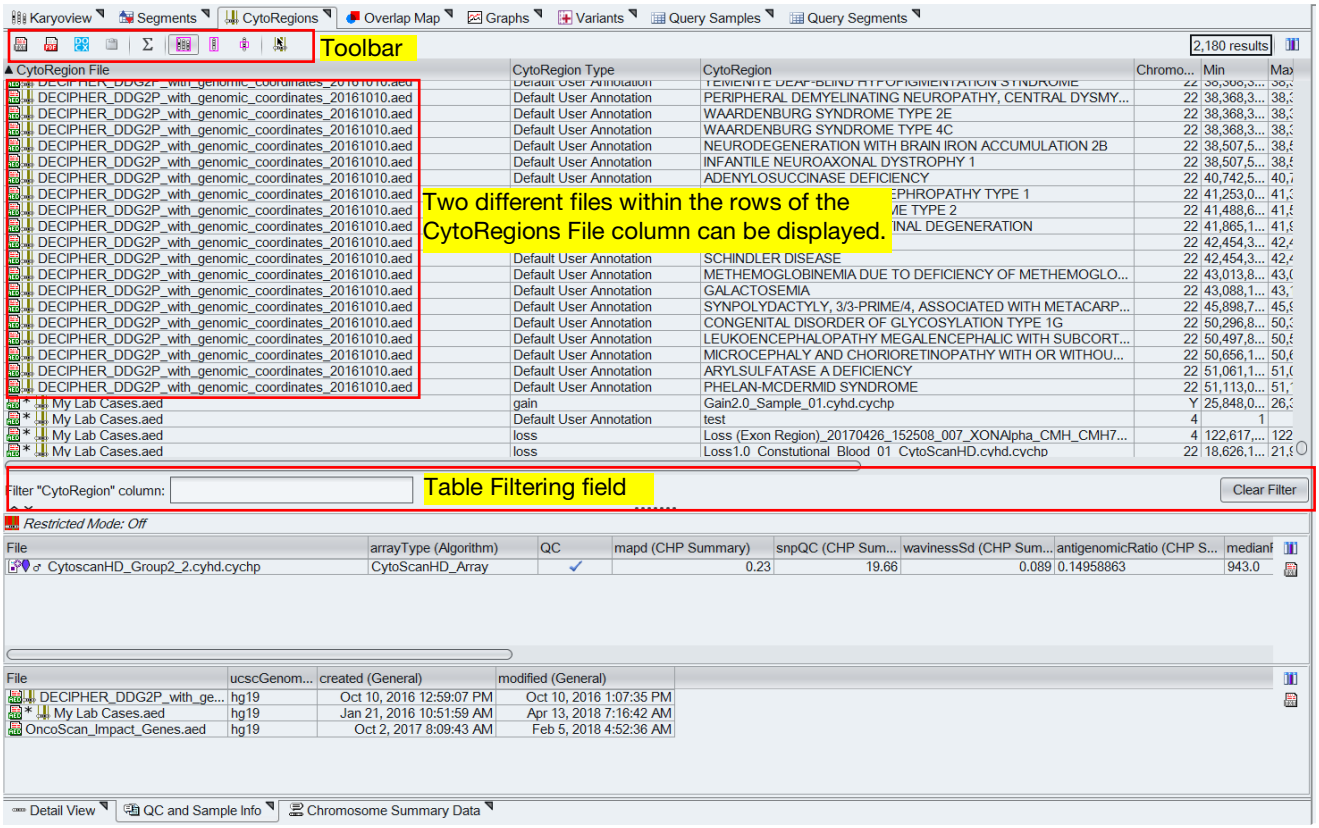


Figure 295 CytoRegions table.

Highlight regions in the CytoRegions table and details view

If the Details View displays the CytoRegions file (the CytoRegions file is check marked in the Files list), you can conveniently find and view items.

- Click a row in the CytoRegions table to select the corresponding annotation from the CytoRegions file. All of the lines for that region are highlighted in the table. The Details View zooms to the currently selected region.

  Note: If the Details View does not automatically zoom to the selected region, confirm that the Auto-zoom option is selected (click **View → Auto-zoom to table selection** from the menu bar).

- In the Detail View, click a region or select multiple regions of the CytoRegion file to highlight all of the corresponding rows in the CytoRegions table. The CytoRegions table automatically scrolls to show at least one of the highlighted rows.
- To quickly find a particular Segment in the CytoRegions table, first double-click that segment in any of the views or in the Segments table (the current region is set to that segment), then press the toolbar icon in the CytoRegions table to show only the cytoregions in the current region.

CytoRegions toolbar

The toolbar (Figure 296) provides quick access to table functions. The standard functions are described in "Standard toolbar controls" on page 306.



Figure 296 CytoRegions table toolbar.

The toolbar has one specialized icon.



Select CytoRegions file (See "Select a CytoRegions information file" on page 252.)

Table 30 CytoRegions table columns.

Column	Description
CytoRegion File	Name of the AED/BED file that contains the region.
CytoRegion Type	Type of file or element from which the CytoRegion is derived. Default User Annotations are annotations derived from AED or BED file.
CytoRegion	Identifier for region.
Chromosome	Chromosome in which the region is located.
Min	Starting position of the region.
Max	Ending position of the region.
Size (kbp)	Size of the region.
Segment Label	Label assigned to the detected segment by ChAS.
Segment Name/ID	Name/ID of the Segment.
Segment File	Sample File that the segment was detected in.
Segment Type	Type of segment: • CN loss or gain • XON loss or gain • Mosaic loss or gain • LOH
Segment Min	Starting position of segment.
Segment Max	Ending position of segment. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).
Segment Size (kbp)	Size of the segment.
CN State	Copy Number State (Displayed for Gain, Loss, and Mosaicism segment types).
Shared Size	Size of the contact between segment and cytoregion.
% of CytoRegion touching Segment	How much of the CytoRegion is contacted by the Segment.
% of Segment touching CytoRegion	How much of the detected segment is contacted by the CytoRegion.

You can:

- Use the common table functions to control the display of data in the table. See “Common table operations” on page 305.
- Export data in various formats. See “Export table data” on page 393.
- Search CytoRegions to display only regions of interest. (See "Search CytoRegions" on page 259).

Search CytoRegions

The Search CytoRegions feature enables you to search the "CytoRegion" column for text strings that match a search string.

1. Enter the search string in the Search CytoRegions box at the bottom of the CytoRegions table.
2. Press **Enter**.

   The table displays only annotations that match the search string.
3. To restore the table, click **Clear Search Field**.

Use filters with CytoRegions

If you have CytoRegions information loaded into ChAS, you have the option to apply different segment filtering parameters to the parts of the genome that are defined as CytoRegions and the parts of the genome that are not within these regions (Genome). For segment filtering to function as described, the CytoRegion segment filters should always be set identical to or less stringent than the counterpart Genome segment filter settings.

The filter settings for CytoRegions and for the rest of the genome interact in different ways, depending upon whether:

- A cytoregion information file is selected
- Restricted Mode is selected

If a cytoregion information file is selected, but Restricted Mode is not selected:

- Segments wholly within a cytoregion are filtered using the CytoRegions filter settings.
- Segments that don't touch a cytoregion are filtered using the Whole Genome settings.
- A segment that touches both Genome and CytoRegions must pass both the CytoRegions filter thresholds and Whole Genome filter thresholds, otherwise it will not be shown.

If Restricted Mode is selected:

- Segments in or straddling cytoregion boundaries are filtered using the CytoRegions filter settings.

These rules also apply when a region information file has been selected as an Overlap Map and the Overlap filters are used.

Use restricted mode

The Restricted mode enables you to view detected segments and graph data only in regions you have defined in advanced in the CytoRegions file.

Note: Restricted mode is not available unless a region information file or one of the Reference Annotations is selected for the CytoRegions function.

Select/De-select Restricted Mode

- From the **View** menu, select **Restrict to CytoRegion**.

Or

- Click the **Restricted Mode** icon  from the main toolbar.

Note: Segments and graph data that are not in the defined CytoRegions are concealed in the display. Segments in or straddling CytoRegions boundaries are filtered using the CytoRegions filter settings. (Figure 297, Figure 298)

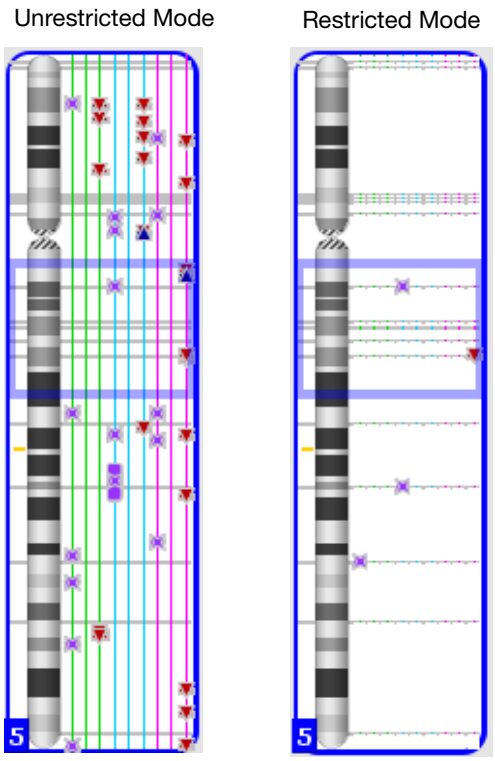


Figure 297 Karyoview example shown in unrestricted and restricted mode.

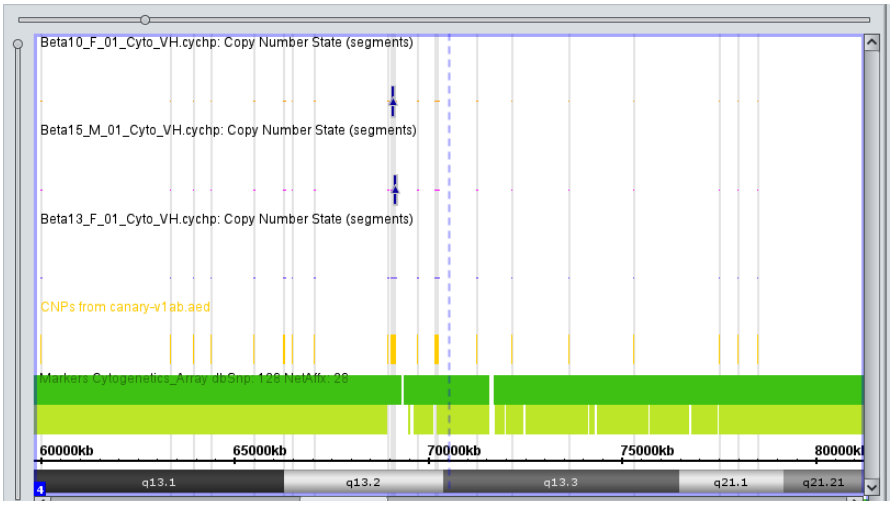


Figure 298 Detail View example in Restricted Mode.

When you deselect Restricted Mode, an Exit Restricted Mode window appears. (Figure 299)



Figure 299 Exit Restricted Mode window.

Click **Yes** to exit Restricted Mode.

Assign a CytoRegion for targeted XON analysis

This option is designed for use with CytoScan™ XON array data. Assigning a CytoRegion for a Targeted XON analysis combines these 2 steps:

1. Assigns the file as the CytoRegion.
2. Automatically sets the appropriate filters for Targeted XON analysis.
   - Hides all segments in the Genome region and colors all the data gray.
   - Turns on all levels in CytoRegions to reveal any XON Region segment call and colors the data the assigned sample color.

Create an AED File from a gene list

To create an AED file from a Gene List for use with the CytoRegion for Targeted XON analysis or other region file (CytoRegion or Overlap Map) do the following:

1. Create a text tab delimited file containing your list of Genes for which you would like to generate an AED file. The gene coordinates are pulled from a selected **NetAffx Genomic Annotation File**. The Gene List should just be a list of gene names with no header and saved as a text tab delimited file.
2. Click **Exports → Create and Export an AED file from a Gene List**.

   The following window appears: (Figure 300)

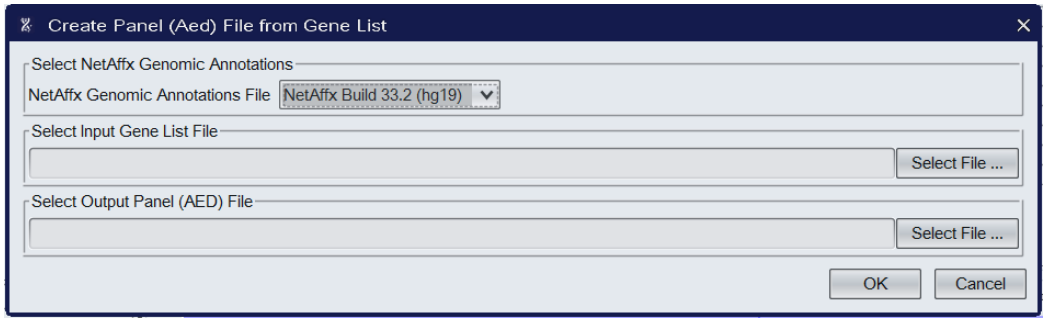


Figure 300 Create Panel (AED) File from Gene List window.

3. Select the **NetAffx Genomic Annotation File** from the dropdown list. The annotation database files visible are available files from the Library file folder. If you do not see a **NetAffx Genomic Annotation File** that you want to use, copy the file into C:\Affymetrix\ChAS\Library and restart the ChAS Browser.
4. Select an Input Gene List File: Click the **Select File** button and browse to the location of your TXT tab-delimited Gene List file.
5. Select an Output AED File: Click the **Select File** button, select the location to save the output file, name the AED file, click **Save**.
6. Click **OK** to generate the AED file.

Note: If entries in the Gene List are not found in the **NetAffx Genomic Annotation File**, they will be listed in a message box. Any skipped entries can be added to the AED file. See "Create and edit AED files" on page 269 and "Add annotations to an AED file" on page 271.

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13 Use the overlap map and filter

Overlap map and filter overview

- The Overlap Map enables you to show or hide segments in areas of the genome that you are not interested in. For example, in regions of Benign Copy Number Polymorphism, you can:
  - Specify regions of the genome in an Overlap Map File.
  - Optionally filter out detected segments that are overlapped by these regions. You can also specify the percentage of the segment (between 1 and 100%) that must be overlapped by the Overlap Map items before being filtered from display.
- The Overlap Map filter operates separately from the CytoRegions features, but you can apply different overlap map filtering parameters to CytoRegions and to areas outside of CytoRegions.
- Protect an Overlap Map file. See “Protect an AED file” on page 288..

Use the overlap filter

1. Select a region information file for the Overlap Map. For supported file types, see "Select the overlap map file" on page 262.
2. Set Percent Overlap value for each segment type you want to filter in the Segment Filters window. See "Use the overlap filter" on page 268.
3. Choose how you want to view the Overlap Map regions.
   - "View overlap map regions in the graphic displays" on page 265
   - "View the overlap map table" on page 266

Select the overlap map file

Use the following file types for the Overlap Map:

- Region information files in AED and BED format.
- Position information from Reference annotation files.

The software automatically checks the hg version of an AED or BED file before loading (see Figure 301 for an example BED file). The file will not be loaded if the hg version does not match what is loaded in the ChAS Browser. If a hg version is not found for the AED or BED file, a warning message appears.

```
File  Edit  Format  View  Help
# Sample BED File.
#
# UCSC Genome Browser and ChAS should both accept this file.
#
# Lines beginning with "#" are ignored.
#
# The UCSC genome version is hg38
#
# The columns are:
# Chromsome     Min     Max     Name
#
# The "browser" line is ignored by ChAS, used by UCSC Genome Browser
#
# The "track" line is used by both ChAS and UCSC Genome Browser.
# ChAS ignores all parameters except "db".
# UCSC Genome Browser also uses "name", "description" and others.
#
# Specify genome version on the track line as: db="hg38"
#
browser position chr6:160460899-160501368
track  db="hg38" name="MyBEDFILE" description="IMAGE306 2022-06-07T11:55:07.782-07:00"
chr1    818811   3685376 Gain3.0_H22000776r3.hg38.OSCHP
chr1    6579786 7622177 Gain3.0_H22000776r3.hg38.OSCHP
chr1    10330637        12770000        Gain2.5_Sample1.hg38.OSCHP
chr1    83550636        121852400       Gain4.0_Sample1.hg38.OSCHP
chr1    143549777       248918679       Gain6.0_Sample1.hg38.OSCHP
chr2    21493   10668078        Gain6.0_Sample1.hg38.OSCHP
chr2    10678600        17653487        Gain4.0_Sample1.hg38.OSCHP
```

hg version

Figure 301 Example BED file.

Do either of the following to select an Overlap Map:

1. In the files list, right-click the file and select **Set File as Overlap Map** on the shortcut menu. (Figure 302)

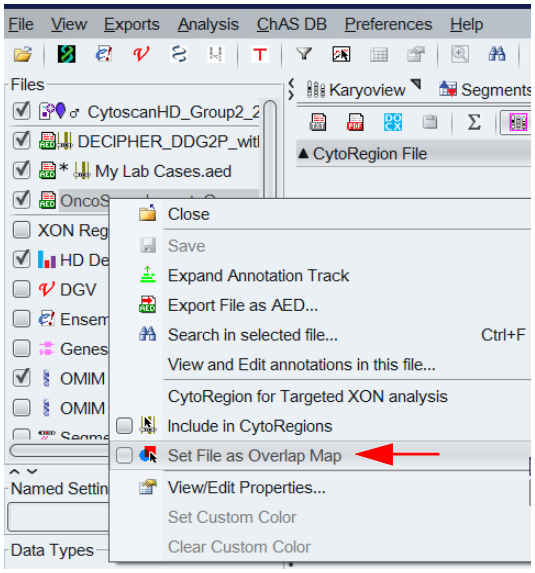


Figure 302 Select an Overlap Map from the files list.

Or

1. **On the menu bar, click Select View → Overlap Map**.

   Alternatively, in the Overlap Map tab in the upper display, click the **Select Overlap Map** toolbar icon.

   The **Select Overlap Map** window appears. (Figure 303)

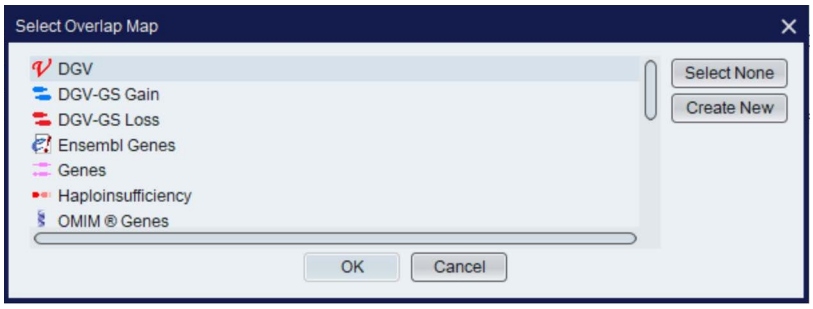


Figure 303 Select Overlap Map window.

   The window displays a list of the region and annotation files you can select for an overlap map.

2. Select the file you want to use, then click **OK**.

   The region file loads and its regions are displayed with overlap information in the **Overlap Map** tab.

   The Overlap Map icon appears next to your selected file within the **File List**.



Note: To clear an Overlap map, click **Select None**. (Figure 303) Alternatively, right-click the file in the files list, then select **Set File as Overlap Map** from the shortcut menu.

View overlap regions

You can view the Overlap Map regions:

- In the graphic display views. See “View overlap map regions in the graphic displays” on page 265.
- In the Overlap Map window tab. See “View the overlap map table” on page 266.

Note: Overlap Map items that are covered by a detected segment are listed in a column of the Segments table. "Segments table" on page 315.

View overlap map regions in the graphic displays

In the Karyoview and Selected Chromosome View, regions specified in the Overlap Map file are displayed as short rectangles to the immediate left of the Cytobands. (Figure 304)

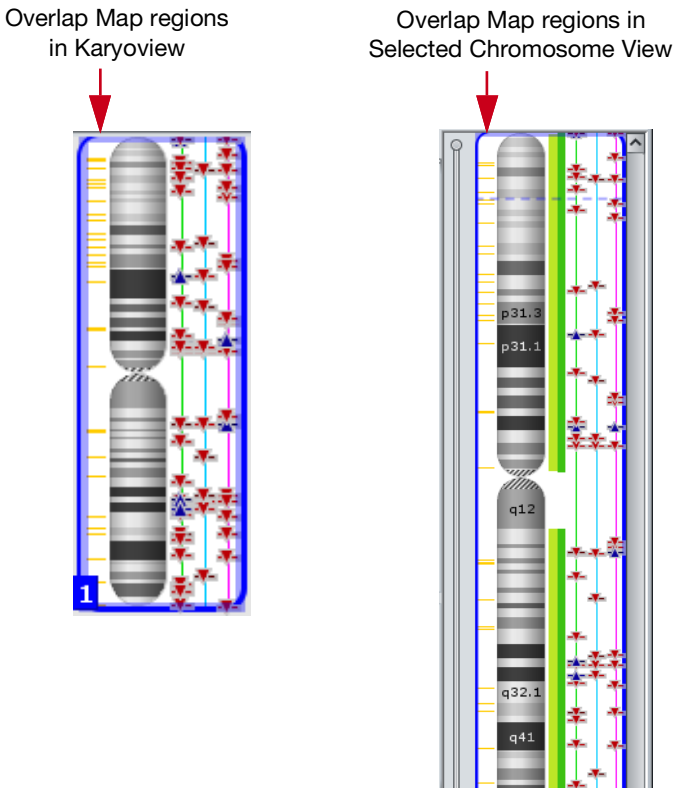


Figure 304 Karyoview and Selected Chromosome View with Overlap Map Regions Displayed.

In the Detail View, the overlap map regions are displayed as rectangles below the Cytobands (Figure 305). The default color is yellow, but you may select different colors for displaying regions in a region information file.

You can change the colors used to display items by using:

- Specifying color when adding the item to an AED file. See "Enter general information" on page 279.
- Using the Color Rules. See "AED/BED color rules" on page 289.

Overlap Map regions

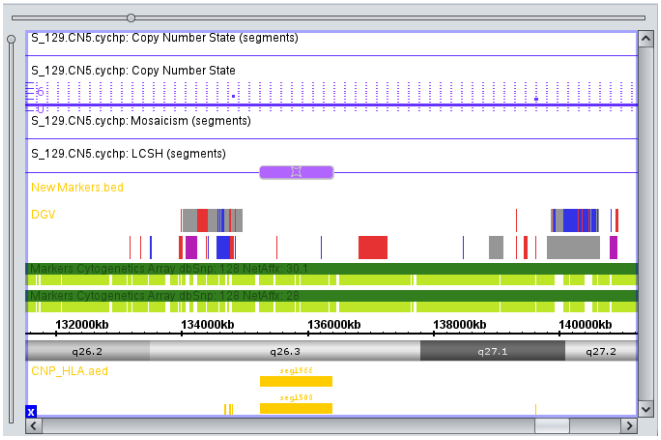


Figure 305 Detail View with Overlap Regions displayed.

View the overlap map table

The Overlap Map table (Figure 306 on page 266) displays a list of overlapping items from the overlap map file and the Segments table. Each region in the Overlap Map file is listed on at least 1 row of the table, even if it does not overlap any segments. For those regions which overlap one or more segments, there will be one for each overlap. Depending on the columns which have been used to sort the Overlap Map table, these rows may or may not be near each other. A segment that overlaps more than one region in the Overlap Map file will appear multiple times in the Overlap Map table, one row for each overlap.

Highlight overlap regions in the overlap map table and details view

If the Details View displays the Overlap Map file (the Overlap Map file is check marked in the Files list), you can conveniently find and view items.

- Click a row in the Overlap Map table to select the corresponding annotation from the Overlap Map file. All of the rows for that region are highlighted in the table. The Details View zooms to the currently selected region.

  Note: If the Details View does not automatically zoom to the selected region, confirm that the Auto-zoom option is selected. (Click **View → Auto-zoom to table selection** from the menu bar.)

- In the Detail View, click a region or select multiple regions from the Overlap Map file to highlight all of the corresponding rows in the Overlap Map table. The Overlap Map table automatically scrolls to show at least one of the highlighted rows.
- To quickly find a particular Segment in the Overlap Map table, first double-click that segment in any of the views or in the Segments table (the current region is set to that segment), then press the toolbar icon in the Overlap Map table to show only the overlaps in the current region.

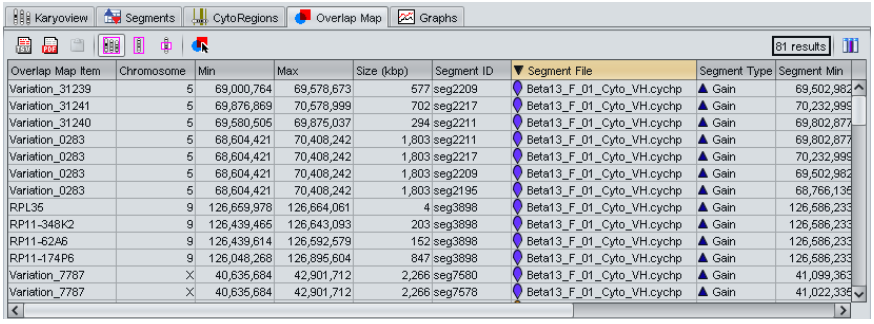
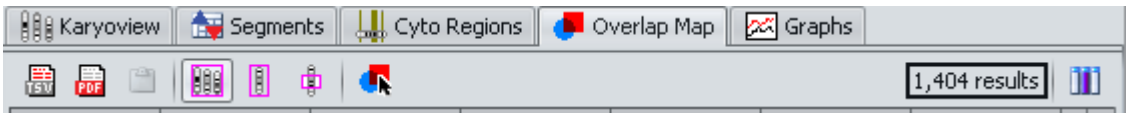


Figure 306 Overlap Map table.

Overlap map toolbar

The toolbar provides quick access to table functions. The standard functions are described in "Standard toolbar controls" on page 306.



Select Overlap Map file (see "Select the overlap map file" on page 262).

Table 31 Overlap Map table columns.

Column name	Description
Map Item Type	Source of the position information (CN Gain or Loss segment, reference annotation, etc.)
Overlap Map Item	Identifier for the item.
Chromosome	Chromosome in which the item is located.
Min	Starting position of the item.
Max	Ending position of the item.
Size (kbp)	Size of the item.
Segment Label	Label assigned to the detected segment by ChAS.
Segment Name/ID	Name/ID of the Segment.
Segment File	Sample File that the segment was detected in.
Segment Type	Type of segment: • CN loss or gain • Mosaicism • LOH
Segment Min	Starting position of segment.
Segment Max	Ending position of segment. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).
Segment Size	Size of the segment.
CN State	Copy Number State (Not displayed for other segment types).
% Overlap	How much of the detected segment is covered by the Overlap Map item. A Segment which has 20% of its length somehow encompassed within the boundaries of an Overlap Map item has an Overlap value of 20%. This percentage value is used to filter segments out of the displays and tables when filtering segments by “Overlap” in the filter slider dialogs.
% Coverage	How much of the Overlap Map item is covered by the Segment.
Shared Size	Size of the overlap between segment and Overlap Map item. Coverage values are not presently used in filtration of Segments from the displays or tables.

Use the overlap filter

After selecting an Overlap Map Region file, you have the option to set Overlap filters for the different segment types.

1. Select a region information or Reference Annotation file for the Overlap Map.
2. Click the icon to open the **Segment Filters** window. (Figure 307)

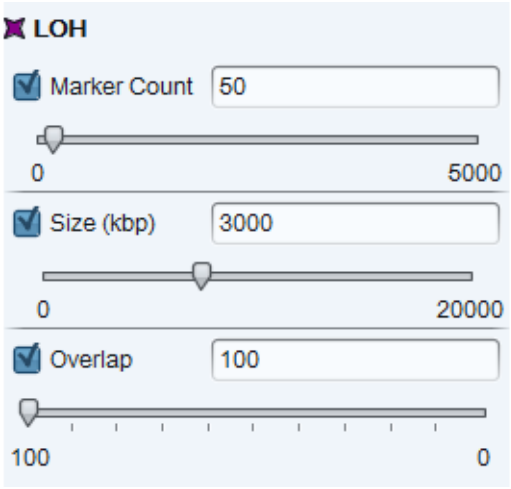


Figure 307 LOH filter settings, Overlap selected.

3. Select the Overlap checkbox(es) for the segment types you want to filter.
4. Use the slider to set the parameter's value or enter a value in the adjacent text box.
   - As you move the slider farther to the right (or enter smaller values in the box) more and more of the Overlapped segments are removed from the display.
   - The detected segments must share at least the specified percentage of their length with the Overlap Map region to be filtered out and hidden from display. A Segment which has 20% of its length somehow encompassed within the boundaries of an Overlap Map item has an Overlap value of 20%.
   - The minimum value of a setting is 1%.
   - The results of filtering are seen instantly in all tables and graphs.
   - See "Use filters with CytoRegions" on page 259. for information about the interactions of the Overlap Map filter with the CytoRegions.

14 Create and edit AED files

Create an AED file of annotations

You can create AED files to include:

- User-selected annotations. The annotations in an AED annotation file can be edited in ChAS.
- Position information on regions of the chromosome, as well as additional annotation information on the regions.

Note: AED region information files can be used for CytoRegions or Overlap Map functions.

- Third party reference annotations converted to AED file format.
- User-generated AED region information files can be used for CytoRegions or Overlap Map functions.

Note: You can add a region to a new or previously created AED file by selecting these feature types:

- Detected Segments
- Reference Annotations
- Regions in previously loaded files

1. In the Detail View, select the non-AED annotation(s) for the AED file using one of the following methods:
   – Right-click an annotation and select **Add to a File** on the shortcut menu. (Figure 308)

   Or

   – Draw a box around multiple annotations, right-click the selection, and select **Add to a File** on the shortcut menu.
2. In the window that appears (Figure 308), click **Create New**.

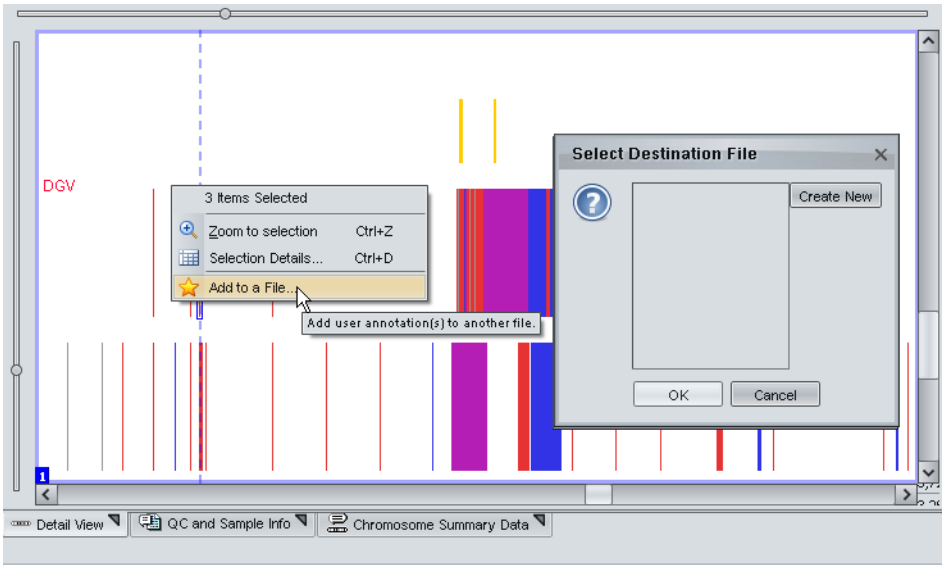


Figure 308 Detail View, create a new AED annotation file.

3. In the **Create** window (Figure 309), click to select a folder, then enter a file name.

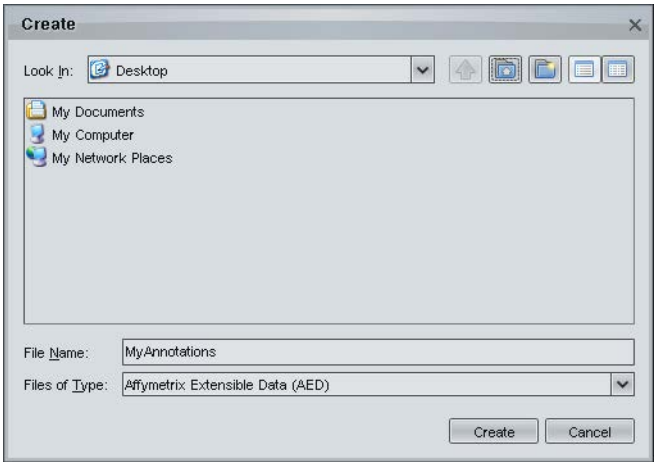


Figure 309 Create window.

4. Click **Create**.

   The **Select Destination File** window appears and displays the name of your new AED file. (Figure 310)



Figure 310 Select Destination File window.

5. Click **OK**.

   The Details View shows the new annotation (AED).

Note: The AED file is automatically assigned the same genome assembly version (that is, "hg18", "hg19", etc.) as the currently loaded NetAffx™ annotations.

Add annotations to an AED file

1. In the Detail View, use one of the following methods to select the annotation(s) you want to add to an AED file:
   – Right-click an annotation and select **Add to a File** on the shortcut menu
   Or
   – Draw a box around multiple annotations, right-click the selection, and select **Add to a File** on the shortcut menu.
2. In the **Select Destination File** window, select an AED file, then click **OK**.

Note: Adding annotations to an AED file does not modify the genome assembly version. If the AED file does not specify a genome assembly version, none is automatically assigned to the AED file when annotations are added.

AED file of regions

Note: You may want to incorporate new segments of features from a set of samples in a new Regions file. You can also use the Export feature to export data in existing files to an AED file. See "Export information in AED or BED format" on page 296.

Create a new CytoRegions file in AED file format

1. From the View menu, choose **Select CytoRegions** file or **Select Overlap Map**.
   The appropriate Select File window appears. (Figure 311)

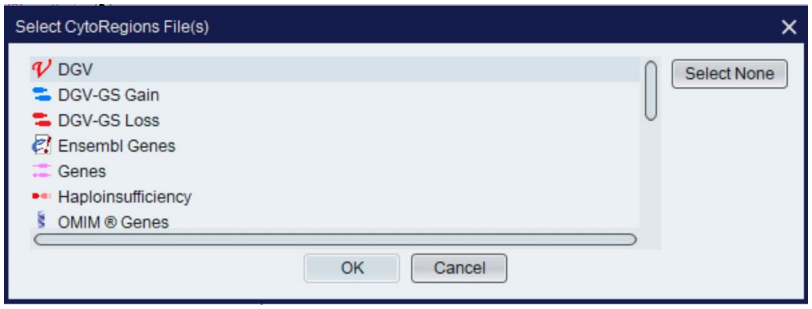


Figure 311 Select CytoRegions file window.

Note: You can also create a new AED file when adding a region to an AED file.

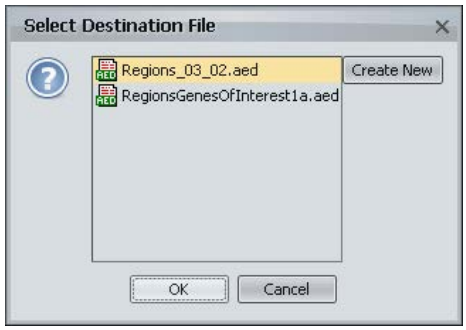


Figure 312 Select Destination File window.

2. Click **Create New** in the Select File window.

   The **Create** window appears. (Figure 313)

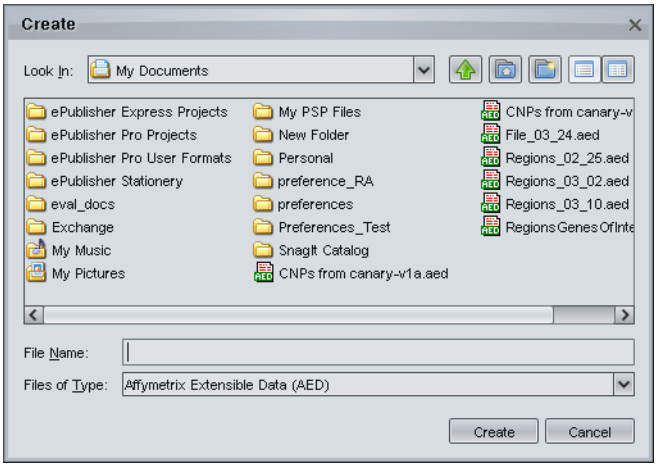


Figure 313 Create window.

3. Use the window controls to browse to a folder for the AED file.
4. Enter a file name.
5. Click **Create** in the Create window.

   The Select File window appears with the new file selected. (Figure 314)

Note: The AED file is automatically assigned the same genome assembly version (that is, "hg18", "hg19", etc.) as the currently loaded NetAffx™ annotations.

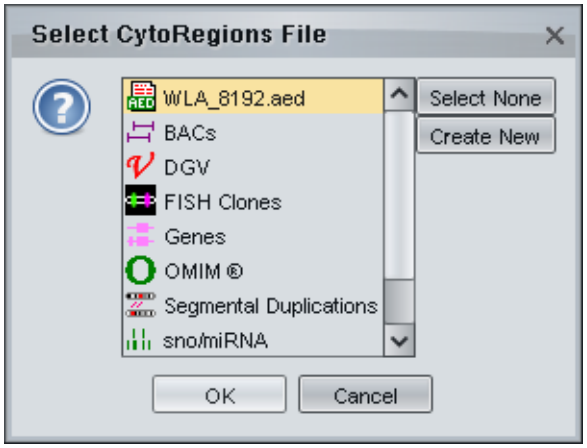


Figure 314 Select Destination File.

You can select the new file or add regions to it, depending upon what function you were performing initially.

Note: To open an AED file, click the icon or select **File → Open** on the menu bar.

Add regions to a new or existing AED file

You can add a new region to either an existing AED file or by creating a new AED file by selecting the following features in the ChAS graphic views:

- Detected Segments
- Reference Annotations
- Regions in previously loaded files.

Note: You can edit the color of annotations that have been added to an AED file by creating a color rule (see "Create a color rule" on page 291). Alternatively, you can edit the color of a particular DGV annotation added to an AED file, in the Detail View (see "Enter general information" on page 279).

Add a section to a new region (AED) file

1. Right-click any of the following feature types in the graphic displays and select **Add to a Copy to a File...** from the right-click menu (Figure 315):
   - Segment
   - Reference Annotation (including Cytobands)

   Note: You should expand the reference annotations before selecting one to add to an AED file to avoid selecting multiple annotations. (See "Expand and contract annotations" on page 183.)

   - Region

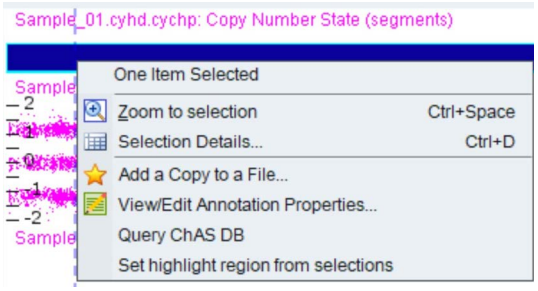


Figure 315 Right-click menu.

The **Select Destination File** window appears. (Figure 316)

Note: Some options may not be available, depending upon the number of type of items you have selected.

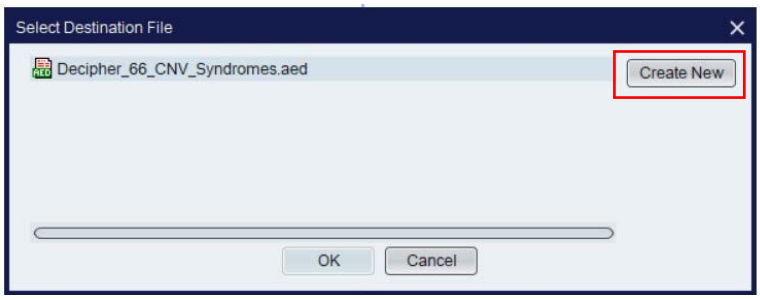


Figure 316 Select Destination File window.

The Select Destination File window displays a list of the currently existing AED files to which you may add the segment.

2. Select the region file you want to use, then click **OK**.
3. Optional: To start a new AED file, click the **Create New** button (Figure 316), then enter a name, choose a destination to save the new AED file, then click **Create**.

Note: The **Annotation Properties** window opens if you have selected a single item (Figure 317). If you have selected multiple items, the **Annotation Properties** window does not open.

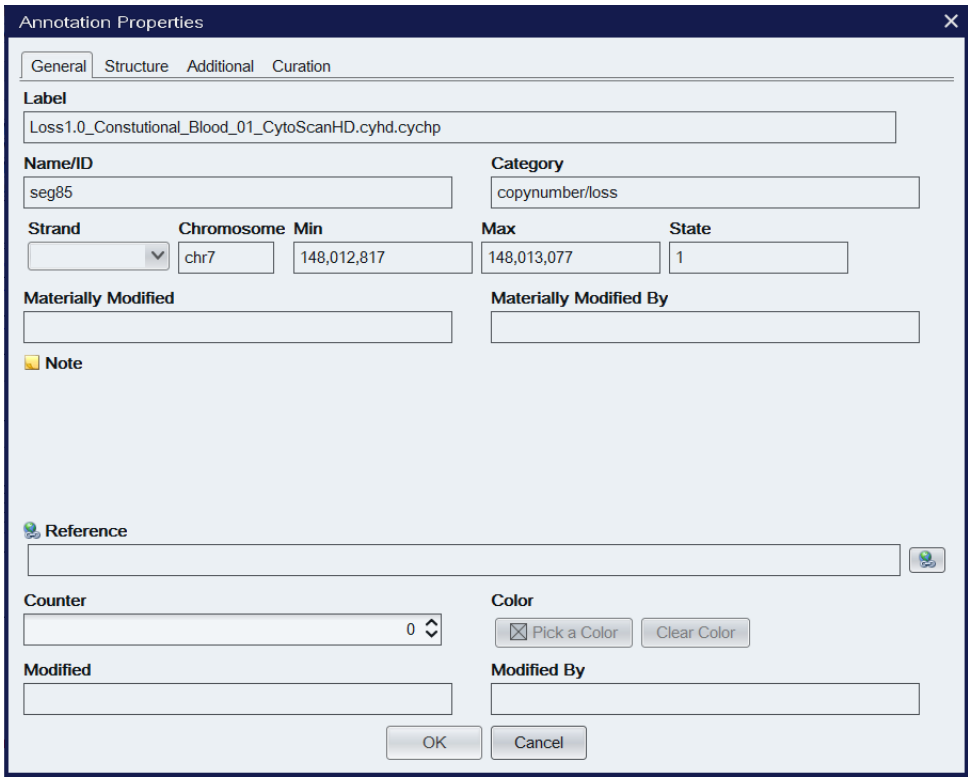


Figure 317 Annotation Properties window.

See "View and edit annotations" on page 278 for information on editing the annotations.

4. After editing the annotation properties, click **OK**.

The section is saved in the Region file.

Delete regions from an AED file

1. Right-click a region in a region file and select **Delete Annotations(s)** (Figure 318).

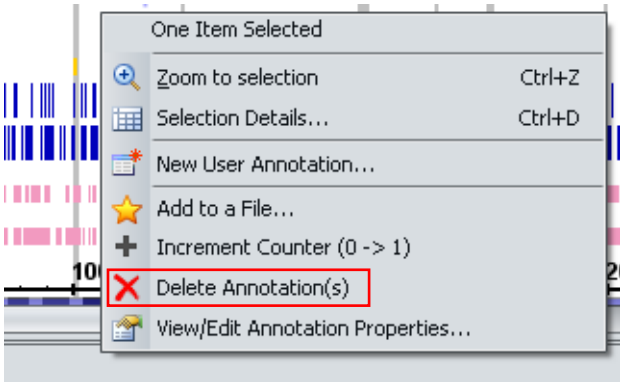


Figure 318 Right-click menu for region.

The region is deleted from the Region (AED) file.

View or edit AED file properties

In ChAS, you can:

- View AED or BED file properties
- Edit AED file properties (modify, add, or delete properties)

Note: BED files cannot be edited in ChAS. For more information on editing BED files, see "Edit BED files" on page 447.

View file properties

1. Right-click a file, then click to select **View/Edit Properties**.

   The **File Properties** window appears.

2. Click the **Extended** tab.

   A table with the file properties appears. (Figure 319 on page 276)

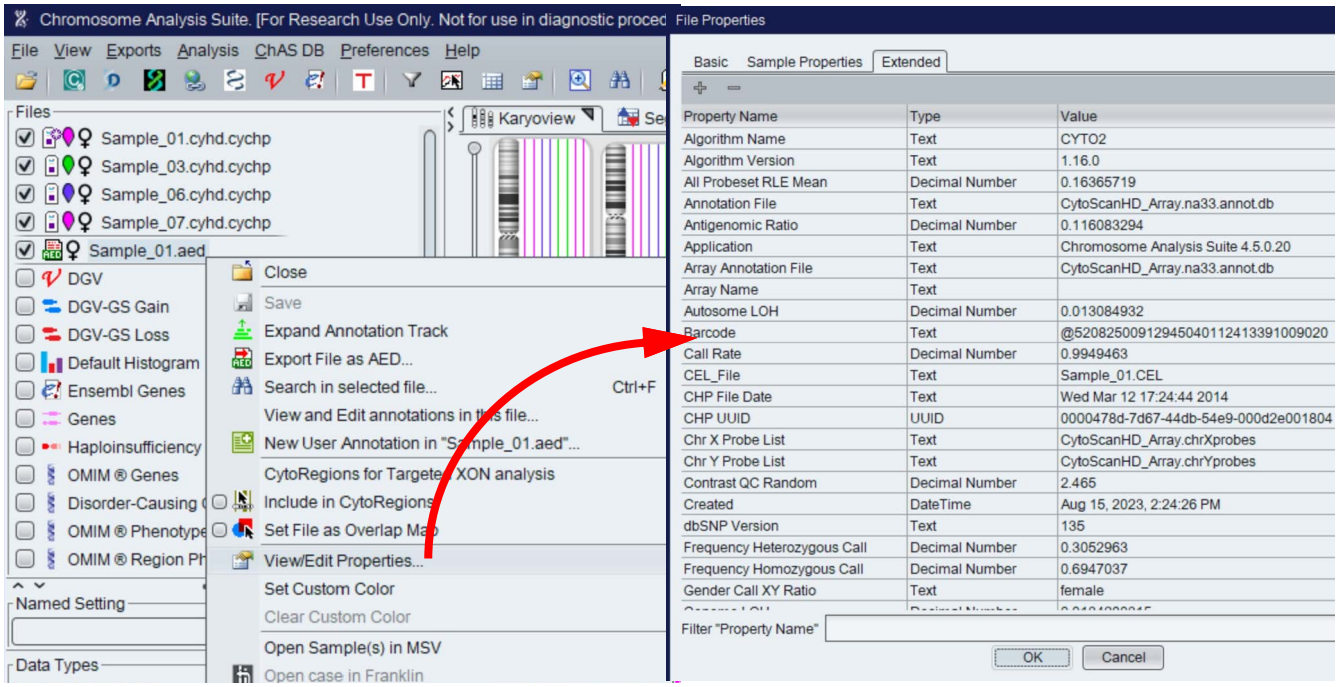


Figure 319 View AED or BED file properties.

View the genome assembly version

The assigned genome assembly version of a loaded file can be viewed in the Properties box (Figure 319). The property, if it has been set for the file, is shown as "ucscGenomeVersion(Affx)". An AED file that is created within ChAS is automatically assigned the same genome assembly version as the loaded NetAffx™ annotations (for example, "hg19"). If you add annotations to an existing AED file, its genome assembly version will not be modified; and if no version is specified for the AED file, no version is assigned to it.

Note: When you save a file as AED or BED, the current value of the genome assembly version property, if present, is saved in the file. If 2 or more files are merged into an AED or BED file, the current value of the genome assembly version, if present in at least 1 of the files, is saved in the merged file.

If an AED file does not include a genome assembly version, you can manually set it. To do this, in the Properties window:

1. Click **+** to add a new property row, as shown in Figure 320 on page 277.
2. Select the Property Name **ucscGenomeVersion(Affx)** from the dropdown list.
3. Select **Text** under the Type dropdown list.
4. In the **Value** column, enter the genome assembly version (for example, "Constitutional").

Note: You can manually set the genome version of an AED file by editing the "ucscGenomeVersion(Affx)" property. For more details on editing a property value, see "Edit a property value" on page 278.

Add a property

1. In the **Properties** window, click the ✚ symbol to create a blank row (Figure 320).
2. In the **Property Name** field, enter a property name or make a selection from the Property Name dropdown list. (Figure 320)

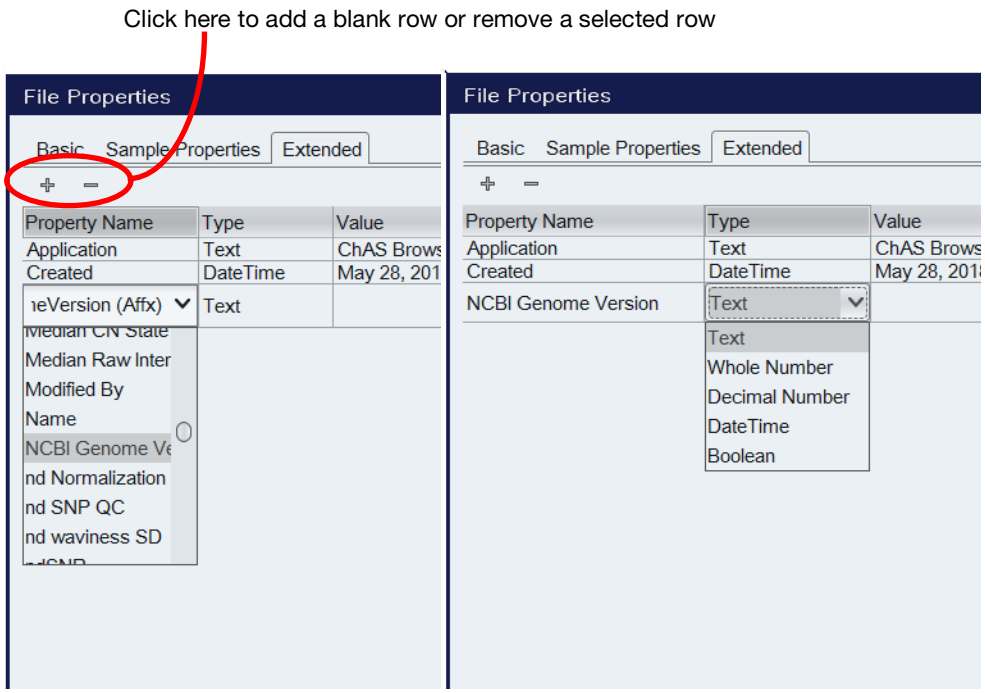


Figure 320 Specify a property name and type.

3. Click **Type**, then make a selection from the dropdown list.
4. Click **Value**

   A cursor appears inside the Value field. (Figure 321)

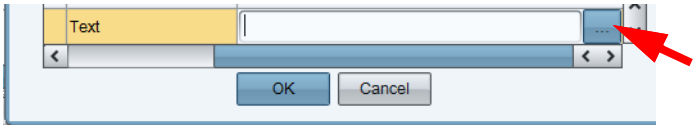


Figure 321 Value field.

5. Enter a value, then click **OK**. For significantly longer values, click ••• (Figure 321) to open a Value editing window. Enter your (longer) value in this window, then click **OK**.

   The new value is entered in the File Properties table.
6. Click **OK**.

Remove a property

1. In the Properties window, select the row that you want to delete.
2. Click the ▬ icon.

Edit a property value

1. Right-click the file and select **View/Edit Properties** on the shortcut menu.

   The File Properties window appears. (Figure 322)
2. Click a row to select it.

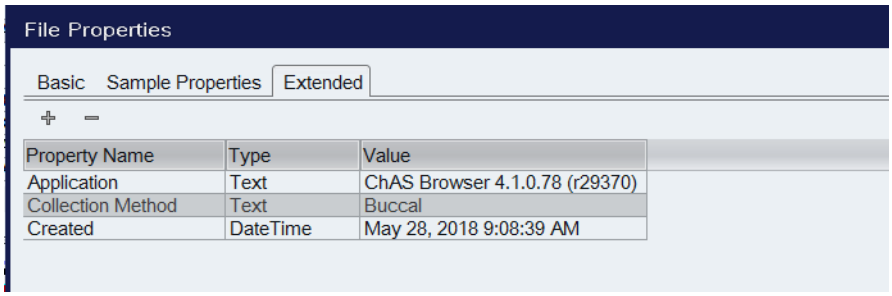


Figure 322 Select a property to edit.

For example, click the **Value** field. (Figure 323).

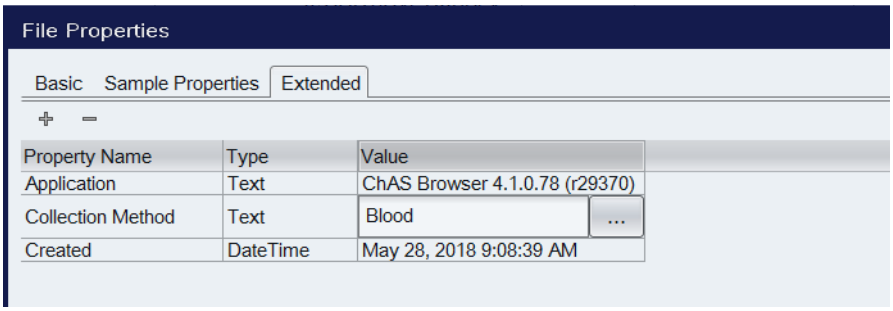


Figure 323 Set a new property value.

3. Enter a value, then click **OK**. For significantly longer values, click  to open a Value editing window. Enter your (longer) value in this window, then click **OK**.

   The new value is entered in the **File Properties** table.
4. Click **OK**.

View and edit annotations

The Annotation Properties window opens:

- When adding a region to a Region (AED) file
- When you select View/Edit Annotation Properties from the right-click menu (Figure 324) for the following types of features:
  - Detected Segments
  - Reference Annotations (view annotations only)
  - AED Annotations (view and edit annotations)

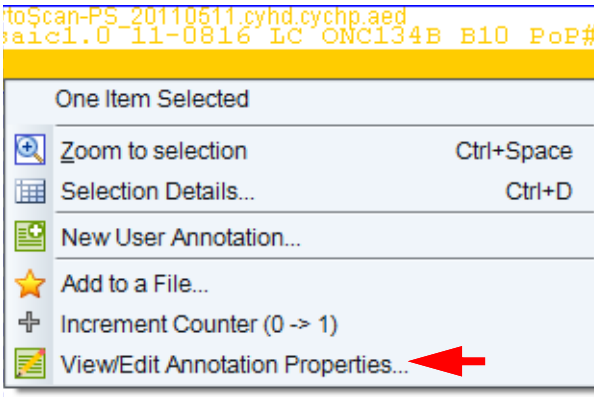


Figure 324 Right-click menu for View/Edit Annotation Properties in the Detail View.

Note: The **View/Edit Annotation Properties** menu option is not available if you have more than 1 feature selected in the Detail View.

The Annotation Properties window has 3 tabs:

- **General** (See "Enter general information" on page 279.)
- **Additional** (See "Add Properties" on page 280.)
- **Curation** (See "Add a curation (optional)" on page 284.)

You can also create new user annotations if you select an element. This feature enables you to create a region that is not based on a segment or reference annotation. See "Create a New User Annotation" on page 285.

Enter general information

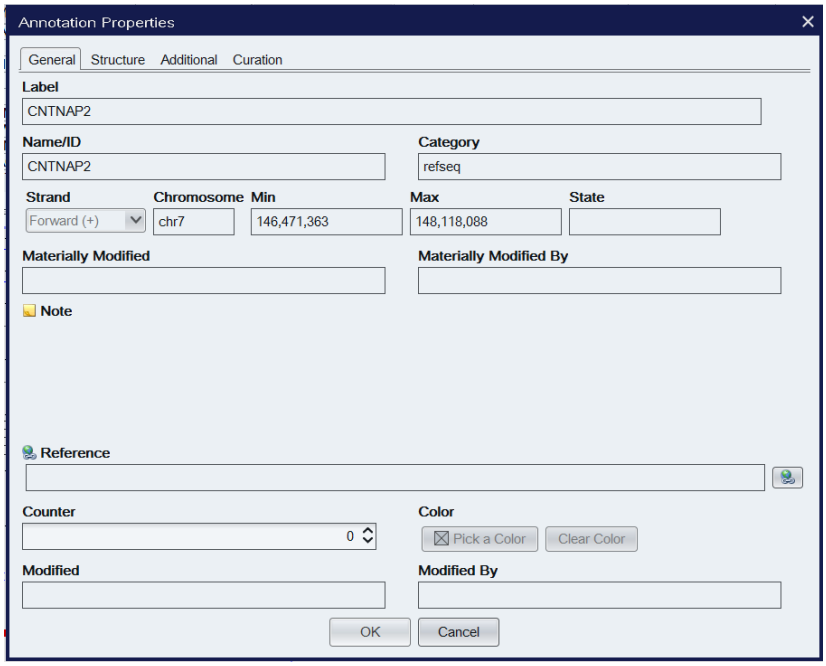


Figure 325 Annotation properties window of CHP Segment exported to AED.

Edit the General tab properties in AED annotations

1. In the **General** tab (Figure 325), enter, edit, or select:

Table 32 AED File Annotations.

Annotation	Description
Label	Label given to the AED, CHP, or other annotation. For annotations originating from CHP segments, can be the Type, State, and Filename of the CHP file. The Label is not editable.
Name/ID	Name/ID assigned to the annotation in the AED, CHP or other file.
Category	Information on the source of the region. If the region was added by selecting a CHP segment, the segment type is saved.
Strand	The Sequence Strand of the item.
Chromosome	Cannot be edited in Annotation Properties box. See "Create a New User Annotation" on page 285.
Min	The smallest of the annotation's chromosomal coordinates.
Max	The largest of the annotation's chromosomal coordinates.
Materially Modified Time	Time stamp of when the start or end coordinate, type, or state of the segment or BED/AED annotation was last altered.
Materially Modified By	The Operating System user and ChAS User IDs of the Modifier who last changed a Material property (start or end coordinate, type, or state) of the segment or annotation.
Note	Information and comments about the region. **Note:** Always use alphanumeric characters and underscores. Avoid the use of odd characters. (Examples: & + ( ) [ ] { } ~ ^ and commas.)
Reference	URL/web address associated with the annotation. Click  to link directly to the Reference from the Annotation Properties window. Internet connection is required.
Modified	The time stamp of the last modification of the annotation.
Modified By	The Operating System user and ChAS User IDs of the Modifier who last changed the annotation.
Counter	enables you to track the number of times something has been seen.
Color	Allows assignment of a hard-coded color to the Annotation in ChAS's graphical views.

Customize properties

Add Properties

In the **Additional** tab you can enter new annotation information for an AED annotation. The information is displayed in the:

- Tool tip when you position the mouse arrow over an annotation in the Details View
- Selection Details window

Add custom properties

1. In the Annotation Properties window, click the **Additional** tab. (Figure 326 on page 281)

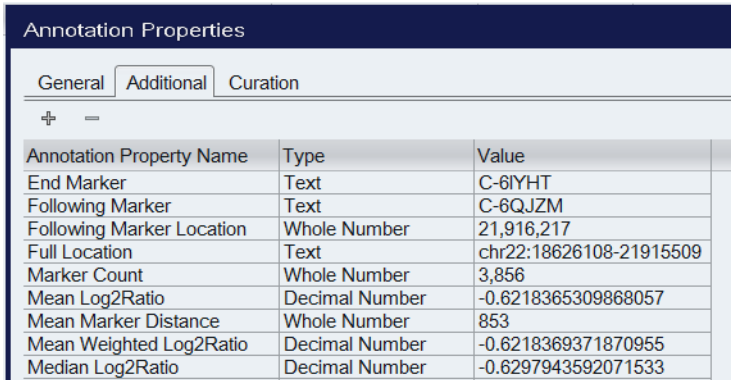


Figure 326 Additional Annotation Properties tab.

Additional annotations	Description
Annotation Property Name	Name assigned to the property.
Type	Type assigned to the property.
Value	Value assigned to the property.

Note: Different default properties may already be assigned to the annotations added from reference annotations.

2. Click the + button at the top of the table.

   A new row appears in the table. (Figure 327)

   If needed, click a column's edge, then drag it (right) to make it wider.

   You can delete a property by selecting a row, then clicking the − button.

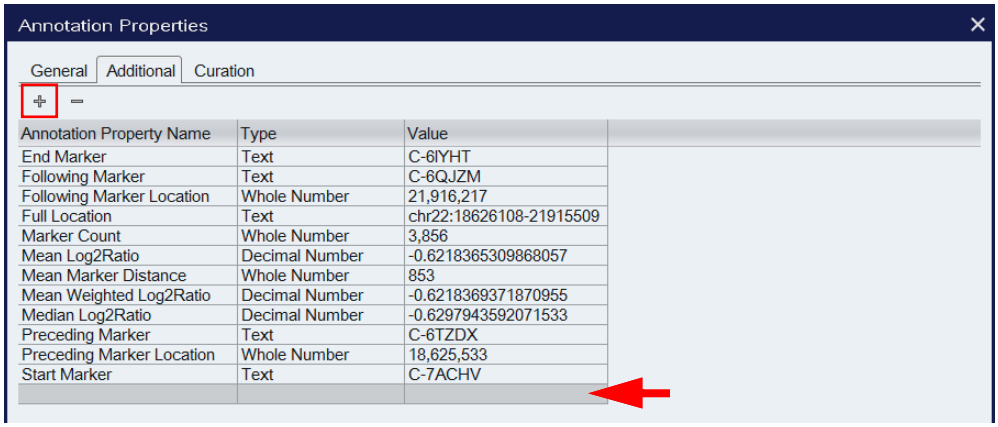


Figure 327 New property row added.

3. Click in the row under the Property Name column and enter a name for the property. Note that your new entry is followed by the text "(custom)". (Figure 328)

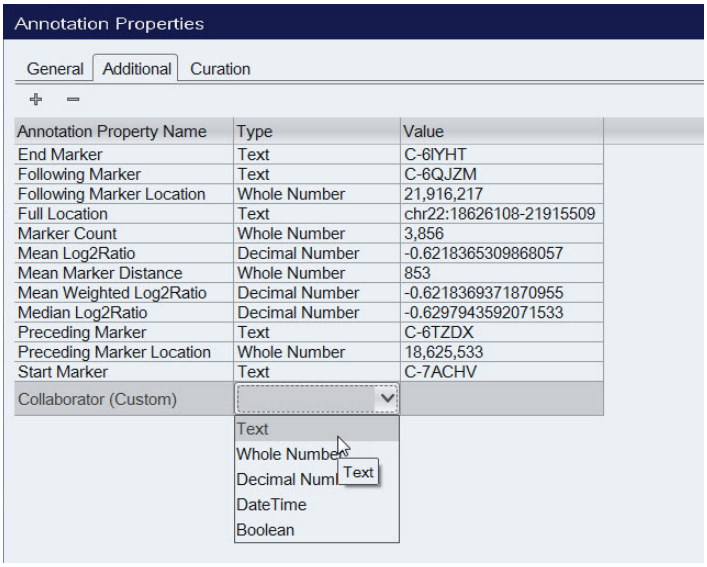


Figure 328 Entering a custom property name and selecting its Type.

4. Click in the row under Type and select a property type from the dropdown list (Figure 329).

   Choose from:

   - Text
   - Whole Number
   - Decimal Number
   - Date Time
   - Boolean

5. Click in the row under **Value** and enter the value (Figure 329).

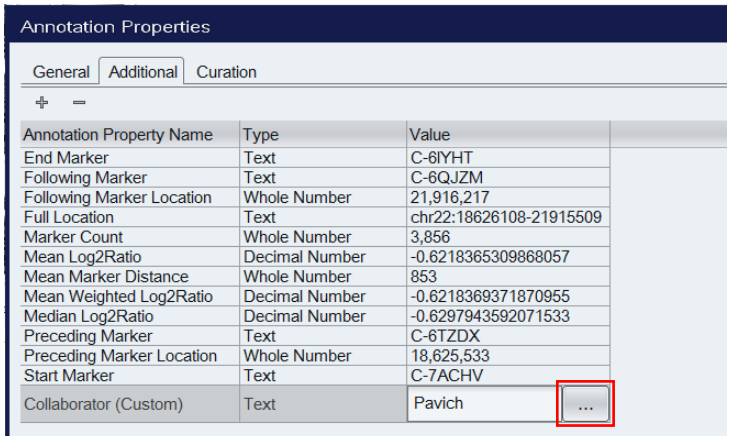


Figure 329 Enter a value for the property type.

Alternatively, click the **Browse** button (Figure 329), then in the **Value** window (Figure 330) that appears, enter the property value, then click **OK.**

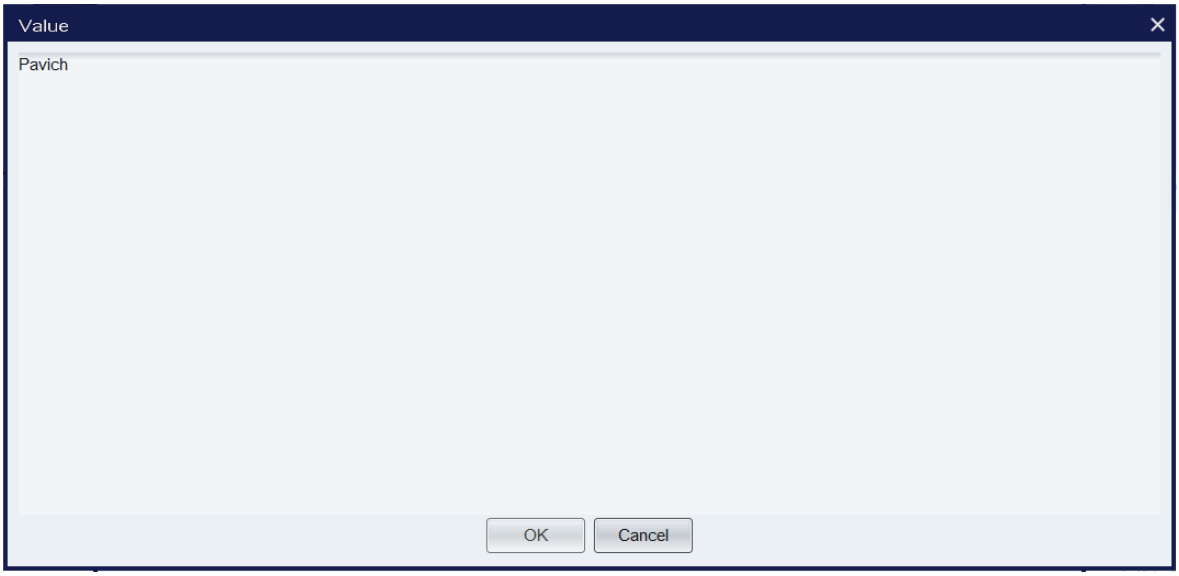
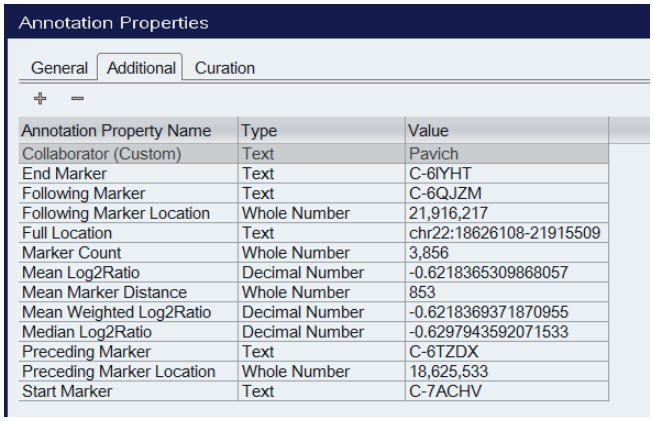


Figure 330 Value window.

The property entry is completed. (Figure 331)



Annotation Property Name	Type	Value
Collaborator (Custom)	Text	Pavich
End Marker	Text	C-6IYHT
Following Marker	Text	C-6QJZM
Following Marker Location	Whole Number	21,916,217
Full Location	Text	chr22:18626108-21915509
Marker Count	Whole Number	3,856
Mean Log2Ratio	Decimal Number	-0.6218365309868057
Mean Marker Distance	Whole Number	853
Mean Weighted Log2Ratio	Decimal Number	-0.6218369371870955
Median Log2Ratio	Decimal Number	-0.6297943592071533
Preceding Marker	Text	C-6TZDX
Preceding Marker Location	Whole Number	18,625,533
Start Marker	Text	C-7ACHV

Figure 331 Property entry completed.

6. Click **OK** in the Annotation Properties window.

Add a curation (optional)

1. Click the **Curation** tab. (Figure 332)

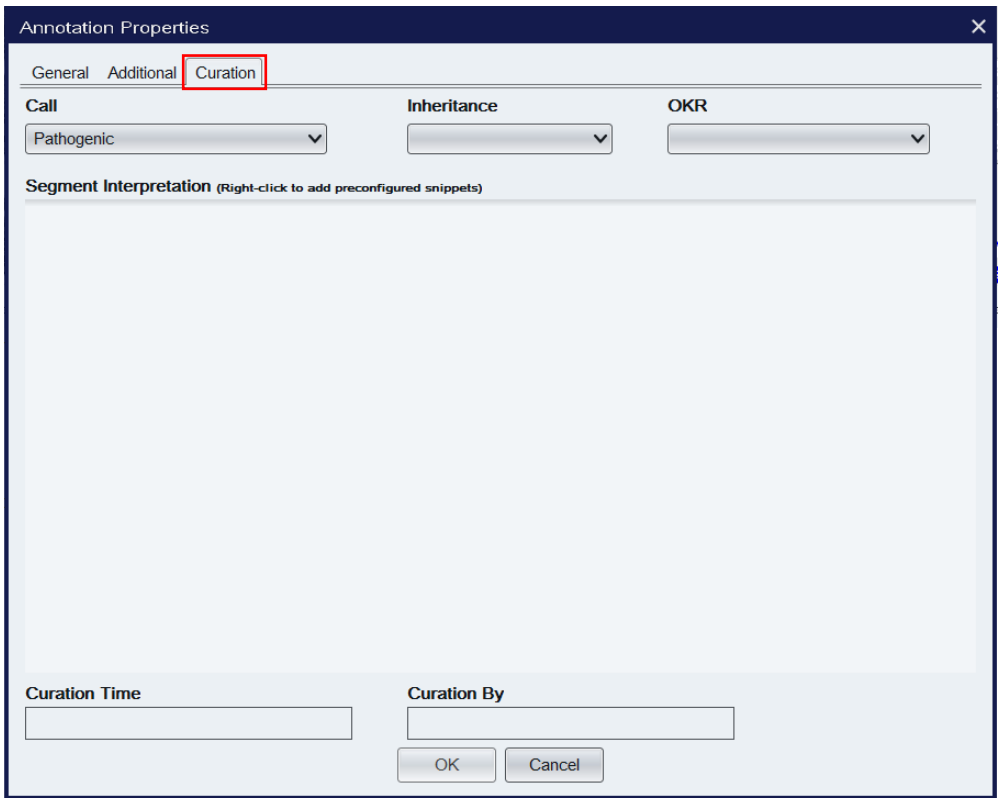


Figure 332 Curation tab.

2. Click the **Call** dropdown list to select a Call. (Figure 333)

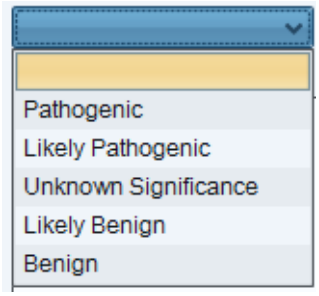


Figure 333 Call dropdown list.

3. Click to select an appropriate Call.
4. If needed, (in the Interpretation field) type in your interpretation or right-click to add a pre-configured snippet.
5. Click the **Inheritance** dropdown list to select an Inheritance mode.
6. Click the **OKR** dropdown list to select an OKR annotation.
7. Click **OK**.

Create a New User Annotation

You can create a new annotation from an AED annotation.

1. In the Details view, right-click an AED annotation and select New User Annotation on the shortcut menu. (Figure 334)

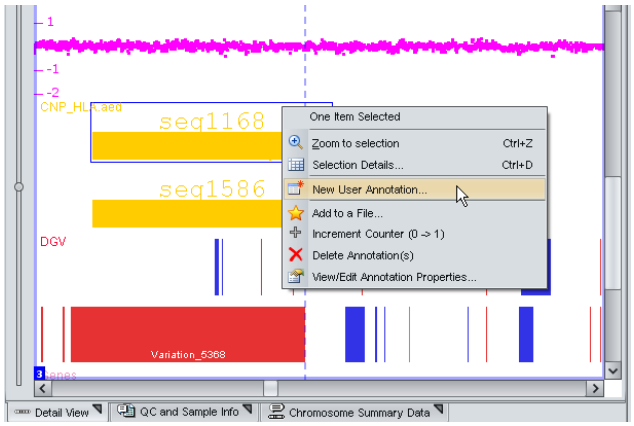


Figure 334 Detail View.

2. In the window that appears, enter the annotation information (Figure 335). For more details, see "Enter general information" on page 279 and "Customize properties" on page 280.

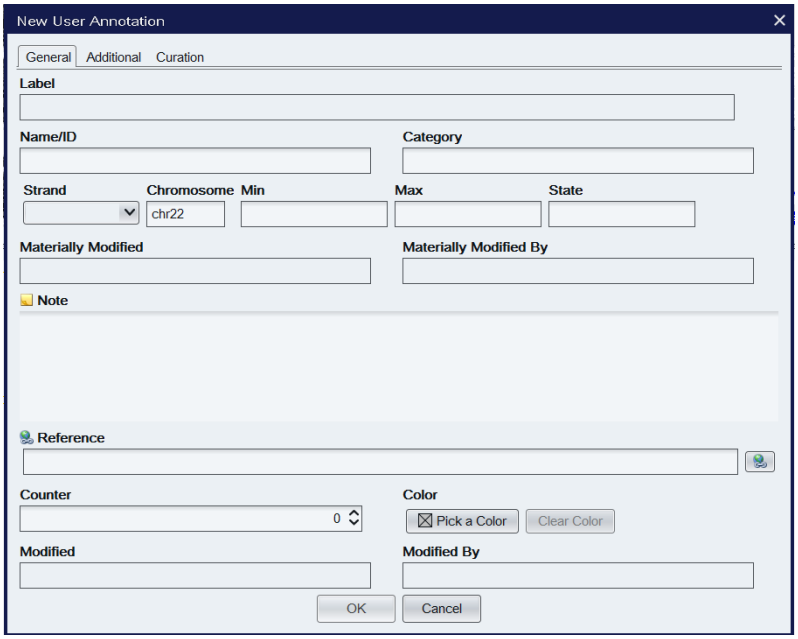


Figure 335 Enter information for the new annotation.

3. Click **OK**.

The new user annotation is created and saved in the AED file.

Note: The default New User Annotation information includes only the chromosome number. It does not include any information or properties associated with the AED annotation.

View and batch edit AED file contents

The AED Editor enables you to view multiple AED annotations and their properties in a table format and edit the values of properties easily - for single annotations or to edit a property for multiple annotations in a batch - to a new value.

1. At the Files pane (top left), right-click the AED file you want to view/edit.

   A pop-up menu appears. (Figure 336)

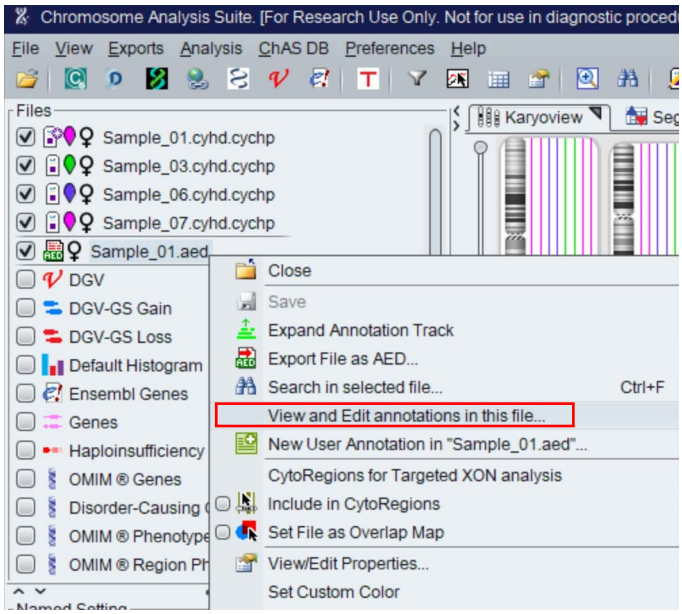


Figure 336 AED file - Right-click menu.

2. Click **View and Edit annotations in this file**.

   The **AED File Editor** table window appears. (Figure 337)

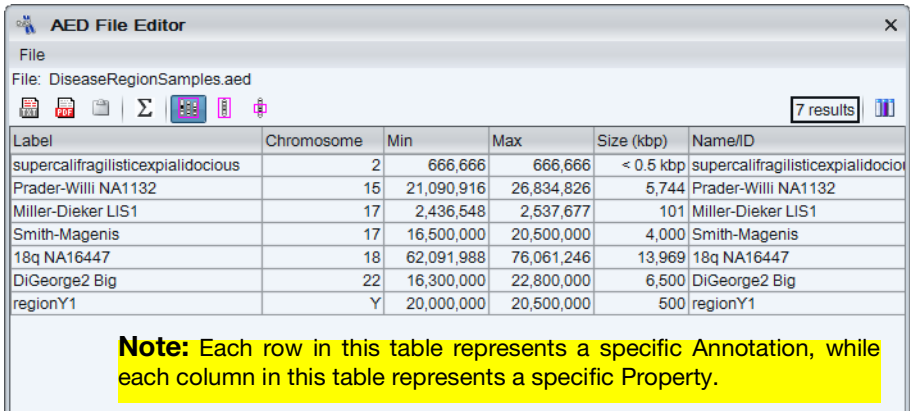


Figure 337 AED File Editor table.

Note: The AED File Editor table displays ALL properties and tabs of every annotation contained in an AED file.

Use the scroll bar to move the table to the right to see more column entries and/or click, then drag the AED File Editor table window to make it wider.

To locate your table data faster, click any appropriate column header to sort in a descending or ascending order. Also, the number of columns displayed in the Select Columns window, varies with each AED file.

3. Locate the group of editable annotations (rows) from a column (property) you want to batch edit (combine together) as a single annotation.
4. Shift+click or Ctrl+click to select (highlight) each annotation (row) entry. (Figure 338).

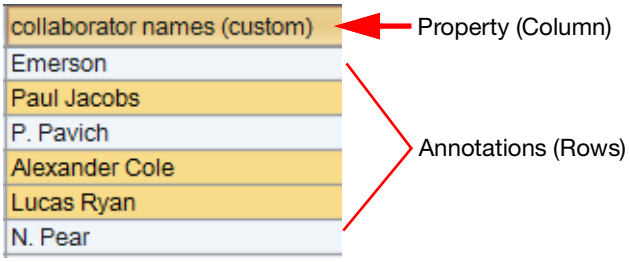


Figure 338 Selection of multiple annotations.

5. Right-click a newly selected annotation.

   The following menu appears: (Figure 339)

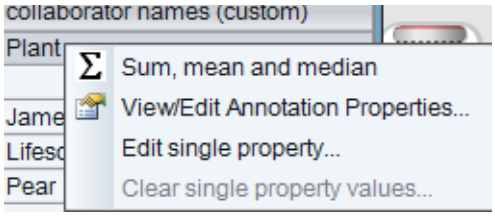


Figure 339 AED table field (right-click menu).

IMPORTANT! Only editable fields can be edited. If a field is non-editable, the Annotations Property Window pops-up. This window displays which fields are not editable (grayed out) versus those that are editable. Also, not all user-editable AED file fields may currently be edited from within the AED Editor. Some basic values (start, stop, type) cannot be edited in the AED Editor table directly. You MUST use "View/Edit Annotation Properties..." for editing the particular field in the annotation of interest.

6. Click **Edit single property**.
7. The following window appears: (Figure 340)

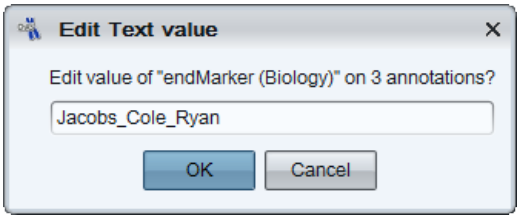


Figure 340 Edit Text Value.

8. Enter an appropriate label to distinguish your new batch annotation entry, then click **OK**.

9. Your batch (group) of annotations appear as follows: (Figure 341)

Before Batch Editing

collaborator names (custom)
Emerson
Paul Jacobs
P. Pavich
Alexander Cole
Lucas Ryan
N. Pear
Thorberg, Southkey

After Batch Editing

collaborator names (custom)
Emerson
Jacobs_Cole_Ryan
P. Pavich
Jacobs_Cole_Ryan
Jacobs_Cole_Ryan
N. Pear
Thorberg, Southkey

Figure 341 Batch edit results - Before and After.

Protect an AED file

Protecting an AED file provides a warning prior to adding an annotation to an AED file. This is an optional field and is intended to double-check the intention of adding an annotation to the selected AED file.

1. Right-click the AED file in the File Tree, select **View/Edit Properties**.
2. In the **Basic** tab window, select the **Protect File** checkbox, as shown in Figure 342.

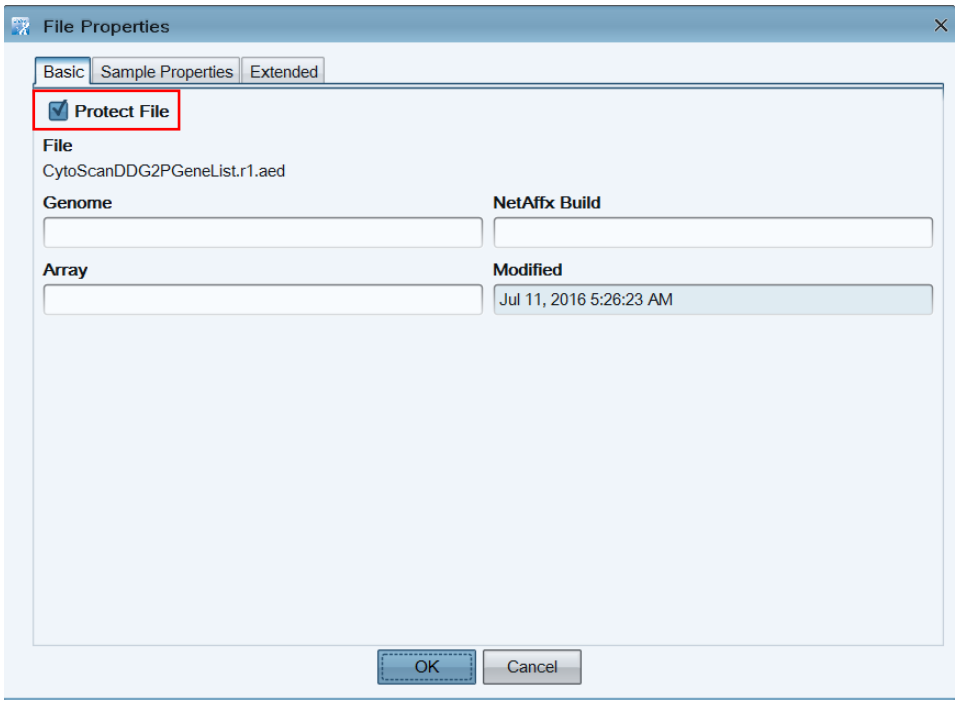


Figure 342 Basic window tab - Protect File option.

When adding annotations to a Protected AED file, the following warning message appears. (Figure 343)

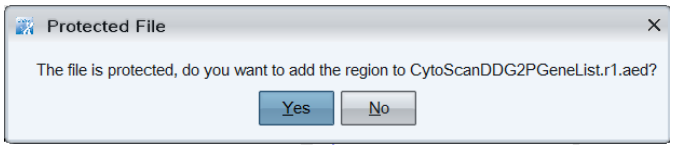


Figure 343 Protected File message.

3. Click **Yes** to acknowledge the message, then click **OK** to add the annotation to the AED file.

   Click **No** to return to the browser window without adding the annotation to the AED file.

Note: Protected AED files are noted with an asterisk, as shown in Figure 344.

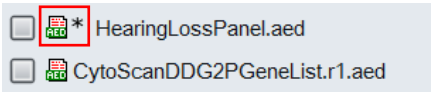


Figure 344 Protected AED file.

AED/BED color rules

The Color Rules feature enables you to set display colors for annotations in AED files by various annotation properties, depending upon the original source of the region (detected segment, reference annotation, etc.). You can color annotations using the properties of the annotations, including:

- name
- category
- markerCount
- confidence

Using the color rules, you can assign a different color to annotations with different properties, making it easier to track the different types of segment data stored in AED files. For example, you could assign different colors to different categories:

- GainMosaic
- LossMosaic
- Gain
- Loss
- LOH

You can also perform comparisons for numerical values, coloring only values above or below a certain level.

By default, ChAS displays the regions in an AED or BED file in a single color. Do one of the following to edit annotation color:

- Select a new default color for all AED or BED files (page 290).
- Create a color rule that specifies a color for annotations which meet user-specified requirements. See "Create a color rule" on page 291.
- In the Detail View, choose a color for a particular annotation in the Annotation Properties window. See "Change an annotation color" on page 295.

Note: An AED annotation color set in the Annotation Properties window (accessed in the Detail View) takes precedence over a color rule and the default AED/BED file color. A color rule can overwrite the AED or BED file default color.

Select a new default color for loaded AED or BED files

1. Open the **User Configuration** window (click the icon or select **Preferences** → **Edit User Configuration** on the menu bar).
2. In the Color Rules tab (Figure 345), click the AED/BED Annotation Color button.
3. In the window that appears, choose a color swatch or use the color controls to specify a color.
4. Click **OK** in the AED/BED Annotation Color window.

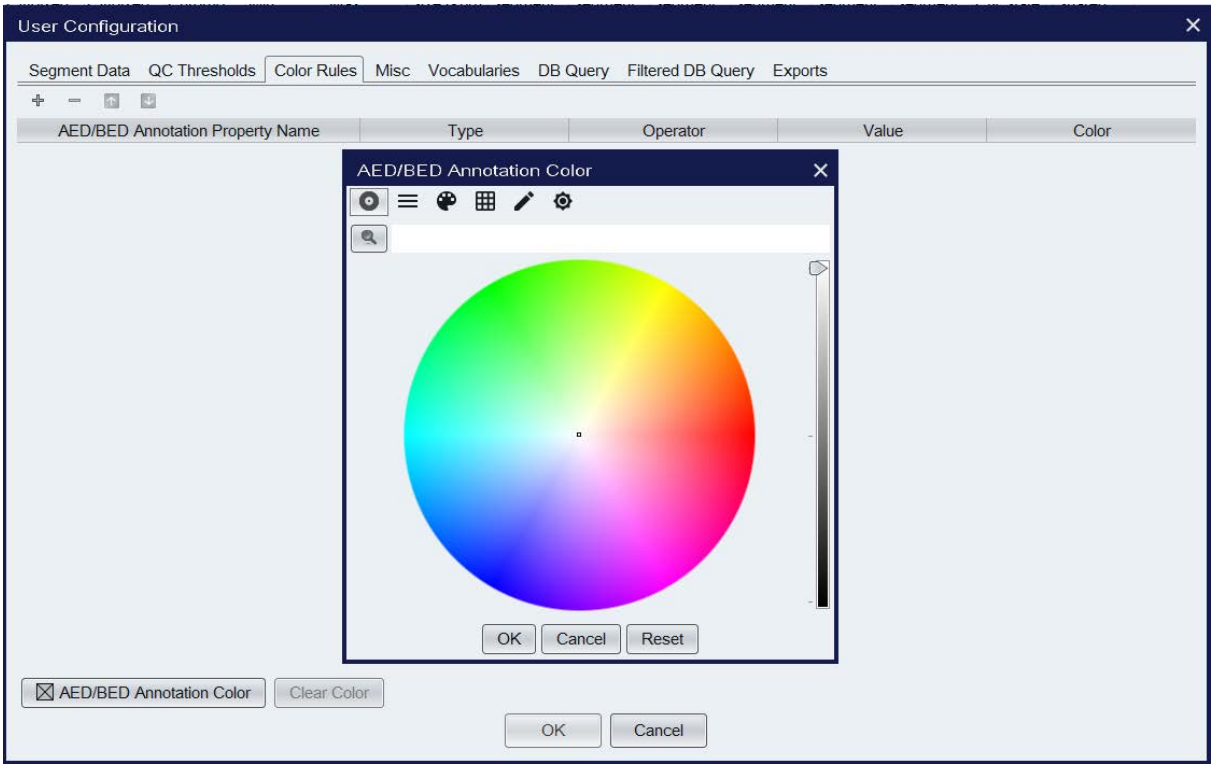


Figure 345 AED/BED Annotation Color window.

Create a color rule

1. Click the button or select **Preferences** → **Edit User Configuration** on the menu bar.
2. The **User Configuration** window appears. (Figure 346)
3. Click the **Color Rules** tab.

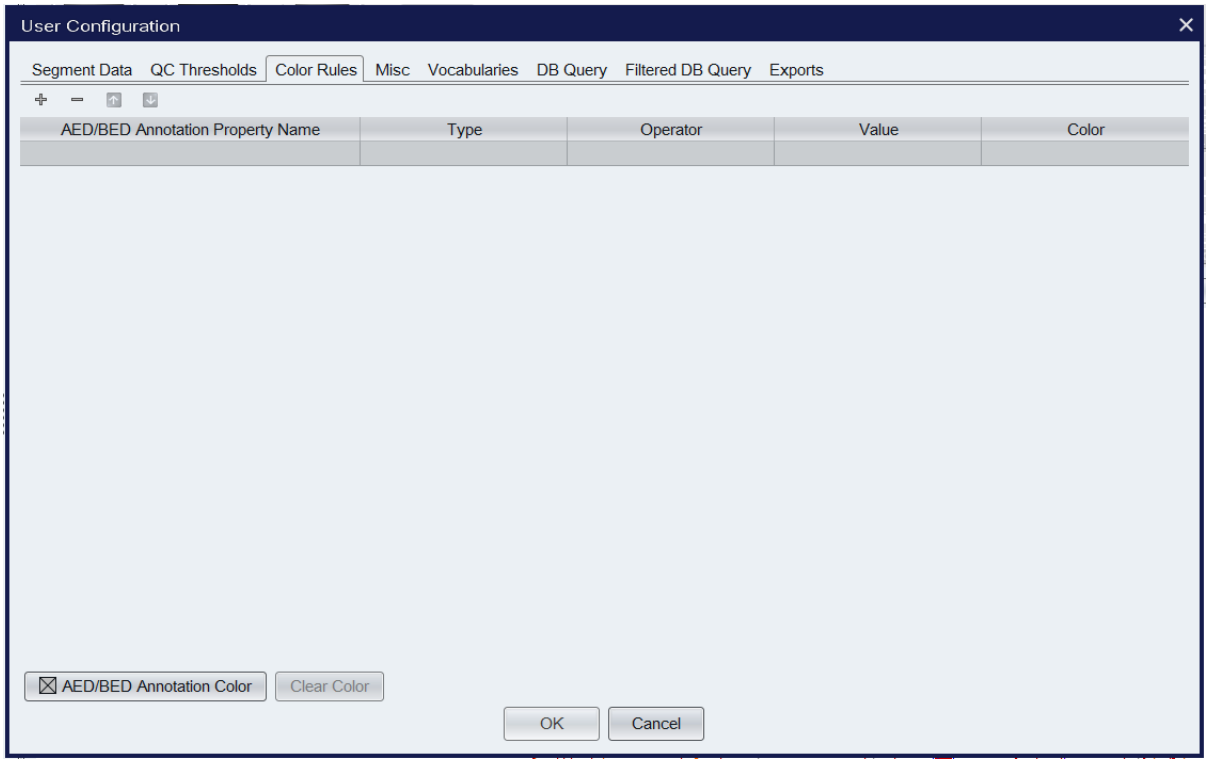


Figure 346 User Configuration window - Color Rules tab.

The **Color Rules** tab has 5 columns:

Column	Description
AED/BED Annotation Property Name	Name assigned to the property.
Type	Type assigned to the property (for example, text).
Operator	Type of comparison with value performed.
Value	Value assigned to the property.
Color	Color assigned to the region property and value.

4. Click the **Add** button at the top of the table (Figure 347).

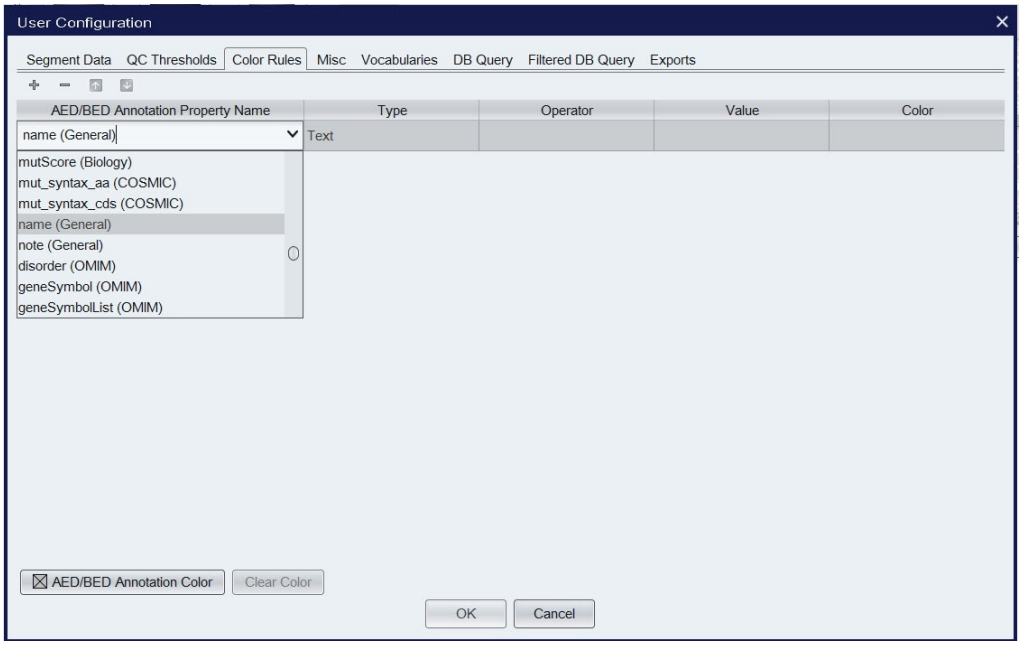


Figure 347 New row added to the Color Rules table.

A new row appears in the table.

5. Click in the row under the Property Name column and enter a name for the property or select a property from the dropdown list (Figure 348).

   To delete a property, select the appropriate row, then click the **Remove** button.

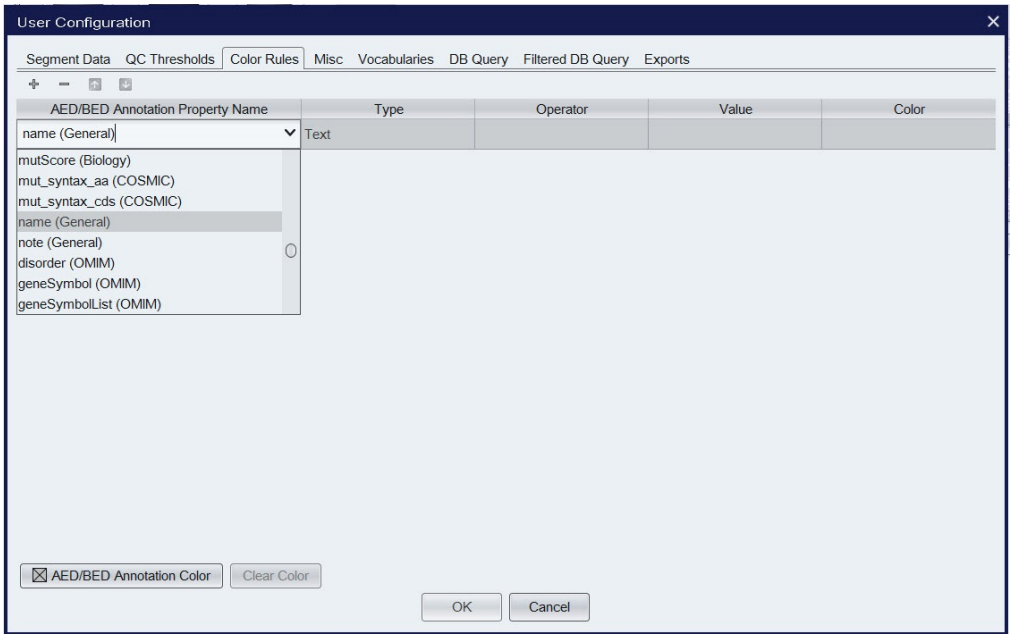


Figure 348 Enter or select a property name.

6. Click in the row under **Type**, then select a property type from the dropdown list.
7. Click in the row under **Operator**, then select an appropriate operator from the dropdown list. (Figure 349)

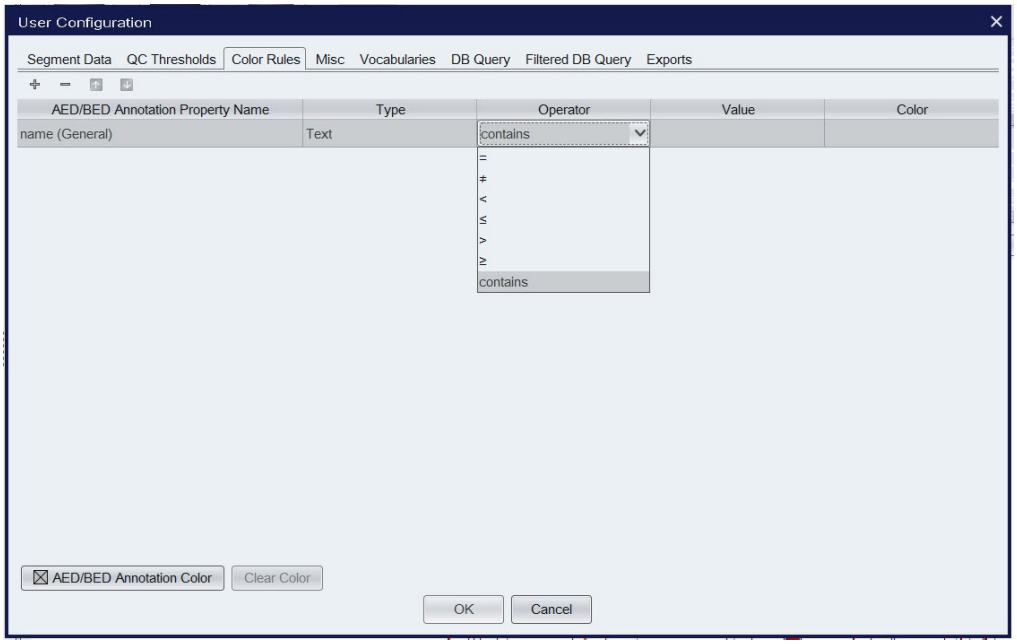


Figure 349 Selecting property type and operator for the comparison.

8. Click in the row under **Value**, then enter a value for the property.
9. Click in the row under **Color**.

   The **Pick a Color** window appears. (Figure 350)
10. Select a color, then click **OK**.

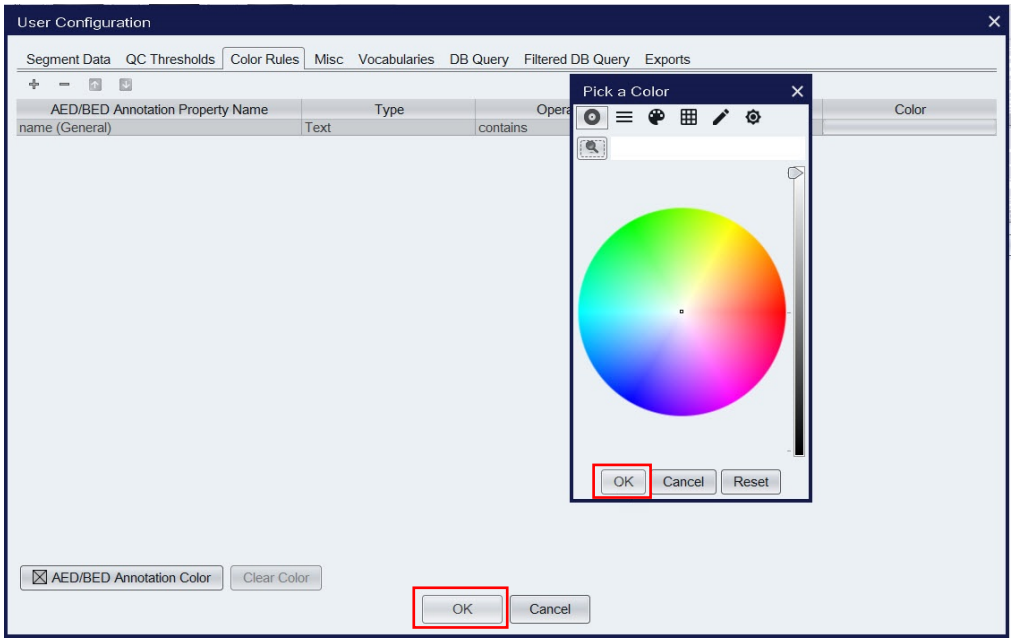


Figure 350 Property entry completed.

Your property entry is completed.

11. Click **OK** to close the **Color Rules** window.

Regions that satisfy the property comparison are displayed in your selected color for all views, as shown in Figure 351 on page 294.

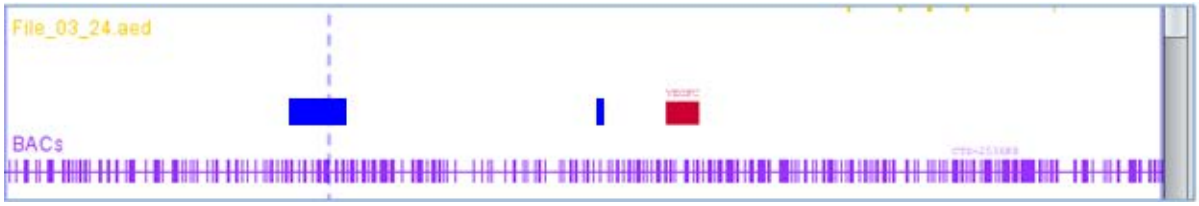


Figure 351 Regions displayed in color.

Change an annotation color

1. Click **Choose Color.**

   The **Pick a Color** window appears. (Figure 352)



Figure 352 Pick a Color window.

2. Choose a color in the Swatches tab, or click the HSB or RGB tab to define a color.
3. Click **OK**.

Note: The color set in the Annotation Property window overrides the colors specified by a Color Rule created in the User Configuration window. For more details, see "AED/BED color rules" on page 289.

Export information in AED or BED format

You can export position data for the different features to AED or BED file format. The exported BED file contains only the names and locations of the detected segments. The exported AED file contains additional information, such as header information, feature ID, and hg version (which is the same as the NetAffx Genomic Annotations Database file loaded in the Browser at export).

For more information on the AED file format, see page 439.

Note: AED or BED files created in ChAS 1.0, 1.0.1, or 1.1 do not automatically include the hg version.

Position data	Export to AED file	Export to BED file
Detected Segments for xxCHP files	Regions, names, and properties	Regions and names
Annotation Features in Reference Annotation files	Regions, names, and properties	Regions and names

Export position data as an AED or BED file

1. From the File menu, select **Export as AED…**

   Alternatively, right-click the file in the Files list and select **Export File as AED** on the menu. (Figure 352)

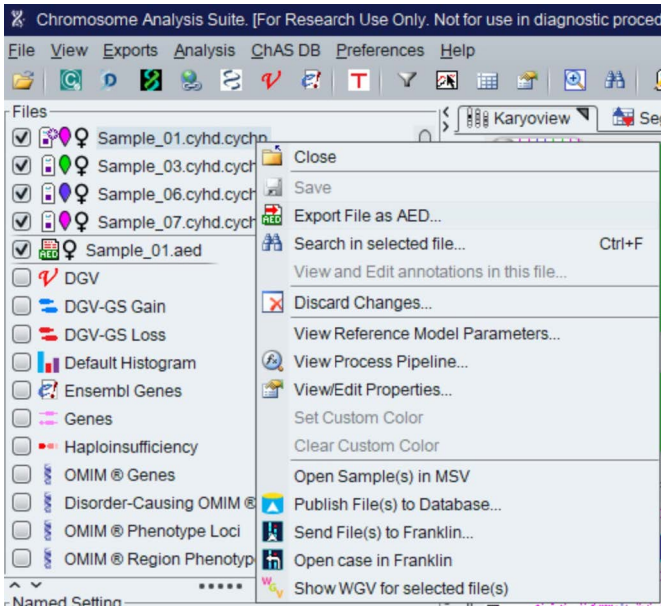


Table 33 Right-click menu.

   The **Export** window appears. (Figure 353)

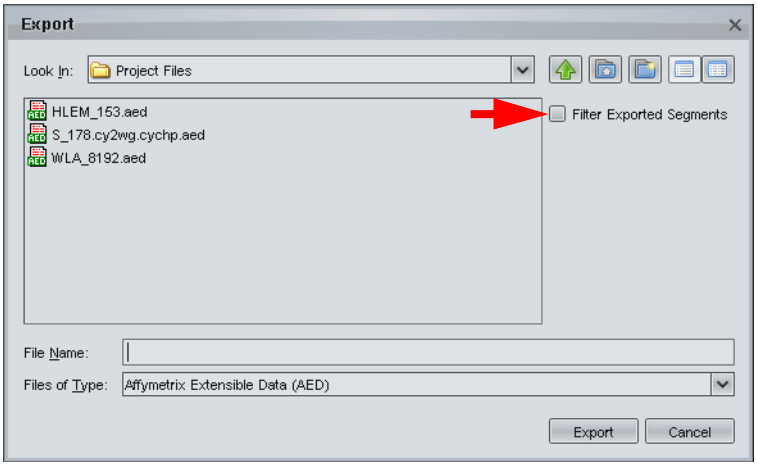


Figure 353 Export window, AED file format selected.

2. Select the **Filter Exported Segments** checkbox to restrict the export to the contents of the Segments table. If filters have been applied to the data, only the retained segments are exported. Graph data and Chromosome Summary data are not exported.

   Note: If this option is not selected, all segments which were loaded with the CxCHP file are exported along with header information, regardless of whether filters are applied. The export includes all segment data, regardless of check mark status (ON or OFF) in the Files window pane.

3. Select a folder location for the file, as you normally would.
4. To export to AED file format, enter a name for the file.

   To export to BED file format (Figure 354), enter a name for the file, then select **Browser Extensible Data (BED)** from the **Files of Type** dropdown list.

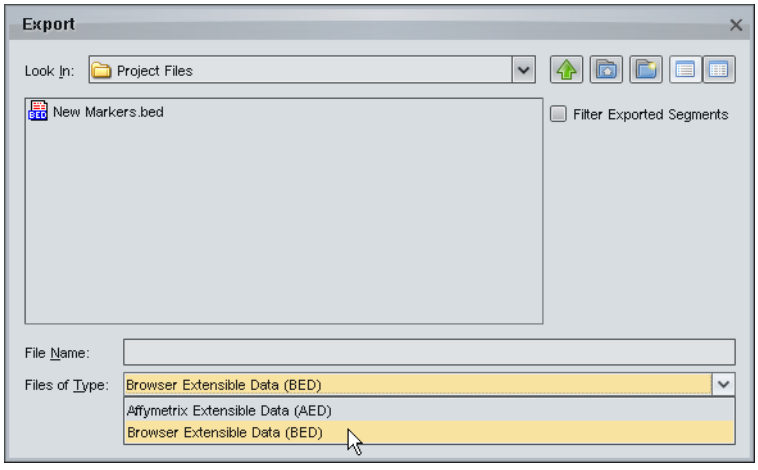


Figure 354 Export window; BED file format selected.

5. Optional: Select the Filter Exported Segments window to export only segments that meet filter criteria.
6. Click **Export** in the Export window.

   The AED file is exported and can be loaded as a region information file or sent to another user.

You can also merge feature position data from multiple different files.

Merge and export feature position information for multiple files

1. Select files in the File List by clicking them while pressing the CTRL key.
2. Right-click the selected files, then click **Merge Files to AED**. (Figure 355)

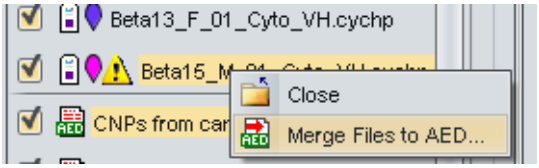


Figure 355 Files selected for merging AED outputs.

The **Export** window appears. (Figure 356)

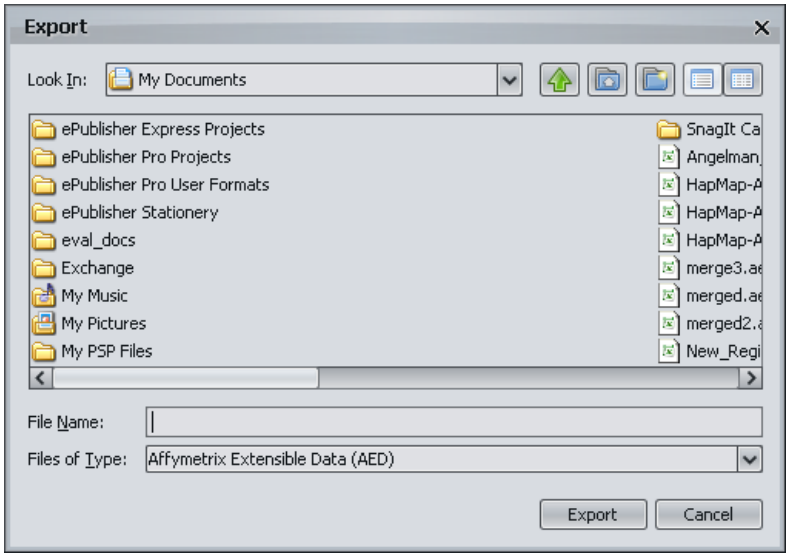


Figure 356 Export window.

3. Use the navigation controls to select a folder for the merged AED file and enter a file name for the file.
4. Click **Export**.

   The file with the merged AED region information is created and can be used as a region information file in ChAS.

IMPORTANT! After 2 AED files are merged, the original metadata in the header is not retained. Also, when 2 or more files are merged into AED/BED format, the current value of the genome assembly version property, if present in at least 1 of the files, is saved in the merged file.

Expression analysis AED file generation

AED files containing Gene Expression information and data can be created using Thermo Fisher Scientific Human Gene Expression arrays analyzed in the Transcription Analysis Console (TAC) 3.0 or higher.

For details on how to create an AED file containing Gene Expression/miRNA data, see the Gene Expression Copy Number Analysis Quick Reference Card.

View a Gene Expression AED file in ChAS

1. Load the AED file exported from TAC using **File → Open**.

   The AED is now visible in the Detail View. (Figure 357)

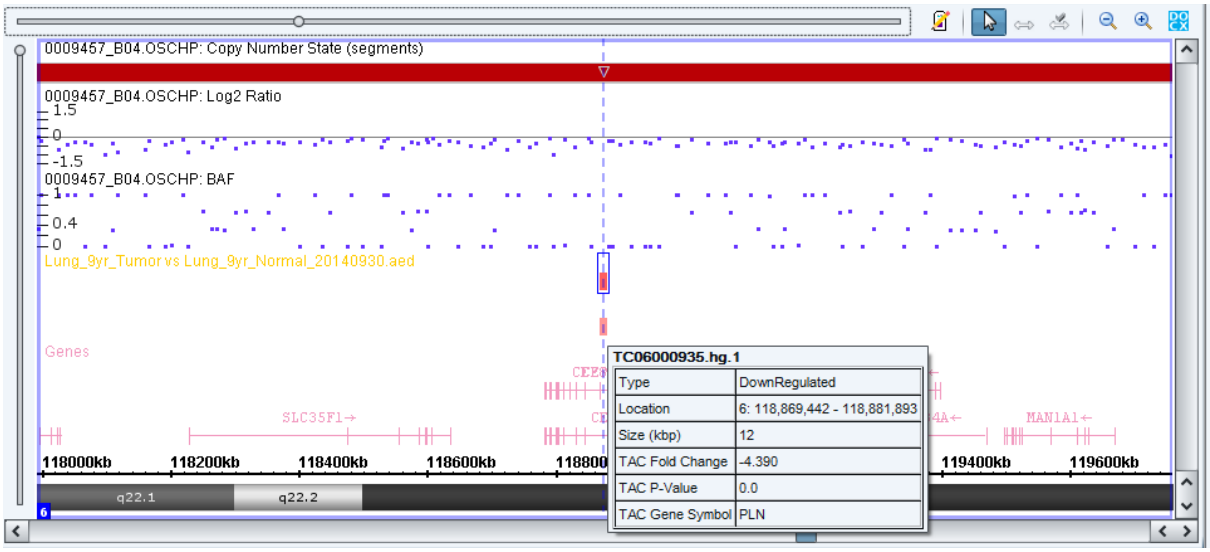


Figure 357 AED file in Detail View.

You can simultaneously view fold change from your Gene Expression dataset with copy number data from xxCHP files. Positive gene expression fold changes are represented by blue transcript cluster IDs. Negative fold changes are represented by red transcript cluster IDs. The deeper the color (blue or red) the larger the fold change.

15 VCF files

Load VCF files

VCF files containing genotyping, copy number and structural variants can be visualized in the Detail View in conjunction with CHP file data.

VCF files are a flexible format, therefore you must use the following guidelines when formatting VCF files for use in ChAS.

- Each VCF file must contain a single sample only.
- The VCF file will display genotype/indel/copy number/LOH and Structural Variation data.
- The VCF file must contain only 10 columns.
- The chromosome ID should use the format chr1, chr2 or 1, 2.
- Only chromosomes 1–22, X and Y are supported. Information from other sequences or variant chromosome assemblies are ignored.
- The DATA column (column 10) can not be left blank/empty.
- A binary compressed VCF is not supported.
- The **vcf.gz** format can also be viewed in the ChAS Browser.
- VCF entries representing structural variations (CNV, DEL, DUP, etc.) do not require GT values.
- Other VCF entries must contain **GT** in the FORMAT column, preferably in the first position.
- The length of structural variation segments should be indicated with a SVLEN value. If missing, the software auto-checks for LEN and END.

IMPORTANT! If your VCF file(s) do not strictly adhere to the above guidelines, the file(s) will not be compatible with, nor load into ChAS.

---

1. From your software, export your VCF file (based on the above guidelines).
2. Click **File → Open.**

   A File Explorer window appears.
3. Navigate to your VCF file location, click to highlight it, then click **Open**.

   A message appears indicating that the Genome Version could not be detected. If you are certain that the genome build for the VCF file matches the Genome Version loaded in the ChAS Browser, click **OK** to acknowledge the message.

   The VCF file loads, then appears inside the Detail View. Copy number and structural variants are displayed in a upper track, while genotype calls are display on the lower track, as shown in Figure 358.

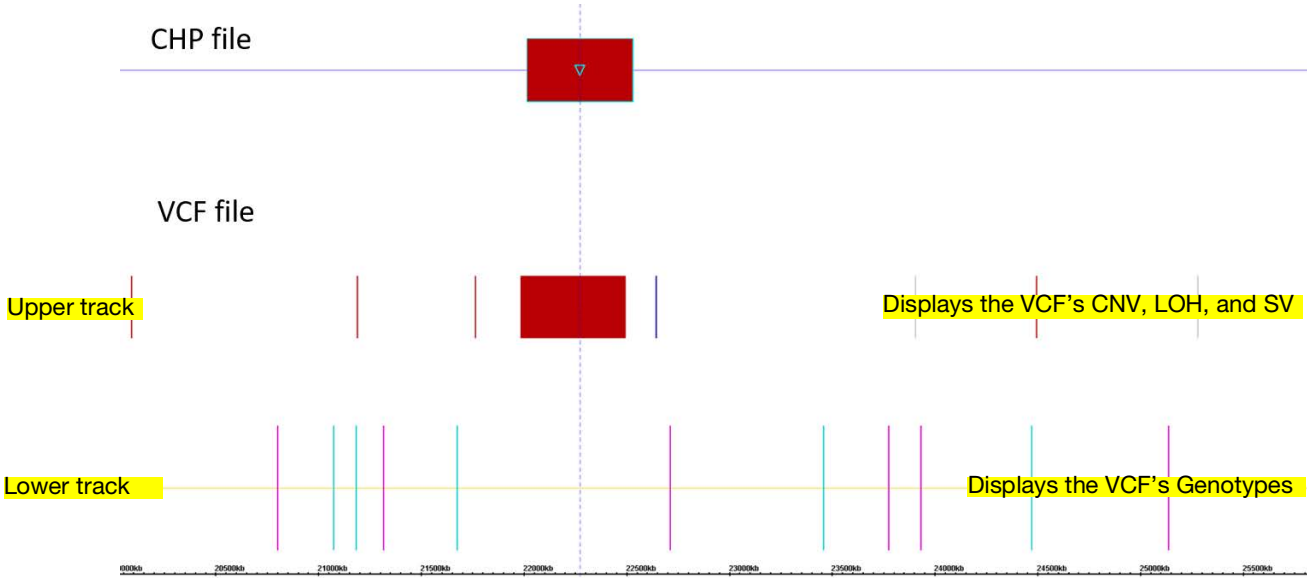


Figure 358 VCF file displayed in Detail View

Genotype Data (Lower track)		
Data Type	Description	Color
GT	Heterozygous Genotype	Cyan
GT	Homozygous Alternate Genotype	Magenta
GT	Homozygous Reference Genotypes	Gray
Segment Data (Upper track)		
Data Type	Description	Color
CNV	Alt allele = DUP	Blue
CNV[1]	Copy Number Value >2 (>1 on Y)	Blue
CNV	Alt allele = DEL	Red
CNV[1]	Copy Number Value <2 (<1 on Y)	Red
LOH	Alt allele = LOH	Purple
INS	Insertion	Gray
INV	Inversion	Gray
DUP:Tandem	Tandem Duplication	Gray

[1] CNV segments on the X and Y chromosomes may appear gray if the gender of the file cannot be determined which in turn, prevents the gain/loss status from being determined.

4. Optional: Mouse over a segment or a genotype in the VCF track to display more detail, as shown in the genotype example. (Figure 359)

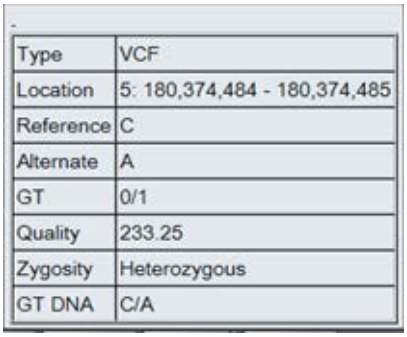


Figure 359 VCF file mouse over details.

Table 34 Mouse-over table definitions.

Annotation	Definition
Type	File Type
Location	Genome location for the SNP (or Indel)
Reference	Reference Base
Alternate	Variant base
GT	Genotype Call, 0 = Reference Base, 1 = Variant Base
Quality	Quality Score assigned from the assay platform
Zygosity	Homozygous or Heterozygous Call
GT DNA	Call in genotyping format (applies to segment data too)

To link to TaqMan™ assays from the VCF file, see "View and order TaqMan™ assays for genotyping" on page 203.

Note: Linking feature applies to hg38 analyses only.

Export VCF files

IMPORTANT! Before you can export VCF files, you must install RHAS on the same workstation.

Export CN and/or variant data as a VCF file (for use in 3rd party browsers)

1. Click **Exports → Export CHP files to VCF**. (Figure 360)

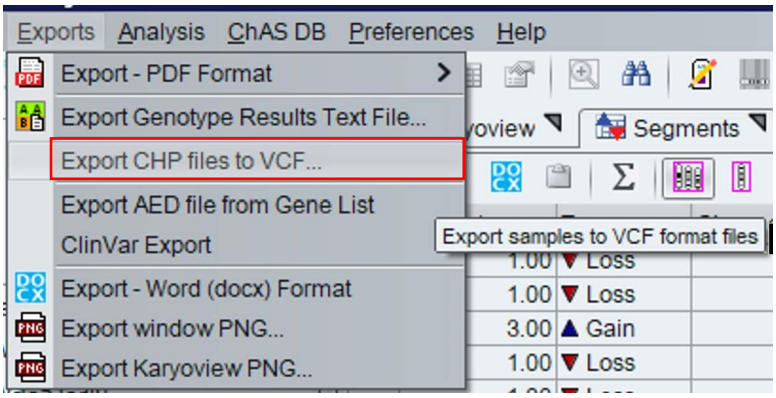


Figure 360 Exports menu.

The Export to VCF window appears. (Figure 361)

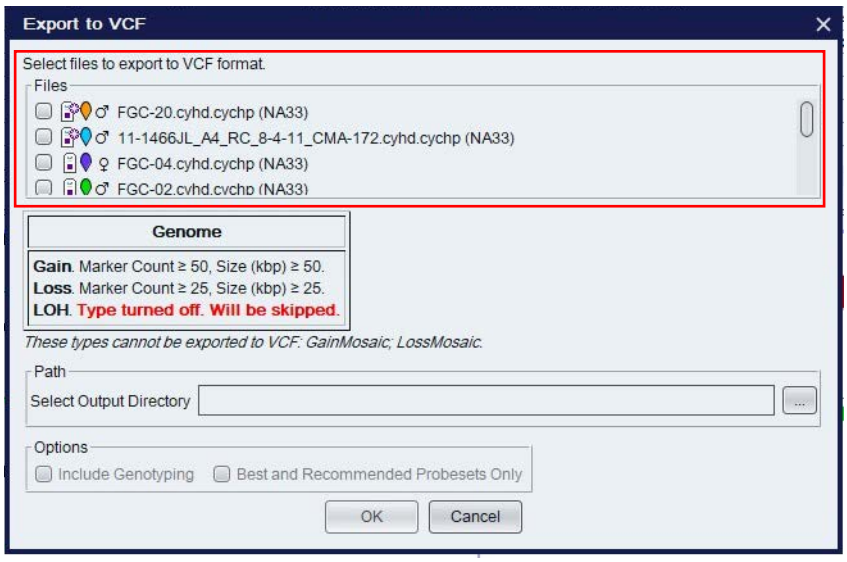


Figure 361 Export to VCF window.

2. Select the checkbox next to the file name for files to be exported in VCF format.
3. Review the Genome filters to ensure that the correct data are exported into the VCF format. If not, click **Cancel**, update the filters accordingly, then repeat Step 1.
4. Click the Path **Browse** button.

   A File Explorer window appears.

5. Navigate to the location where you want to save your exported VCF, then click **Save**.

   Your selected output directory appears.

6. Optional: Select the files to be exported in VCF format by the **Include Genotyping** checkbox to export the copy number segments and genotype data together. For HTCMA arrays, check **Best and Recommended Probesets Only** to export genotypes for those probesets the algorithm selected as the best and recommended.
7. Click **OK**.

16 Display data in table views

Display overview

The data in the xxCHP files can be displayed and exported in tabular format, as well as the graphic representation in the Karyoview, Selected Chromosome View, and Detail View.

- "Selection Details table"
- "CytoRegions table"
- "View the overlap map table"

Common table operations

The controls that are common to all tables are described in this section and include:

- "Standard toolbar controls".
- "Sort by columns".
- "Change the order of table columns".
- "Select columns to display or hide".
- "Sum, mean, and median calculator".

Standard toolbar controls

The toolbar provides quick access to table options.

Left side


Export as TXT file. See "Export tables as TXT file" on page 397.


Export as PDF Report. See "Export table data into a PDF" on page 394.


Export as Microsoft™ Word DOCX file. See "Export as Word™ (DOCX) format" on page 391.


Copy selected cells to clipboard (includes column headers). See "Export a segments table with modified segments to a TXT file" on page 398.

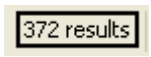

Calculate the sum, median, and mean of the selected values from a numeric column.


Display results for entire genome.


Display results for selected chromosome.


Display results for portion of chromosome displayed in Detail View.

Far Right



The number of rows in the table.


Opens the Select Columns window that enables you to choose the column headers to show or hide.

The Export functions are described in "Export table data" on page 393.

Sort by columns

You can sort a table by a single column's values, or by the values in up to 3 columns.

Note: You may sort on any column except, for reasons of efficiency, the marker name column in the graphs table.

Sorting on certain columns can cause a noticeable decrease in performance. For example, it is recommended that you do not sort a table using the columns in the Segments table that show the overlapping RefSeq, FISHClones, or other items. The data for these table cells is calculated only on an as-needed basis when it needs to be displayed. Using such a column for sorting would force the calculation of the data for all such cells. Since the sorting would be alphabetical, it is unlikely to be useful. Similarly, for reasons of efficiency, sorting based on the marker name column in the Graphs table is not allowed.

Sort a table by a single column

1. Click in the header of the column to sort the table by that column's values.

   A triangle appears in the header.

   – Up triangle 

= ascending order.

   – Down triangle 

= descending order.

2. Click the header to toggle between Ascending, Descending, or no sort order.

Note: The **Type** column in the Segments table sorts segments based on the order that they appear in the **Data Types** window pane, not in an ascending/descending alphabetical order.

Perform a multi-column sort

1. Click in the header of the first column you want to sort on.
2. Click in the header of the second column.
3. Click in the header of the third column, if desired.

   The last selected column has sort priority.

   Sort priority is indicated by the size of the triangle in the header (Figure 362).

CytoRegion Type	CytoRegion	Chromosome	Min	Max	▲ Size (kbp)	▼ Segment ID	▼ Segment File	Segment
Default User Annotation	CNP505	3	163,995,351	164,106,575	111	seg684	Beta10_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP2269	17	41,521,619	41,719,991	198	seg6392	Beta13_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP12506	17	26,040,482	26,043,732	3	seg6312	Beta13_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP2197	16	72,953,795	73,009,537	55	seg6151	Beta13_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP2156	16	21,422,575	21,498,841	76	seg5977	Beta13_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP147	1	194,997,668	195,068,695	71	seg587	Beta13_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP148	1	195,089,940	195,168,372	78	seg587	Beta13_F_01_Cyto_VH.cychp	▲ Gain
Default User Annotation	CNP933	6	32,539,530	32,681,749	142	seg572	Beta15_M_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP901	5	180,311,316	180,350,709	39	seg537	Beta15_M_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP12104	13	69,640,329	69,670,896	30	seg5218	Beta13_F_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP1952	13	68,149,981	68,166,243	16	seg5216	Beta13_F_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP12099	13	66,945,140	66,954,900	9	seg5214	Beta13_F_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP12094	13	65,094,528	65,103,708	9	seg5210	Beta13_F_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP1946	13	63,122,789	63,134,693	11	seg5208	Beta13_F_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP12093	13	64,198,454	64,209,619	11	seg5208	Beta13_F_01_Cyto_VH.cychp	▼ Loss
Default User Annotation	CNP12091	13	63,227,094	63,303,323	76	seg5208	Beta13_F_01_Cyto_VH.cychp	▼ Loss

Figure 362 Table sorted by descending order of Segment ID and ascending order of Size.

Change the order of table columns

1. Click and drag in the column header to move the column to a new location.

Select columns to display or hide

You can select columns using these 2 methods:

- Select Columns window.
- Right-click a column header, then choose **Select columns**.

1. Click the Select Columns toolbar button  . The Select Columns window opens. (Figure 363)

Note: Specific items may vary, as they are dependent upon the type of table and data being displayed.

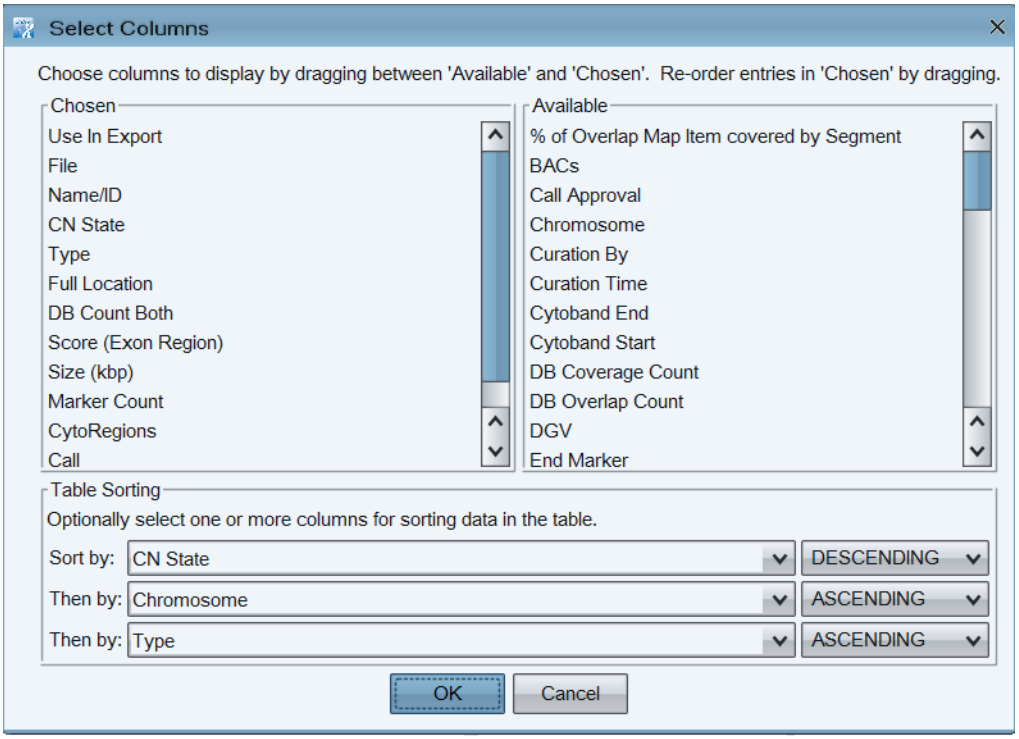


Figure 363 Select Columns window.

The columns in the left side pane are displayed in the table (in their default order).

- To hide columns within the table, move the column entry from the left (Chosen) pane into the right (Available) pane.
- To view different columns in the table, click and drag entries from the right pane to the left pane.
- Column order can be determined by clickingto a column entry, then dragging it into its desired location (inside the left pane).
- Use the **Table Sorting** dropdown lists to sort your columns.

Note: These choices are auto-saved between sessions.

Use the right-click menu

Right-click a column header to perform the following:

- Hide the selected column.
- Show all columns, including hidden ones.
- Expand selected column to display complete heading.
- Auto-size all columns.
- Restore the default selection of columns.
- Select other columns to hide or show.
- Create, select, save, and delete saved table states.

Use a column header's right-click menu

1. Right-click the appropriate column header.

   A menu appears. (Figure 364)

Note: Right-click menu items vary, as they are dependent upon the type of table and data that is displayed.

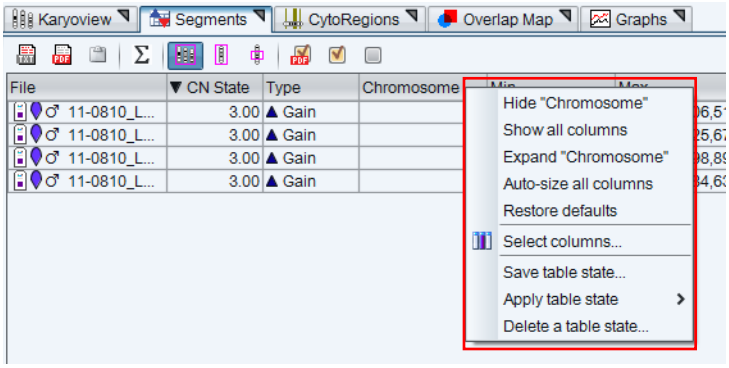


Figure 364 Column heading right-click menu.

2. Click to select the option you want.

Sum, mean, and median calculator

Use this calculator to calculate the sum, mean, and median of the selected or multiple numeric fields.

Calculate multiple numeric values in a column

1. Ctrl+click or Shift+click to highlight multiple numeric fields (*must be* in the same column).
2. Click Σ.

   Your multiple numeric values are calculated and summarized, as shown in. Figure 365.
3. Click **OK.**

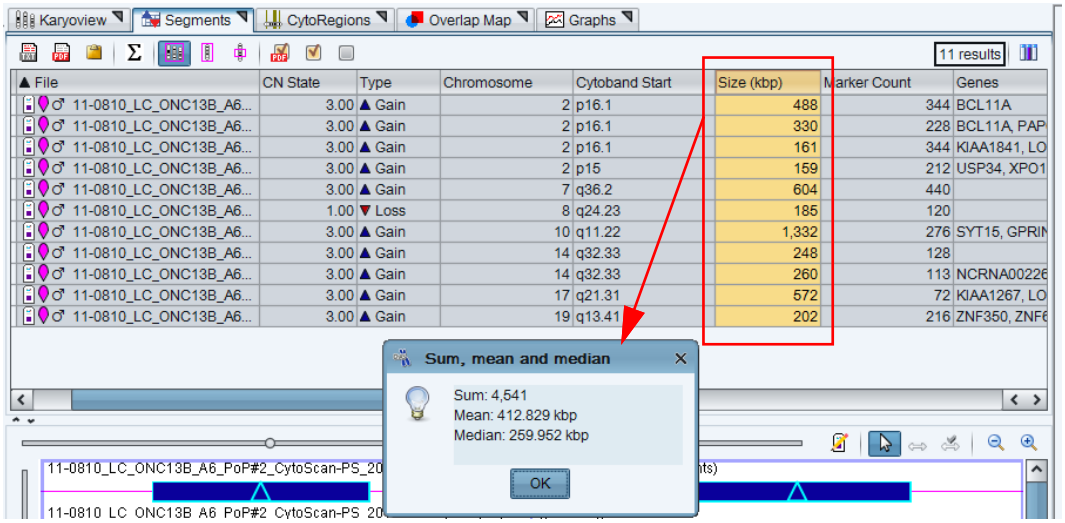


Figure 365 Calculator.

Saved table states

Saving multiple customized tables is a very useful tool, as it enables you to use a large number of columns in a table for interpreting the biology of a particular sample. On the other hand, saving a table state with a smaller subset of columns in a different order - may be more appropriate for including in a report.

In either case, having various Saved Table States enables you to switch back and forth between multiple sets of tables/columns without having to painstakingly recreate interpreting and reporting columns for each of your sample selections.

In the Segments Table, there are 6 pre-loaded Table States available for use in ChAS:

- Cytogenetic
- Default
- Oncology
- Oncomine Reporter
- ReproSeq
- ClinVar

There are 2 pre-loaded Table States available for use in the Variants table. See "Variants table" on page 326 for details.

- CytoScan HTCMA
- OncoScan

There are 6 pre-loaded Table States in the QC & Sample Info table based on relevant metrics for the array type loaded.

- CytoScan QC View
- Default
- CytoScan HTCMA QC View
- OncoScan QC View
- ReproSeq QC View
- SMN Sample Info View

IMPORTANT! You can restore your default settings at any time by right-clicking a table column header and selecting Restore Defaults from the menu or selecting the Default Table State under Apply Table State.

Save the current segment table state to its default

1. Right-click any column header.

   A menu appears.

2. Click **Save table state**.

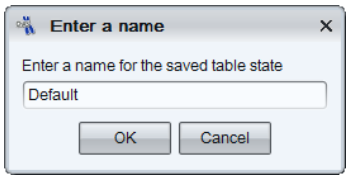


Figure 366 Save Table State - Enter a name.

3. Type **Default**.
4. Click **OK**.

   The table is now saved (as Default) to your User Profile for future use and/or reference.

Add columns to table states

1. Click the Select Columns icon. (Top right of Segments table)

   A list of available columns appears. (Figure 367)

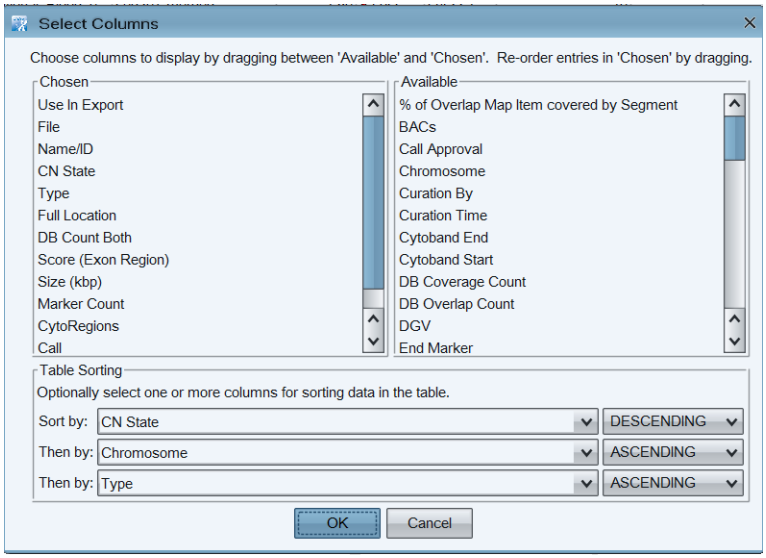


Figure 367 Save Table State - Available Columns.

2. Move the scroll bar downward to reveal more available columns.
3. Click and drag the column header inside the right pane into the desired location inside the left pane.
4. Click **OK**.

   The additional column(s) is now added to your the left pane location. If the column is not in the position you want, click the column, then drag and drop it into its correct spot.

Remove (hide) columns in a table for report use

1. Right-click a column header you want to remove (hide) from the table.

   The following menu appears: (Figure 368)

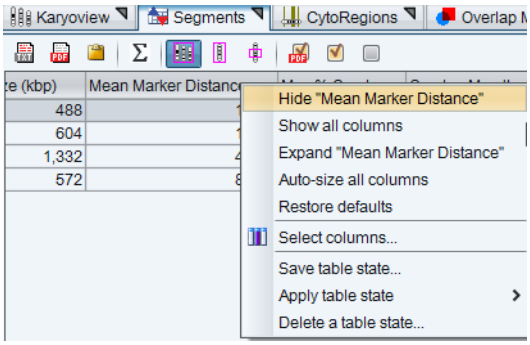


Figure 368 Save Table State - Remove (Hide) Column.

2. Click to select **Hide "Column Name"**.

   The column is now removed (hidden) from your table.
3. Repeat steps 1–2 to remove (hide) additional columns that are not required for a report.

Save a newly edited segment table state

1. Right-click any column header.

   A menu appears.

2. Click **Save table state**.

   A pop-up window appears. (Figure 366)

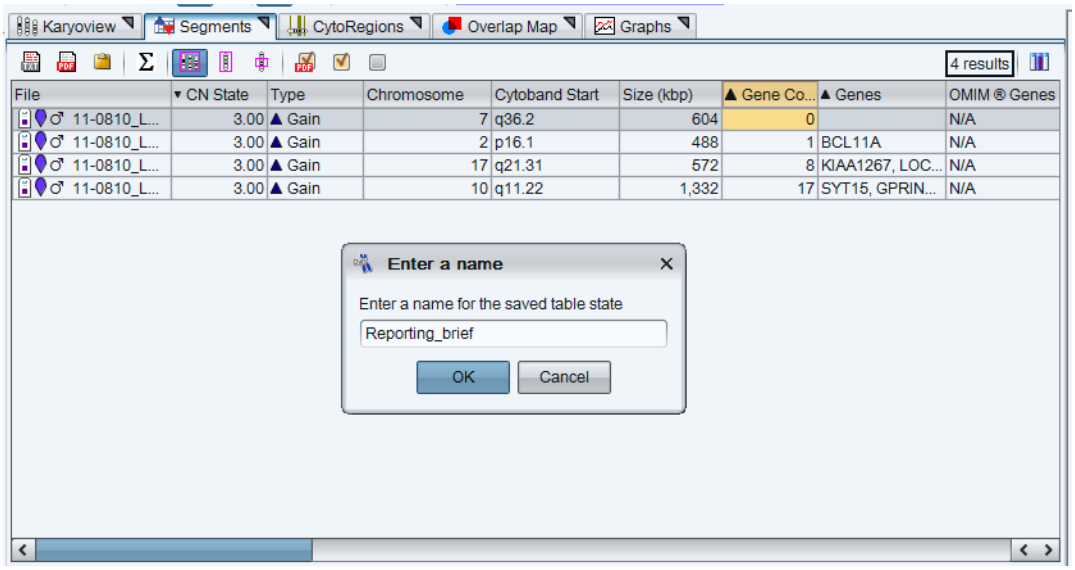


Figure 369 Saved Table State - Example: Table used for reporting.

3. Type a name for your new table state. (Example: **Reporting_brief**)

   The saved table state remembers the columns that you selected, their order, their widths, and which ones were used for sorting.

4. Click **OK**.

   The edited table is now saved to your User Profile for future use/reference.

Apply previously saved table states

There are 6 Table States that are created automatically in ChAS.

- **Default**: Commonly used columns in any xxCHP file analysis
- **Oncomine Reporter**: Simplified Table State for export and use with Oncomine Reporter Software.
- **Cytogenetic**: Commonly used columns when analyzing constitutional samples.
- **Oncology**: Commonly used columns when analyzing cancer samples.
- **ReproSeq**: Standard columns for use with analyzing ReproSeq™ samples.
- **ClinVar**: All required columns for using the ClinVar export.

1. Right-click any column header.

   A menu appears.

2. Click **Apply table state**.

   A sub-menu appears displaying your saved tables, as shown in Figure 370.

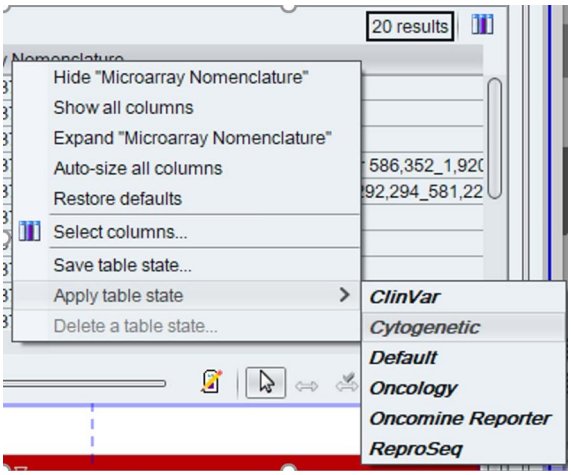


Figure 370 Apply Table State - Saved tables list.

3. Click the table you want to display.
   The table is now displayed.
4. Optional: Repeat steps 1–3 to apply (open) other previously saved tables.

Delete previously saved table states

1. Right-click any column header.
   A menu appears.
2. Click **Delete table state**.
   The following dialog window appears: (Figure 371)

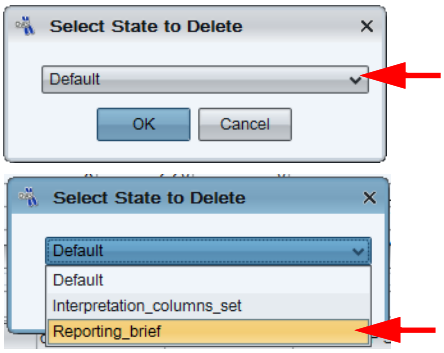
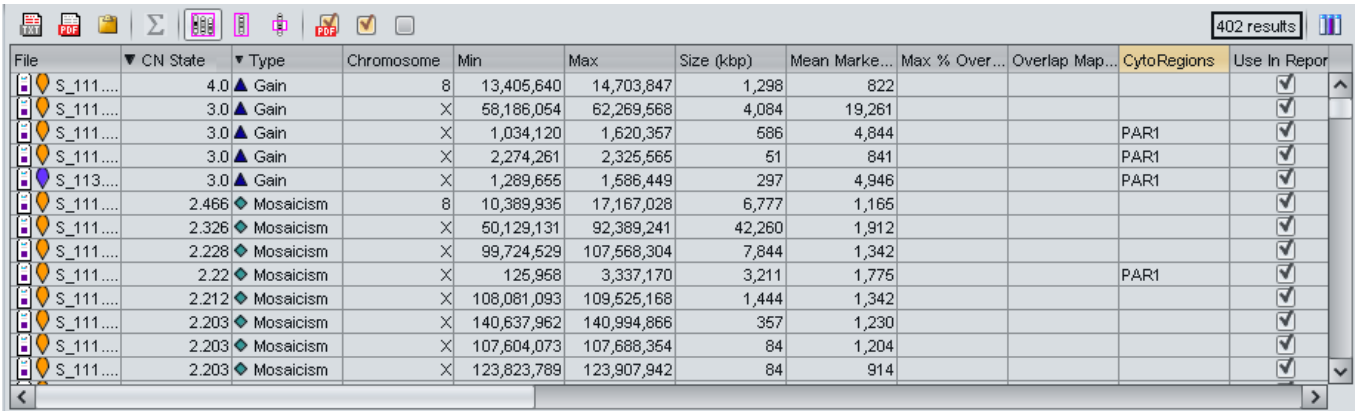


Figure 371 Apply Table State - Deleting saved tables.

3. Click the dropdown list to reveal your saved tables.
4. Click to select the table you want to delete.
5. Click **OK**
6. Optional: Repeat steps 1–5 to delete other saved tables.

Segments table

The Segments table (Figure 372) displays a list of the detected segments in the loaded sample data files.



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File	CN State	Type	Chromosome	Min	Max	Size (kbp)	Mean Marke...	Max % Over...	Overlap Map...	CytoRegions	Use In Repor
S_111....	4.0	Gain	8	13,405,640	14,703,847	1,298	822				☑
S_111....	3.0	Gain	X	58,186,054	62,269,568	4,084	19,261				☑
S_111....	3.0	Gain	X	1,034,120	1,620,357	586	4,844			PAR1	☑
S_111....	3.0	Gain	X	2,274,261	2,325,565	51	841			PAR1	☑
S_113....	3.0	Gain	X	1,289,655	1,586,449	297	4,946			PAR1	☑
S_111....	2.466	Mosaicism	8	10,389,935	17,167,028	6,777	1,165				☑
S_111....	2.326	Mosaicism	X	50,129,131	92,389,241	42,260	1,912				☑
S_111....	2.228	Mosaicism	X	99,724,529	107,568,304	7,844	1,342				☑
S_111....	2.22	Mosaicism	X	125,958	3,337,170	3,211	1,775			PAR1	☑
S_111....	2.212	Mosaicism	X	108,081,093	109,525,168	1,444	1,342				☑
S_111....	2.203	Mosaicism	X	140,637,962	140,994,866	357	1,230				☑
S_111....	2.203	Mosaicism	X	107,604,073	107,688,354	84	1,204				☑
S_111....	2.203	Mosaicism	X	123,823,789	123,907,942	84	914				☑

Figure 372 Segments Table.

Segment Table components:

- "Segments Table toolbar" on page 314
- "Segments table" on page 315

There are certain columns in the Segments table which dynamically compute intersections of reference annotations with the segments. The data in these columns is computed on an as-needed basis for each cell. You may see text such as "<Working…>" in these cells while the data is being calculated. The results for all cells are calculated when exporting to PDF or TXT, or copying to the clipboard. Hiding columns that are not needed may improve performance, particularly during export operations.

Note: Sorting the table based on the dynamically computed columns may be slow.

To highlight segments in the views or the table:

- Double-click in a row of the table to zoom to the segment in the Karyoview, Selected Chromosome and Detail Views.
- Click a segment in the Karyoview, Selected Chromosome or Detail View to highlight the segment in the Segments table.

Segments Table toolbar

The toolbar (Figure 373) provides quick access to table functions. The standard functions are described in "Standard toolbar controls" on page 306.

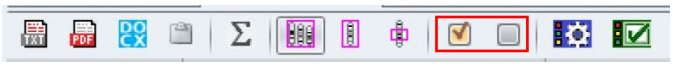


Figure 373 Segments Table toolbar.

Use the **Use in Export** checkboxes to ☑ select all or ☐ deselect all segments displayed in the table.

Note: If the **Use in Export** column is hidden from the Segments table, then all rows are exported.

Segments table

In the Segments table, "N/A" can mean that the information (for example, FISH Clones or sno/mRNA) is not available in the NetAffx™ database because the information has not yet been mapped. For example, FISH Clones or sno/mRNA files will not appear in the Files list for the NA31 (hg19) ChAS Browser NetAffx Genomic Annotation File. "N/A" can also mean that a column which has been persisted to appear from a previous user profile, no longer has data in the current NetAffx Genomic Annotation File that is loaded.

Annotations which share genomic coordinates with a segment are listed in order of start coordinate value, smallest values first (that is, from left to right in the Details View). For annotations with the same start coordinate (for example, isoforms of a single gene), the one with the smallest end coordinate is listed before others with larger stop coordinates.

If a column in the Segments table contains more than 10 items, "…" is displayed after the 10th item to indicate that some data are not displayed to save calculation time. For example, "…" will follow the 10th name in the Genes column. However, a complete list of the genes are included when the information is copied to the system clipboard or exported to reports. For gene isoforms with identical names, only 1 instance of the gene locus is listed in the Segment table to reduce duplicate gene names.

The table can display each segment with the following information (the default set of columns in a new user profile may include only a subset of the total columns listed. For instructions on how to switch quickly between table column sets for a particular table, see "Saved table states" on page 310.

Materially Modified Segments (merged, created *de novo*, segments with edited start or end coordinates) and deleted segment have a different appearance in the Segments Table and export to TXT differently depending on the software settings. For more information, see "Export a segments table with modified segments to a TXT file" on page 398.

Table 35 Segment table columns.

Column	Description
% of Overlaps Map Item covered by Segment	Overlap Map Item and the percentage by which it is covered by the segment.
Call	User-editable field populated by a user-configurable drop list of Calls
Call Approval	Can be used as a bookmark for segments that have been reviewed.
Call From Prioritization	The Call term assigned based on Tier or Score Classification. For more information, see "View segment prioritization in the segments table" on page 354.
Chromosome	Chromosome on which the segment was found.
CN State	Copy Number State (not displayed for LOH segment types). The expected Copy Number State on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes. See "LOH segments on X and Y chromosomes" on page 46 for more information.
Confidence (ReproSeq)	The log likelihood that the called copy number state is not normal ploidy, example 2 on autosomes (reflects the likelihood of the region's ploidy number being different than the normal ploidy 2).
Curation By	The current computer Operating System login ID and ChAS user profile name at the time that the Curation field was last edited.
Curation Time	The time and date when the Curation field was last edited.
Cytoband End	Cytoband in which the segment ends.

Table 35 Segment table columns. (Continued)

Column	Description
Cytoband Start	Cytoband in which the segment begins.
CytoRegions	Names of the CytoRegions with which the segment shares coordinates.
DB Both	The number of segments in the database meeting BOTH the Minimum Percent Overlap and the Minimum Percent Coverage. This number can change depending on whether the “match only same gain/loss type” box is selected. Right-click the a row in the Segment Table. From the menu, click DB Count Both. See "Query a segment from the segment table" on page 365.
DB Coverage	The number of segments in the database meeting the Minimum Percent Coverage. This number can change depending on whether the “match only same gain/loss type” box is selected. Right-click the a row in the Segment Table. From the menu, click DB Coverage Count. See "Set up a ChAS DB query" on page 363.
DB Overlap	The number of segments in the database meeting the Minimum Percent Overlap. This number can change depending on whether the “match only same gain/loss type” box is selected. Right-click the a row in the Segment Table. From the menu, click DB Overlap Count. See "Set up a ChAS DB query" on page 363.
DGV	List of DGV variations that share coordinates with the segment.
DGV-GS	List of curated Database of Genomic Variants considered "Gold Standard" that share coordinates with the segment.
End Marker	The array marker name which marks the end of the segment.
Estimated Mosaic Percentage	The reported value represents the estimated percentage of the region that is mosaic. This estimate is approximate and should be interpreted with a margin of ±10%.
Evidence	Provides information on which annotations the segment overlapped. For more information, see "View segment prioritization in the segments table" on page 354.
File	File the segment was detected in.
Filtered DB Both	The number of segments in the database meeting the Minimum Percent Overlap and Minimum Percent Coverage and the selected Filter Criteria.
Filtered DB Coverage	The number of segments in the database meeting the Minimum Percent Coverage and the selected Filter Criteria.
Filtered DB Overlap	The number of segments in the database meeting the Minimum Percent Overlap and the selected Filter Criteria.
Following Marker	The array marker just below the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Following Marker Location	The coordinate location of the array marker just below the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Full Location	Chromosome Start and Stop in a user-friendly format for use in external databases.
Gene Count	A count of the gene names listed in the Genes column
Genes	List of RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Inheritance	User-editable field populated by a user-configurable drop list of Inheritance.

Table 35 Segment table columns. (Continued)

Column	Description
Marker Count	Number of markers in the segment.
Materially Modified By	The current computer Operating System login ID and ChAS user profile name at the time that the segment was last materially modified.
Materially Modified Segment	Indication that segment was previously merged, deleted, or had its start or end boundary, type, or state altered by a ChAS user. (ChAS-based processes of Smoothing and Joining are not “Modifications”, nor are making Calls or Interpretations, in this context).
Materially Modified Time	The time and date when the Segment was last materially modified.
Max	End position of segment. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).
Max % Coverage	The highest percentage by which a segment covers some item(s) in the Overlap Map.
Max % Overlap	The highest percentage by which some item(s) in the Overlap Map overlaps the segment. Segments completely overlapped by an Overlap Map item are 100% overlapped. This number is used for Filtering Segments out by “Overlap”.
Mean Log2 Ratio	The mean of all the Log2 Ratio values contained in the segment.
Mean Marker Distance	Length of the segment in base pairs divided by the number of markers in the segment.
Mean Weighted Log2 Ratio	The mean of all the Weighted Log2 Ratio values contained in the segment.
Median Log2 Ratio	The median of all the Log2 Ratio values contained in the segment.
Microarray Nomenclature	An ISCN-based description of the segment.
Microarray Nomenclature ISCN 2013	ISCN 2013 based description of the segment.
Min	Start position of segment.
Number of Overlap Map Items	Number of Overlap Map items which share genomic coordinates with the segment.
OMIM Gene Count	A count of the OMIM™ Gene names listed in the OMIM Genes column.
OMIM Genes	List of OMIM™ Genes that share coordinates with the segment.
OMIM Phenotype Loci	List of OMIM™ Phenotype Loci that share coordinates with the segment.
OMIM Region Phenotype	Lists the Gene Title of any region that overlaps with a copy number or LOH segment.
Oncomine Report	A dropdown list of annotations compatible with the Oncomine™ Reporter Software. For details on this application, go to: https://www.thermofisher.com/order/catalog/product/A34298

Table 35 Segment table columns. (Continued)

Column	Description
Overlap Map Items (% of Segment overlapped)	Item(s) in the Overlap Map which overlap the segment, followed by the percentage by which the segment is overlapped by that Item.
Preceding Marker	The array marker just above the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Preceding Marker Location	The coordinate location of the array marker just above the segment in the data track used as input for the segment. **Note:** This column is only applicable to CNState Gain and Loss segments.
Precision (ReproSeq)	The log likelihood that the called copy number state is different than next closest copy number state (reflects the likelihood that the precise ploidy number is correct).
Protein Coding Genes	List of protein coding RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Protein Coding Genes Count	Number of Protein Coding RefSeq genes that shapre coordinates with the segment.
Protein Coding Ensembl Genes	List of protein coding Ensembl Genes annotations that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Protein Coding Ensembl Genes Count	Number of Protein Coding Ensembl Genes that share coordinates with the segment.
Sample UUID	Unique identifier for the CHP file.
Segment Interpretation	User-editable field for free-text interpretation on the segment
Segment Label	A label comprised of the segment's Type, State, and Filename.
Segment Name/ID	File-specific identifier assigned to the detected segment.
Segmental Duplications	List of Segmental Duplications that share coordinates with the segment.
Size (kbp)	Size of the segment.
Smoothed/Joined/ XON merged	Indication that the segment was created by smoothing, joining or merging 2 or more segments in the initial segment detection.
sno/miRNA	List of sno/miRNA features that share coordinates with the segment.
Start Marker	The array marker name which marks the beginning of the segment.
Summarized Log 2 Ratio	The median of the LR, after transformation to adjust for individual marker responsiveness.
Tier or Score	The assigned Tier or Score value based on the Segment Prioritization method selected. When using Tier based, the column will display the assigned Tier. When using Score based, the column will display the score value based on the annotations the segment overlaps. For more information, see "View segment prioritization in the segments table" on page 354.
Type	Type of segment, for example, LOH. When sorting by this column, the segments of a particular sample are listed in the same order that they appear in the Data Types window pane.

Table 35 Segment table columns. (Continued)

Column	Description
Use in Report	Allows manual selection of Segments for export to a Segments Table PDF, DOCX, or Text rather than all segments in the table.
XON Region Level	The annotation Level assigned to this region of the genome.

Note: For normal samples with no segments, the Segments Table can be exported withs the nomenclature for normal sex call when applicable.

- Male Sex Call: arr (X,Y)×1,(1–22)×2
- Female Sex Call: arr (X,1–22)×2
- Unknown Sex Call: arr (1–22)×2

Obtain all annotations associated with a segment

1. Select a segment in the Segments table, Karyoview, Detail View, or Selected Chromosome View, then press **Ctrl+Space** or right-click the segment to select **Zoom to selection** from the menu.

   The exact length of the selected segment fills the entire width of the Detail View (Figure 374).

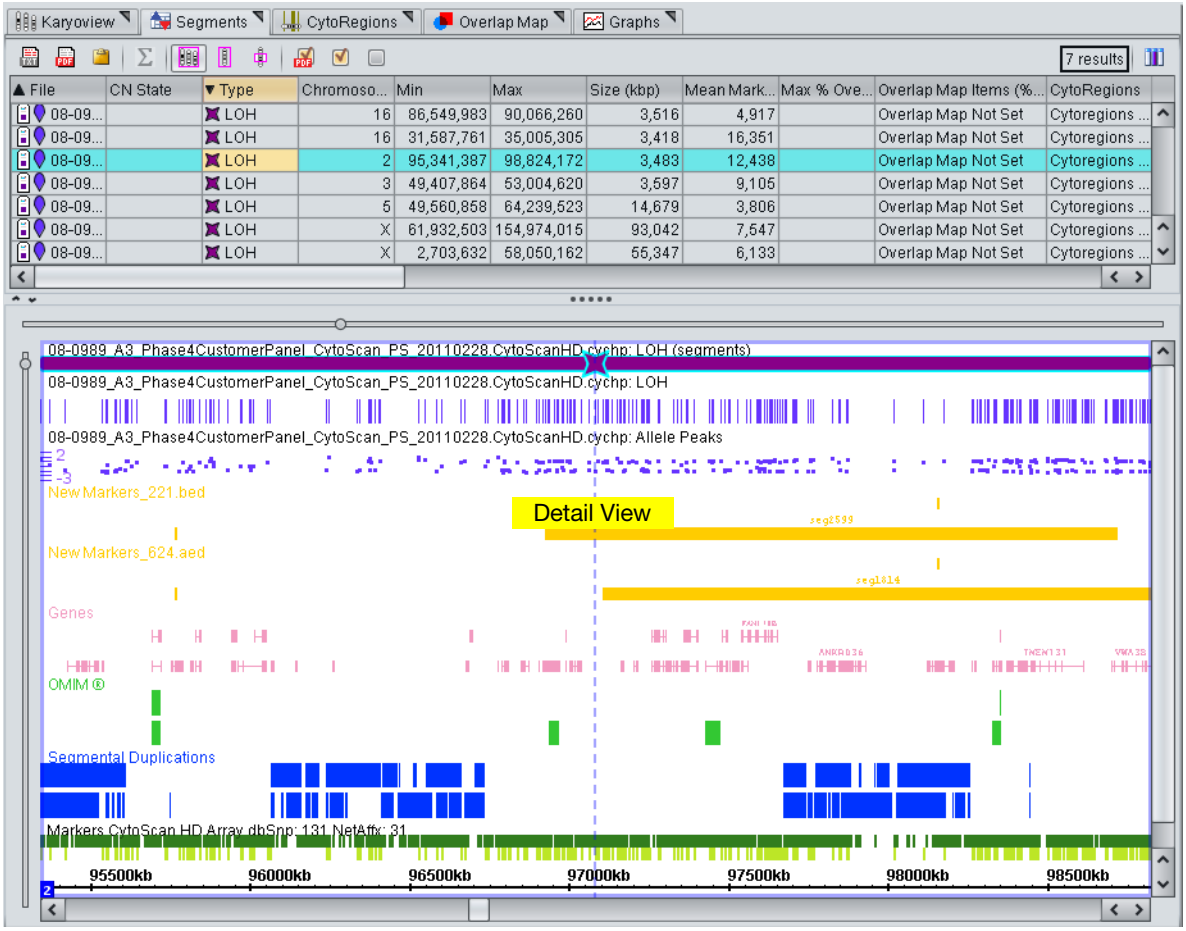


Figure 374 Detail View zoomed in on the segment selected in the Segments table.

2. Click the toolbar button to expand all annotation tracks.

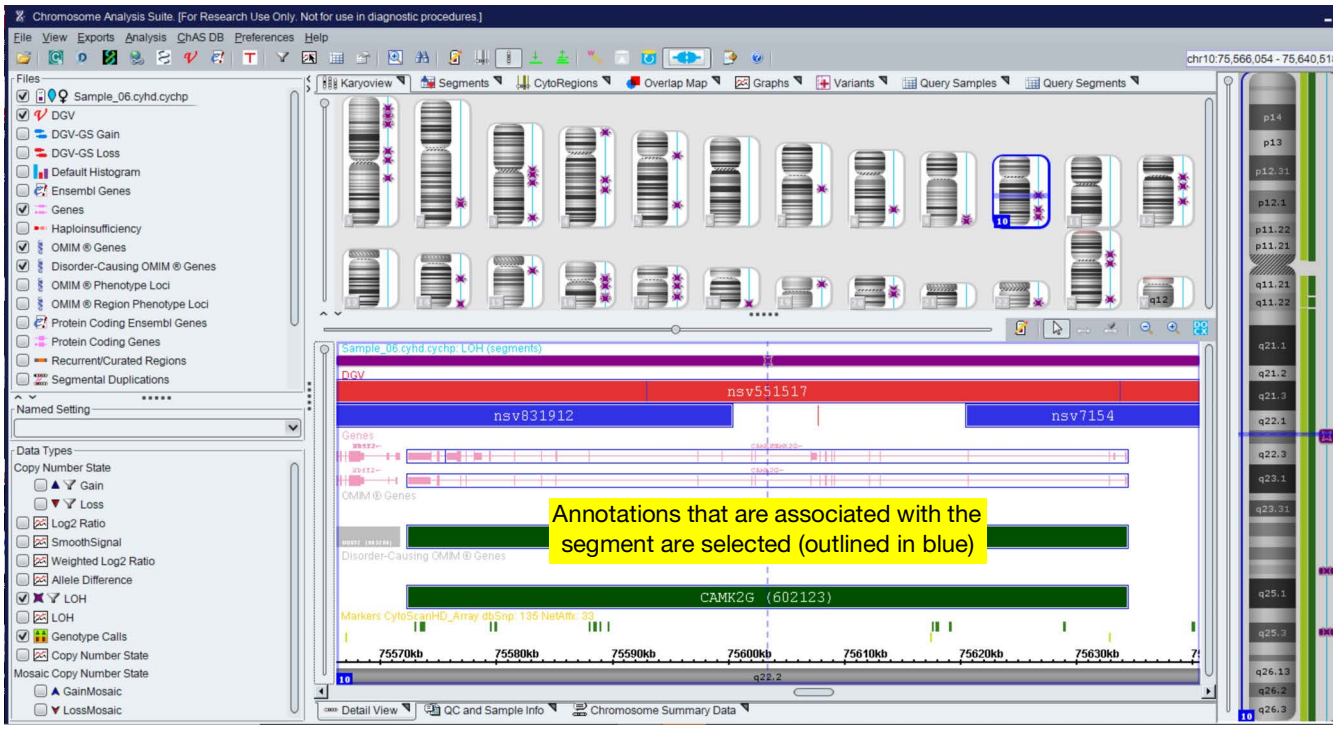
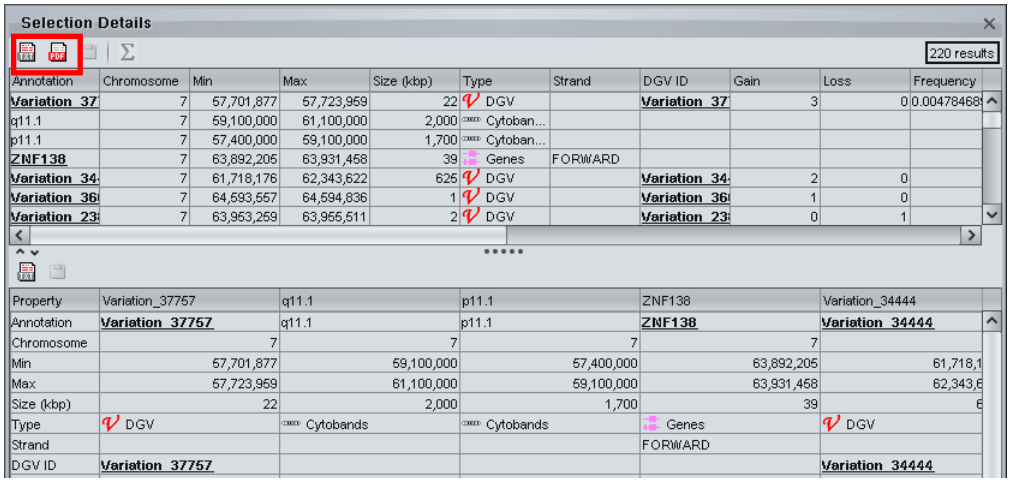


Figure 375 Detail View, annotations have been expanded and selected.

3. Using the mouse, draw a box around all of the genes and annotations of interest. When you release the mouse button, a blue box outlines the selected items in the Detail View. (Figure 375)
4. Click the toolbar icon.

   The Selection Details table appears (Figure 376). It includes all of the items selected in the Detail View. For more details on the table, see "Selection Details table" on page 195.

Click a button to export the information



Annotation	Chromosome	Min	Max	Size (kbp)	Type	Strand	DGV ID	Gain	Loss	Frequency
Variation_37	7	57,701,877	57,723,959	22	DGV		Variation_37	3	0	0.00478468
q11.1	7	59,100,000	61,100,000	2,000	Cytoban...					
p11.1	7	57,400,000	59,100,000	1,700	Cytoban...					
ZNF138	7	63,892,205	63,931,458	39	Genes	FORWARD				
Variation_34	7	61,718,176	62,343,622	625	DGV		Variation_34	2	0	
Variation_36	7	64,593,557	64,594,836	1	DGV		Variation_36	1	0	
Variation_23	7	63,953,259	63,955,511	2	DGV		Variation_23	0	1	

Property	Variation_37757	q11.1	p11.1	ZNF138	Variation_34444
Annotation	Variation_37757	q11.1	p11.1	ZNF138	Variation_34444
Chromosome	7	7	7	7	
Min	57,701,877	59,100,000	57,400,000	63,892,205	61,718,1
Max	57,723,959	61,100,000	59,100,000	63,931,458	62,343,6
Size (kbp)	22	2,000	1,700	39	6
Type	DGV	Cytobands	Cytobands	Genes	DGV
Strand				FORWARD	
DGV ID	Variation_37757				Variation_34444

Figure 376 Selection Details table.

For details on exporting from the Segment table, see "Export table data" on page 393.

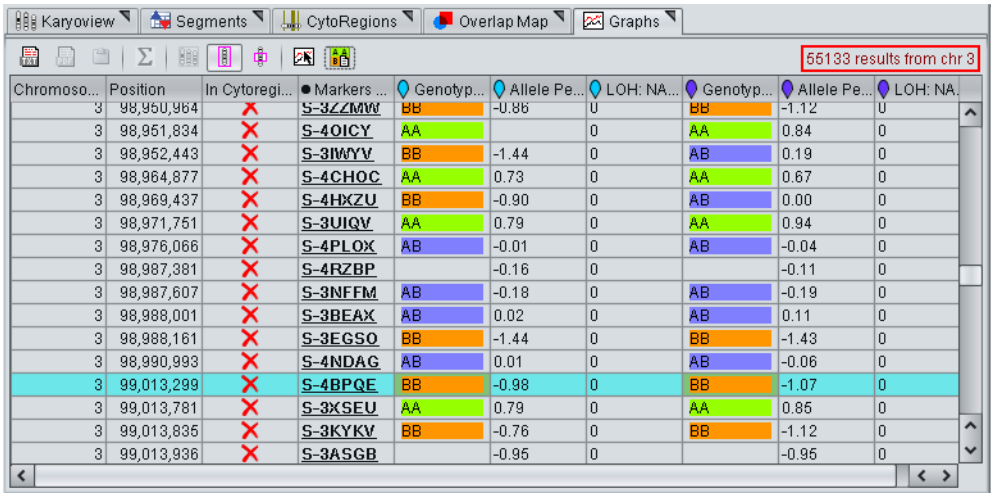
Graphs table

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

The Graphs table displays the marker data used to create the graphs in the Detail view. Markers that are not used for the graphs currently displayed do not appear in this table. As in the Detail View, only markers from a single chromosome are displayed.

The column headings are colored according to the tracks used for the Karyoview, Selected Chromosome View, and Details View.

The Graphs table includes genotype SNP calls for CytoScan array results (Figure 377).



Chromoso...	Position	In Cytoregi...	Markers ...	Genotyp...	Allele Pe...	LOH: NA...	Genotyp...	Allele Pe...	LOH: NA...
3	98,950,964	✗	S-3ZZMW	BB	-0.86	0	BB	-1.12	0
3	98,951,834	✗	S-4OICY	AA		0	AA	0.84	0
3	98,952,443	✗	S-3IWYV	BB	-1.44	0	AB	0.19	0
3	98,964,877	✗	S-4CHOC	AA	0.73	0	AA	0.67	0
3	98,969,437	✗	S-4HXZU	BB	-0.90	0	AB	0.00	0
3	98,971,751	✗	S-3UIQV	AA	0.79	0	AA	0.94	0
3	98,976,066	✗	S-4PLOX	AB	-0.01	0	AB	-0.04	0
3	98,987,381	✗	S-4RZBP		-0.16	0		-0.11	0
3	98,987,607	✗	S-3NFFM	AB	-0.18	0	AB	-0.19	0
3	98,988,001	✗	S-3BEAX	AB	0.02	0	AB	0.11	0
3	98,988,161	✗	S-3EGSO	BB	-1.44	0	BB	-1.43	0
3	98,990,993	✗	S-4NDAG	AB	0.01	0	AB	-0.06	0
3	99,013,299	✗	S-4BPQE	BB	-0.98	0	BB	-1.07	0
3	99,013,781	✗	S-3XSEU	AA	0.79	0	AA	0.85	0
3	99,013,835	✗	S-3KYKV	BB	-0.76	0	BB	-1.12	0
3	99,013,936	✗	S-3ASGB		-0.95	0		-0.95	0

Figure 377 Example Graphs table with Genotype Calls for CytoScan HD results.

The table displays each data point in the displayed graphs.

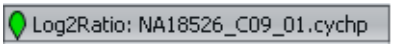
To highlight markers in the views or the table:

- Click in a row of the table to place the cursor on the marker in the Chromosome and Detail Views.
- Click a marker in the Selected Chromosome or Detail View to highlight the marker in the Graphs table.

Graphs table properties

The Graphs columns display:

Table 36 Graphs table columns.

Column	Description
In CytoRegion	Whether marker is located in a CytoRegion or not: In CytoRegion Not in CytoRegion. See "Use CytoRegions" on page 251 for more information.
Markers	Marker ID. Right-click to link to NetAffx™ information about the marker. **Note:** For efficiency reasons, it is not possible to sort the table on this column.
Genotype	The SNP genotype call.
Graph Data types	 The Column header displays: • Color Nib • Data Type • Name of sample file The table cell displays the value for the marker.

Graph table toolbar

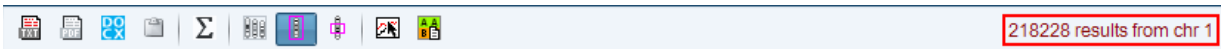


Figure 378 Graphs toolbar.

The toolbar (Figure 378) enables you to:

- Export data in TXT format only. See "Export tables as TXT file" on page 397
- View data for chromosome and selected chromosome region only. You cannot display data from the whole genome in Graphs tab. You can export this data for the whole genome using the "Display and export data from the analysis workflow" on page 84.
- Export genotype results

The Graphs Settings button opens the Graph Settings panel, enabling you to change the style of graph, scale, and other features for the data graphs. See "Change graph appearance" on page 184.

Export genotype calls

Note: Exporting genotypes is not available for OncoScan™ or ReproSeq™ data.

1. Click the toolbar button. Alternatively, select **Reports → Export Genotype Results Text File** from the menu bar.
2. In the window that appears (Figure 379 on page 323), select the array type, results file(s) (CYCHP), and annotation database to use for the export.

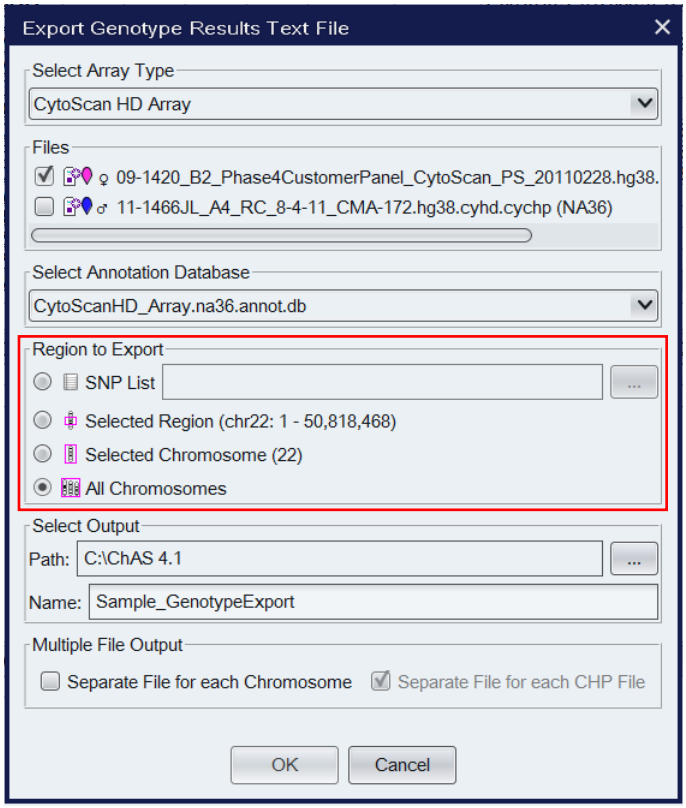


Figure 379 Export Genotype Results.

3. Specify a region to export:
   - **SNP List**: SNPs specified in a user-created SNP list (.txt).
   - **Selected Region**: SNPs included in the chromosome region selected in the Karyoview.
   - **Selected Chromosomes**: SNPs on the chromosome selected in the Karyoview.
   - **All Chromosomes**: SNPs on all 24 chromosomes.

Note: The SNP list should include 1 column header named Probe Set ID or ProbeSet_ID and 1 probe set name per row. (Figure 380)

The export will not proceed without one of these column headers.

	A
1	Probe Set ID
2	S-3FHVO
3	S-3PWJF
4	S-3HSFV
5	S-3IYFW
6	S-4CTUJ
7	S-3APZN
8	S-3UDWU

Figure 380 Example SNP list.

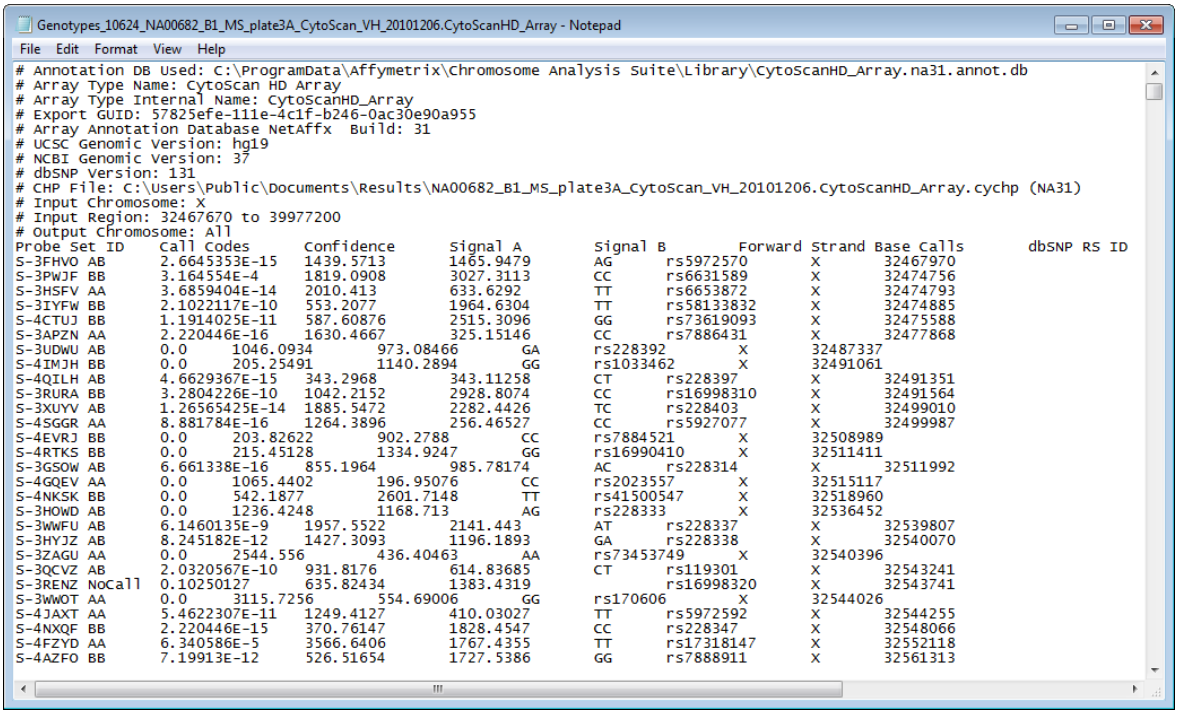
4. Enter the path name or click the **Browse** button to select a folder for the output.
5. Enter a file name prefix. If only 1 output file is created, this is the file name. If multiple files are created, a suffix is added to this string to create the file name. Do not include the file extension here.
6. Select a Multiple File Output option which determines if a separate file is created for each chromosome and/or CYCHP file.

Table 37 Selected Output Option(s).

Option	Files created
None	One output file is exported that contains all chromosome and all CYCHP file data. There are separate data columns for each CYCHP file in the exported file.
Separate File for each Chromosome	Creates a separate file for each chromosome in the output data. If all chromosomes are selected, 24 files are created. There are separate data columns for each CYCHP file in the exported file.
Separate File for each CYCHP File	Creates one text file per CYCHP file. Each file contains genotype calls for all chromosomes.
Separate File for each Chromosome and Separate File for each CYCHP File	Create a separate file for each CYCHP file and for each chromosome. If 3 CYCHP files are selected and all chromosomes are reported on, this will create 72 files.

7. Click **OK**.

Note: Exporting of Genotypes may take several minutes to complete, as this process is dependent on the total number of SNPs selected for export.


Genotypes_10624_NA00682_B1_MS_plate3A_CytoScan_VH_20101206.CytoScanHD_Array - Notepad

File Edit Format View Help

```
# Annotation DB Used: C:\ProgramData\Affymetrix\Chromosome Analysis Suite\Library\CytoScanHD_Array.na31.annot.db
# Array Type Name: CytoScan HD Array
# Array Type Internal Name: CytoScanHD_Array
# Export GUID: 57825efe-111e-4c1f-b246-0ac30e90a955
# Array Annotation Database NetAffx  Build: 31
# UCSC Genomic Version: hg19
# NCBI Genomic Version: 37
# dbSNP Version: 131
# CHP File: C:\Users\Public\Documents\Results\NA00682_B1_MS_plate3A_CytoScan_VH_20101206.CytoScanHD_Array.cychp (NA31)
# Input Chromosome: X
# Input Region: 32467670 to 39977200
# Output Chromosome: All
Probe Set ID    Call Codes      Confidence      Signal A        Signal B        Forward Strand Base Calls       dbSNP RS ID     Chromosome      Chromosomal Position
S-3FHVO AB      2.6645353E-15   1439.5713       1465.9479       AG      rs5972570       X       32467970
S-3PWJF BB      3.164554E-4     1819.0908       3027.3113       CC      rs6631589       X       32474756
S-3HSFV AA      3.6859404E-14   2010.413        633.6292        TT      rs6653872       X       32474793
S-3IYFW BB      2.1022117E-10   553.2077        1964.6304       TT      rs58133832      X       32474885
S-4CTUJ BB      1.1914025E-11   587.60876       2515.3096       GG      rs73619093      X       32475588
S-3APZN AA      2.220446E-16    1630.4667       325.15146       CC      rs7886431       X       32477868
S-3UDWU AB      0.0     1046.0934       973.08466       GA      rs228392        X       32487337
S-4IMJH BB      0.0     205.25491       1140.2894       GG      rs1033462       X       32491061
S-4QILH AB      4.6629367E-15   343.2968        343.11258       CT      rs228397        X       32491351
S-3RURA BB      3.2804226E-10   1042.2152       2928.8074       CC      rs16998310      X       32491564
S-3XUYV AB      1.26565425E-14  1885.5472       2282.4426       TC      rs228403        X       32499010
S-4SGGR AA      8.881784E-16    1264.3896       256.46527       CC      rs5927077       X       32499987
S-4EVRJ BB      0.0     203.82622       902.2788        CC      rs7884521       X       32508989
S-4RTKS BB      0.0     215.45128       1334.9247       GG      rs16990410      X       32511411
S-3GSOW AB      6.661338E-16    855.1964        985.78174       AC      rs228314        X       32511992
S-4GQEV AA      0.0     1065.4402       196.95076       CC      rs2023557       X       32515117
S-4NKSK BB      0.0     542.1877        2601.7148       TT      rs41500547      X       32518960
S-3HOWD AB      0.0     1236.4248       1168.713        AG      rs228333        X       32536452
S-3WWFU AB      6.1460135E-9    1957.5522       2141.443        AT      rs228337        X       32539807
S-3HYJZ AB      8.245182E-12    1427.3093       1196.1893       GA      rs228338        X       32540070
S-3ZAGU AA      0.0     2544.556        436.40463       AA      rs73453749      X       32540396
S-3QCVZ AB      2.0320567E-10   931.8176        614.83685       CT      rs119301        X       32543241
S-3RENZ NoCall  0.10250127      635.82434       1383.4319       rs16998320      X       32543741
S-3WWOT AA      0.0     3115.7256       554.69006       GG      rs170606        X       32544026
S-4JAXT AA      5.4622307E-11   1249.4127       410.03027       TT      rs5972592       X       32544255
S-4NXQF BB      2.220446E-15    370.76147       1828.4547       CC      rs228347        X       32548066
S-4FZYD AA      6.340586E-5     3566.6406       1767.4355       TT      rs17318147      X       32552118
S-4AZFO BB      7.19913E-12     526.51654       1727.5386       GG      rs7888911       X       32561313
```

Figure 381 Example of exported genotypes from a selected region of chromosome X.

The exported text file (Figure 381) includes information about the analysis (for example, array type, NetAffx™ annotation database, hg version, and chromosome).

Note: If the option "Separate File for each CYCHP File" was not selected, many of the headers are repeated for each CYCHP file. The header titles are appended with the CYCHP file name to indicate which file the column belongs to.

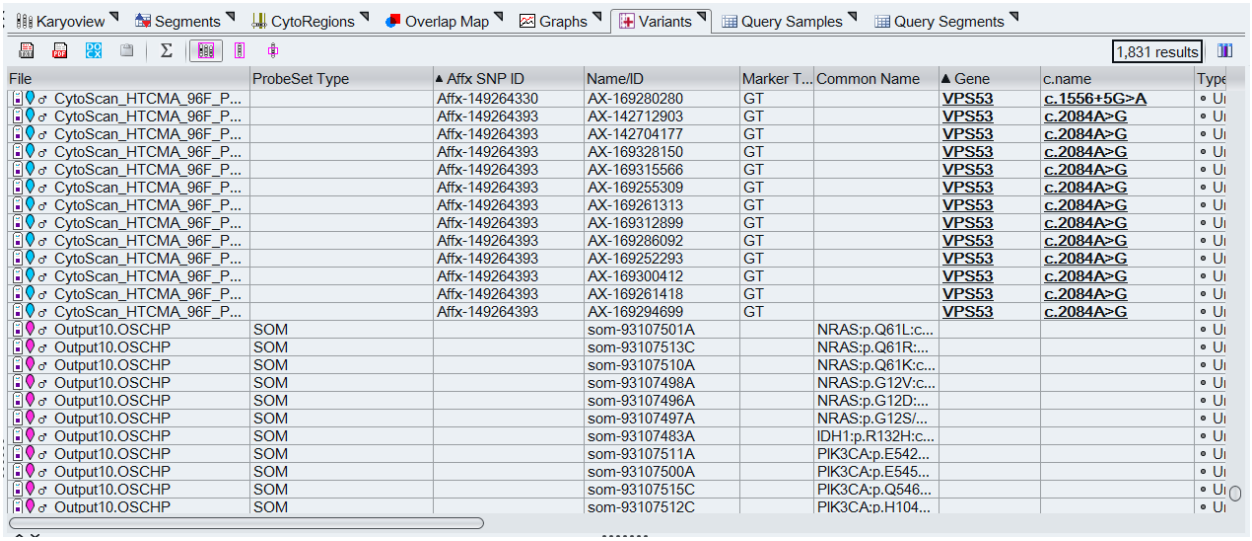
The column headers report the following information:

Table 38 Column header information.

Column	Description
Probe Set ID	Probe set identifier
Call Codes	Genotype call for the SNP.
Confidence	Confidence value for the call.
Signal A	Raw signal value for Signal A on the probe set.
Signal B	Raw signal value for Signal B on the probe set.
Forward Strand Base Calls	Base calls for the forward strand.
dbSNP RS ID	dbSNP RS ID value
Chromosome	Chromosome associated with the probe sets.
Chromosomal Position	Chromosome position of the SNP.

Variants table

For OncoScan™ FFPE and CytoScan™ HTCMA Arrays only. The Variants table (Figure 382) displays the somatic mutation information from OncoScan™ FFPE Arrays and/or the variant information from CytoScan™ HTCMA Arrays.



File	ProbeSet Type	▲ Affx SNP ID	Name/ID	Marker T...	Common Name	▲ Gene	c.name	Type
CytoScan_HTCMA_96F_P...		Affx-149264330	AX-169280280	GT		VPS53	c.1556+5G>A	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-142712903	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-142704177	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169328150	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169315566	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169255309	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169261313	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169312899	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169286092	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169252293	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169300412	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169261418	GT		VPS53	c.2084A>G	• U
CytoScan_HTCMA_96F_P...		Affx-149264393	AX-169294699	GT		VPS53	c.2084A>G	• U
Output10.OSCHP	SOM		som-93107501A		NRAS:p.Q61L:c...			• U
Output10.OSCHP	SOM		som-93107513C		NRAS:p.Q61R:...			• U
Output10.OSCHP	SOM		som-93107510A		NRAS:p.Q61K:c...			• U
Output10.OSCHP	SOM		som-93107498A		NRAS:p.G12V:c...			• U
Output10.OSCHP	SOM		som-93107496A		NRAS:p.G12D:...			• U
Output10.OSCHP	SOM		som-93107497A		NRAS:p.G12S/...			• U
Output10.OSCHP	SOM		som-93107483A		IDH1:p.R132H:c...			• U
Output10.OSCHP	SOM		som-93107511A		PIK3CA:p.E542...			• U
Output10.OSCHP	SOM		som-93107500A		PIK3CA:p.E545...			• U
Output10.OSCHP	SOM		som-93107515C		PIK3CA:p.Q546...			• U
Output10.OSCHP	SOM		som-93107512C		PIK3CA:p.H104...			• U

Figure 382 Variants table.

The Variants table components include:

- Toolbar on page 327
- Variants table on page 326

To highlight segments in the views or in the table:

- Click the row of the table to zoom to the somatic mutation in the Karyoview, Selected Chromosome View and Detail View.
- Click the somatic mutation in the Karyoview, Selected Chromosome View or Detail View to highlight the somatic mutation in the Somatic Mutations Table.
  - **Detected**: Large, bright green circles in the graphic views denote somatic mutations of high confidence (OncoScan™ somatic mutations) or variants called het, hom, NoCall, NRP (anything that isn't a major homozygous call)
  - **Undetected**: Small, dark gray dots in the graphic views denote somatic mutations and variants in which the wild type or major homozygous genotype was called.

Note: Calls can be removed from the table (and graph view) by deselecting the Undetected or Detected checkbox(es). in the Data Types window.

Toolbar

The Toolbar (Figure 383) provides quick access to table functions. Its standard functions are described in "Standard toolbar controls" on page 306.



Figure 383 Toolbar.

Variants table

The table can display each mutation with the following information. The default set of columns in a new user profile may include only a subset of the total columns listed in the following tables.

CytoScan™ HTCMA

Table 39 Variants table columns for CytoScan™ HTCMA.

Column	Description
Affx SNP ID	Unique Thermo Fisher Scientific generated identifier for the SNP.
Alt Allele	The call for the first alternate allele associated with a non-normal phenotype.
Alt Code	The Call Code (A,B,C,D,...) of the Alternate Allele.
Alternate Name	Displays the alternative names for the variant.
Associated Phenotype	Displays the Phenotype that is associated with the variant.
c.name	Displays standard variant nomenclature based on coding DNA reference sequences.
Chromosome	The chromosomal location of the variant.
File	Name of the sample file.
g.name	Displays the genomic coordinates for the variant.
Gene	Name of heritable genetic sequence that encodes proteins.
Inheritance Pattern	Method of Inheritance (Example: AR (Autosomal Recessive), XLR (X-linked Recessive).
Marker Type	The type of marker (Indel, SNP, CN).
Max	Ending genomic position for the variant.
Min	Starting genomic position for the variant.
Name/ID	Thermo Fisher Scientific identifier for the probeset.
p.name	Displays the change in protein translation for the variant.
Recommended Probeset	A quality control metric determined by SNP Polisher algorithm that chooses the best probesets querying a SNP.
Ref Allele	The call for the reference allele associated with a normal phenotype.
Ref Code	The Call Code (A,B,C) of the Reference Allele.
RSID	dbSNP ID

Table 39 Variants table columns for CytoScan™ HTCMA. (Continued)

Column	Description
Size	Size in bp of the variant.
Transcript	RefSeq transcript ID associated with the c.name.
Type	Undetected/Detected. Detected are those mutations with any call other than the wild-type or major homozygous genotype.
Variant Status	Status of the variant based on the genotype (that is, Not Detected, Het, Hom, NoCall, NRP).
Variant Status Alt Allele	Severity status for the variant mapped to Alt Allele.
Variation ID	ClinVar ID

OncoScan™ CNV Plus

Table 40 Variants table columns for OncoScan™ CNV Plus.

Column	Description
accession number	RefSeq Gene accession number.
Channel	CEL file from which the signal is measured. “A” is the AT CEL, “C” is the GC CEL.
Chromosome	Chromosome on which the somatic mutation is found.
Common Name	Abbreviated description of the mutations to which this ProbeSet is known to respond. The name has the form [Gene]:[amino acid change for mutation]:[cDNA change for mutation]. In the event that the ProbeSet cannot differentiate among multiple mutations to which it can respond, the slash (/) delimits the multiple known mutations.
COSMIC ID	The identifier of the mutation as listed in the COSMIC database, which is a catalogue of somatic mutations in cancer. More information on these mutations can be found at: http://cancer.sanger.ac.uk
Event	Describes if the probeset is a point mutation, deletion, insertion or sequence variant.
Event Type	Describes if the event is missense, frame-shift, in-frame insertion or deletion.
File	Name of the OSCHP file the somatic mutation is in.
Fwd 3’ flank	Sequence flanking the mutation at the 3' end.
Fwd 5’ flank	Sequence flanking the mutation at the 5' end.
Genes	RefSeq gene that shares coordinates with the somatic mutation.
High Threshold	High confidence MutScore threshold. Measurements equal to or greater than this threshold are called “High confidence,” describing the likelihood that the mutation is present.
Low Threshold	Lower confidence MutScore threshold. Measurements with a MutScore below this value are called “Undetected”. Measurements equal to or greater than this threshold but less than the High Threshold are called “Lower confidence,” describing the likelihood that the mutation is present.
Max	Stop position of the somatic mutation.

Table 40 Variants table columns for OncoScan™ CNV Plus. (Continued)

Column	Description
Min	Start position of the somatic mutation.
Mutation (aa)	Wild type > mutant amino acid change on the coding strand.
Mutation (nt)	Wild type > mutant nucleotide change on the coding strand.
Mutation Syntax (aa)	Encoding of which nucleotide was changed and its location in the CDS.
Mutation Syntax (cds)	Encoding of which amino acid was changed and the location of the codon.
MutScore	Measures somatic mutation probeset response. The stronger the response, the more likely it is that the somatic mutation is present. The MutScore calculation depends on the algorithm version. The newer MutScore calculation also corrects for sample-specific effects, and thereby reduces false positive calls, which were sample specific. For algorithm versions 1.0 - 1.2 (ChAS 3.0 and earlier, OncoScan™ Console 1.2 and earlier): MutScore.old = (measured quantile normalized signal - median signal for this marker in the reference model file) / (95th percentile signal for this marker in the reference model file - median signal for this marker in the reference model file). For algorithm versions 1.3 and newer (ChAS 3.1 and newer, releases of OncoScan™ Console after 1.2): MutScore.new = (MutScore.old - median MutScore.old for this sample) / standard deviation of MutScore.old for this sample (where standard deviation is calculated for all but the num-out-std strongest MutScore.old for this sample, median is calculated for all but the num-out-med strongest MutScore.old for this sample, and the used median is the maximum of zero and the measured median).
Name/ID	Thermo Fisher Scientific identifier for the marker.
ProbeSet Type	Probeset Type is Somatic Mutation.
Size (bp)	The size of the Somatic mutation in base pairs.
Source DB	Cosmic database version.
Strand	Coding strand of the associated gene (Forward or Reverse).
Type	HighConfidence, LowerConfidence or Undetected call as to the presence of the mutation.

Note: Changes made to an OSCHP file in the Somatic Mutation Viewer Application requires the OSCHP file to be reloaded into the ChAS Browser to reflect the change made to the sample.

QC and sample info tab

The QC and Sample Info tab (Figure 384) in the lower pane displays information about the loaded Data and Region files.

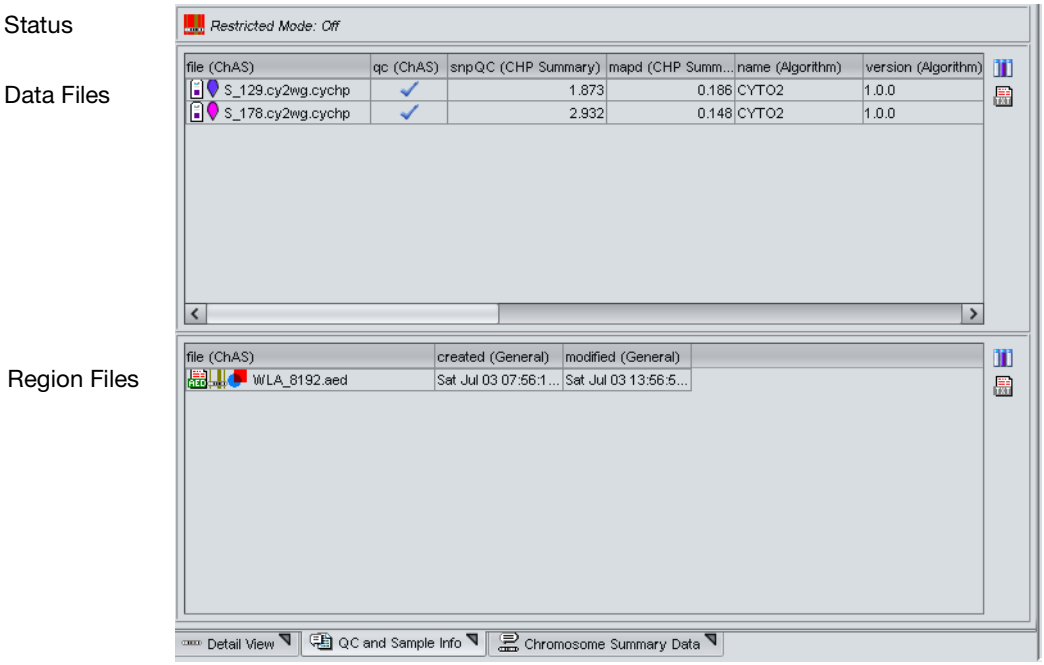


Figure 384 QC and Sample Info table.

The top section displays Status for Restricted Mode (see "Use restricted mode" on page 259)

The tables display information on:

- Loaded data files. "QC and sample information table" on page 330
- Loaded region files. "Loaded AED/BED files table" on page 336

QC and sample information table

The QC table has 6 preloaded Table States allowing you to quickly toggle to the relevant information based on array type. For detailed information, see "Saved table states" on page 310.

File	QC	Gender ...	MAPD	SNP QC	Waviness SD	Program Name	Program Ver
♂ 11-0810_LC_ONC1...	✓	male	0.18	18.462	0.077	Chromosome Analysis Suite	3.1.1
♂ 11-0816_LC_ONC1...	✓	male	0.162	19.628	0.097	Chromosome Analysis Suite	3.1.1
♂ Sample_01.cyhd.cy...	✓	male	0.149	20.562	0.09	Chromosome Analysis Suite	3.2
♂ Sample_02.cyhd.cy...	✓	male	0.153	23.715	0.065	Chromosome Analysis Suite	3.2
♂ Sample_03.cyhd.cy...	✓	male	0.162	19.628	0.097	Chromosome Analysis Suite	3.2
♀ Sample_04.cyhd.cy...	✓	female	0.144	23.293	0.068	Chromosome Analysis Suite	3.2
♀ Sample_05.cyhd.cy...	✓	female	0.183	22.611	0.084	Chromosome Analysis Suite	3.2
♂ POC_WGTriploid...	⚠	male	0.203	7.215	0.196	Chromosome Analysis Suite	3.1.1

Figure 385 Data Files table.

The 6 preloaded QC Table States and their column descriptions are shown in the following tables:

Default QC view

Table 41 Sample information columns for Default QC view.

Column	Description
Algorithm Name	Name of the algorithm used in processing the array.
Algorithm Version	Version of the algorithm used in processing the array.
Annotation File	Name of the Annotation file used to create the xxCHP file.
Array Type	Type of array used in the analysis.
Autosome LOH	The proportion of LOH on chromosome 1 to 22.
Cel Pair Check	Inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel.
Created	Date the xxCHP file was created.
File	File Name
Low Diploid flag	An essential part of the algorithm is the identification of “normal diploid” markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that “normal diploid markers” result in a log2 ratio of 0 (for example, copy number 2). The algorithm might later determine that the “normal diploid” markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of “normal diploid” markers and no “normal diploid calibration occurs. This event triggers “low diploid flag” = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.
MAPD	Median Absolute Pairwise Difference value. See Appendix F for detailed information.
Modified	Date the xxCHP file was last modified.
ndSNPQC	QC metric for SNP probes that is derived from polymorphic SNP probes in normal diploid regions.
ndwavinessSD	Measure of variation of probes in normal diploid regions that are insensitive to short-range variation and focus on long-range variation.
Parameter File	Name of the chasparam file used to create the xxCHP file.
QC	In or Out of QC bounds.
Reference	Reference Model file used in the single sample analysis.
Sex	Gender call for the sample. See "Gender call algorithms" on page 336).
SNPQC	SNP QC value. Median Absolute Pairwise Difference value. See Appendix F for detailed information.
SNPQC Type	ND or non-ND

Table 41 Sample information columns for Default QC view. (Continued)

Column	Description
TuScan %AC	If % AC = 100%, we return “homogeneous” because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.
TuScan Ploidy	The most likely ploidy state of the tumor before additional aberrations occurred. TuScan Ploidy is assigned the median CN state of all markers, provided that %AC could be determined and integer copy numbers are returned. If %AC cannot be determined, NA (Not Available) is reported for both ploidy and %AC.
Waviness SD	A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. See Appendix F for detailed information.

Note: For samples run through the Normal Diploid Analysis for CytoScan, the ndSNPQC and ndwavinessSD metrics can be viewed in the QC Information Tab, but will not flag a sample as pass/fail.

CytoScan™ QC view

Table 42 Sample information columns for CytoScan™ QC view.

Column	Description
Antigenomic Ratio	Ratio of median intensity antigenomic control probes/median intensity all copy number probes.
File	File Name
Genome Version	Build of the genome (that is, hg19, hg38)
MAPD	Median Absolute Pairwise Difference value. See Appendix F for detailed information.
Median Raw Intensity	Pre-processed Median signal of the array.
QC	In or Out of QC bounds.
Sex	Gender call for the sample. See "Gender call algorithms" on page 336).
SNPQC	SNP QC value. Median Absolute Pairwise Difference value. See Appendix F for detailed information.
Waviness SD	A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. See Appendix F for detailed information.
Waviness Segment Count	Number of raw segments without any post-processing.

OncoScan™ QC view

Table 43 Sample information columns for OncoScan™ QC view.

Column	Description
Cel Pair Check	Inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel.
Cel Pair Check Concordance	Percentage of SNPs that match between the AT and GC arrays.
File	File Name
Genome Version	Build of the genome (that is, hg19, hg38)
Low Diploid flag	An essential part of the algorithm is the identification of “normal diploid” markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that “normal diploid markers” result in a log2 ratio of 0 (for example, copy number 2). The algorithm might later determine that the “normal diploid” markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of “normal diploid” markers and no “normal diploid calibration occurs. This event triggers “low diploid flag” = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary.
MAPD	Median Absolute Pairwise Difference value. See Appendix F for detailed information.
ndSNPQC	QC metric for SNP probes that is derived from polymorphic SNP probes in normal diploid regions.
ndWaviness SD	Measure of variation of probes in normal diploid regions that are insensitive to short-range variation and focus on long-range variation.
Number nd	Number of probes called normal diploid by the algorithm.
Percentage nd	Percentage of probes called normal diploid by the algorithm
QC	In or Out of QC bounds.
Sex	Gender call for the sample. See "Gender call algorithms" on page 336).
TuScan %AC	If % AC = 100%, we return “homogeneous” because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.
TuScan Log 2 Ratio adjustment	Log 2 ratio determined from TuScan algorithm needed to “center” the diploid region of the sample (around Log 2 = 0).

CytoScan™ HTCMA QC view

Table 44 Sample information columns for CytoScan™ HTCMA QC view.

Column	Description
DishQC (DCQ)	Measures the amount of overlap between 2 homozygous peaks created by non polymorphic probes. DQC of 1 is no overlap, which is good. DQC of 0 is complete overlap, which is bad.
File	File Name
MAPD	Median Absolute Pairwise Difference value. See Appendix F for detailed information.
QC	In or Out of QC bounds.
QC Call Rate	Percentage of autosomal SNPs with a call other than NoCall measured at the Sample QC step.
QC Het Rate	Percentage of SNPs called AB (that is, the heterozygosity) for autosomal SNPs measured at the Sample QC step.
Sex	Gender call for the sample. See "Gender call algorithms" on page 336).
SMN MAPD	Median Absolute Pairwise Difference value calculated from the CNVMix algorithm for the SMN pipeline.
SMN WavinessSD	A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation from the CNVMix algorithm for the SMN pipeline.
SNPQC	SNP QC value. Median Absolute Pairwise Difference value. See Appendix F for detailed information.
Waviness SD	A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. See Appendix F for detailed information.

SMN Sample Info view

Table 45 Sample information columns for SMN Sample Info view.

Column	Description
DishQC (DCQ)	Measures the amount of overlap between 2 homozygous peaks created by non polymorphic probes. DQC of 1 is no overlap, which is good. DQC of 0 is complete overlap, which is bad.
File	File Name
MAPD	Median Absolute Pairwise Difference value. See Appendix F for detailed information.
QC	In or Out of QC Bounds.
QC Call Rate	Percentage of autosomal SNPs with a call other than NoCall (measured at the Sample QC step).
QC Het Rate	Percentage of SNPs called AB (that is, the heterozygosity) for autosomal SNPs measured at the Sample QC step.
Sex	Gender call for the sample. See "Gender call algorithms" on page 336).
SMN MAPD	Median Absolute Pairwise Difference value calculated from the CNVMix algorithm for the SMN pipeline.

Table 45 Sample information columns for SMN Sample Info view. (Continued)

Column	Description
SMN WavinessSD	A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation from the CNVMix algorithm for the SMN pipeline.
SMN1 (variant)	Genotype call for SMN variant(s) as defined in the SMN.SNP.list For more details, see the *Reproductive Health Research Analysis Software User Guide* (Pub. No. MAN0027973).
SMN1 CN	Copy number for SMN1.
SMN1 Exon7	Copy number for Exon 7 of SMN1.
SMN1 Exon8	Copy number for Exon 8 of SMN1.
SMN2 CN	Copy number for SMN2.

ReproSeq™ QC view

Table 46 Sample information columns for ReproSeq™ QC view.

Column	Description
Application (ReproSeq)	Name of Application from the ReproSeq™ assay.
Batch File (ReproSeq)	Name of the batch file downloaded from Ion Reporter™ Software.
Chr MA Ratio	Ratio of Mitochondrial/Autosome.
File	File Name
MAPD	Median Absolute Pairwise Difference value. See Appendix F for detailed information.
Sex	Gender call for the sample. See "Gender call algorithms" on page 336).
Single File (ReproSeq)	Name of the current file from a Batch File downloaded from Ion Reporter™ Software.
Workflow (ReproSeq)	Name of the workflow run in Ion Reporter™ Software.

Other data from the header of the Sample Data file or the ARR file can also be selected for display in the Select Columns window.

You can only hide or display columns by using the Column Select window, at the right of the table.

Gender call algorithms

The following table explains which algorithm is used to make the gender calls for the different arrays.

The CytoScan™ Array uses the call "Y-gender" which gives a male/female call.

Depending on the version they were created under, various GTC 2.x and 3.x SNP6 CNCHP files use other gender calls present in their CNCHP file header.

These calls used from the CNCHP file header are NOT the same gender calls used for those files in GTC, since the GTC-displayed gender calls were stored in GQC or CN_SEGMENTS files which are not supported in ChAS.

Note: For more details how the array-specific algorithms call LOH segments for the X or Y chromosome, see "LOH segments on X and Y chromosomes" on page 46.

Software/array type	Gender call algorithm	Call	Gender call confidence
ChAS 1.X, 2.X and 3.X/4.X CytoScan Arrays	Y-gender	male/female	Yes
GTC 3.0 to GTC 4.1/ Genome-Wide SNP Array 6.0	affymetrix-chipsummary-Gender	male/female/unknown	No
GTC 2.1/ Genome-Wide SNP Array 6.0	affymetrix-chipsummary-hasY	male/female	No
GTC 2.0/ Genome-Wide SNP Array 6.0	affymetrix-chipsummary-hasY	male/female	No

Loaded AED/BED files table

This table (Figure 386) displays information about the loaded Region (AED or BED) files.

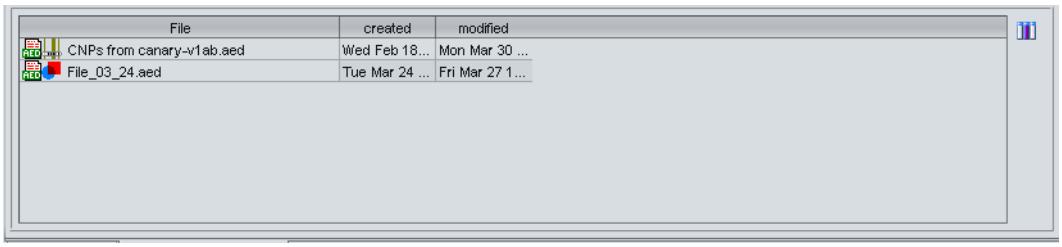


Figure 386 AED/BED Files information.

The Region information files section displays information on:

- **File:** File Name with Icons displayed if selected as Overlap File or CytoRegions File).
- **Created:** Date and time file was created.
- **Modified:** Date and time file was last modified.

You can only hide or display columns by using the Column Select window, at the right of the table.

Chromosome summary data

The Chromosome Summary Data tab has 2 components:

- Chromosome Summary Data
- Auto-Generated Autosome LOH Percentage

The Chromosome Summary Data table (Figure 387) summarizes particular data across each chromosome in the loaded sample data files.

The available data types are:

Note: ReproSeq™ Anueploidy data is not supported within this tab.

- Min Signal – minimum Log2 Ratio value found in the chromosome
- Median Signal – median Log2 Ratio value found in the chromosome
- Max Signal – maximum Log2 Ratio value found in the chromosome
- StandardizedSignal – Standardized Whole Chromosome Log2 ratio value
- Median CN State – median calibrated Log2 Ratio
- Mosaicism – median mosaicism mixture value LOH – proportion of genomic distance of LOH calls per chromosome.

Note: The mosaicism CN state value is not an integer due to cell populations with different CN state values. In the Chromosome Summary Data table, the mosaicism value indicates how much the median CN state value is above or below 2, the normal CN state value for autosomes and X in females. For example, a median mosaicism mixture CN state value of 2.48 is displayed as 0.48 in the Chromosome Summary table.

Note: Mosaicism (median mosaicism mixture value) for normal males is –1.0 for chromosome X and –1.0 for chromosome Y. Mosaicism for normal females is 0.0 for chromosome X and –2.0 for chromosome Y. A mosaic XO female is treated the same as a mosaic autosomal monosomy (that is, the mosaicism level of chromosome X is between –1.0 and 0). A mosaic XXY male is also given a mosaicism level between –1 and 0 for chromosome X.

Note: The Standardized Log2 Raion is generated for autosome (Chromosome 1–22).

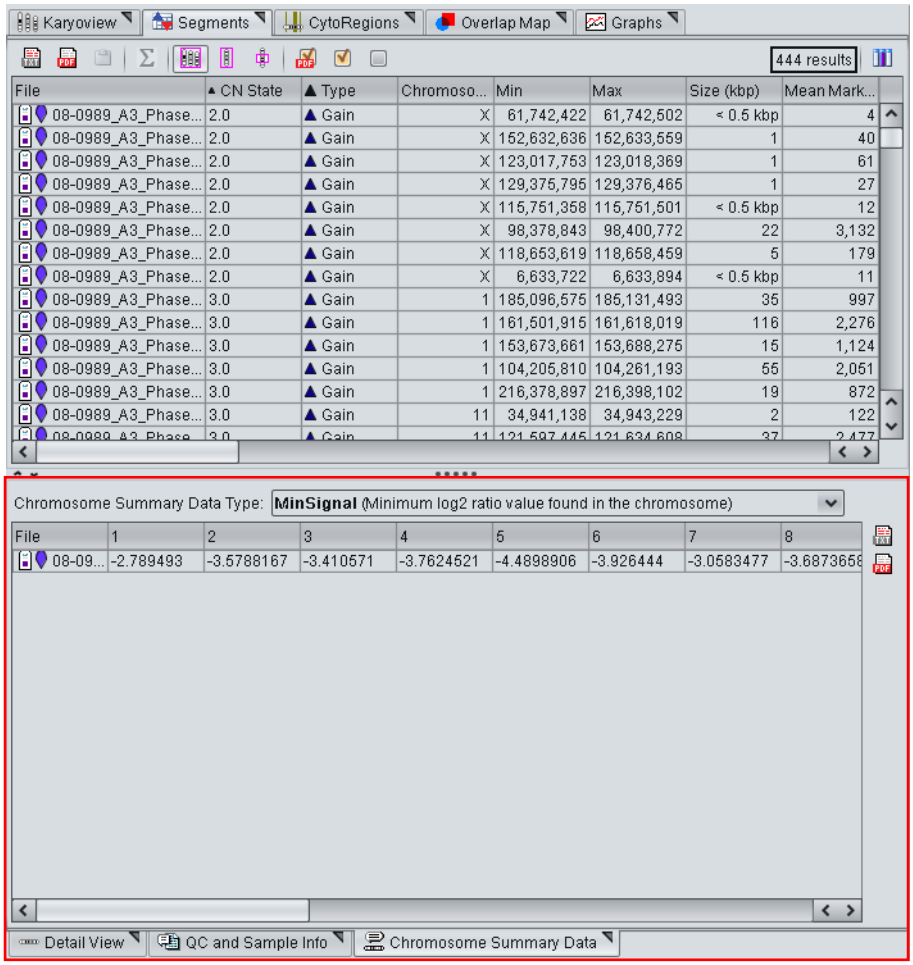


Figure 387 Chromosome Summary Data, Min Signal in each chromosome.

To choose the data type, make a selection from the dropdown list. (Figure 388)

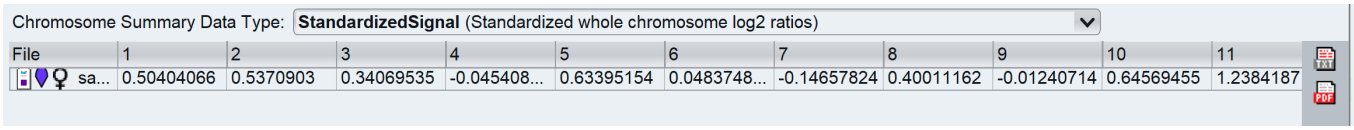


Figure 388 Selecting the data type for the Chromosome Summary table.

Auto-generated Autosome LOH percentage

The percentage LOH displayed is calculated for the Autosome based on the filter size set for LOH in the Filters Window. (Figure 389)

The Covered Autosome Length is the base pairs of the Autosome covered by probes.

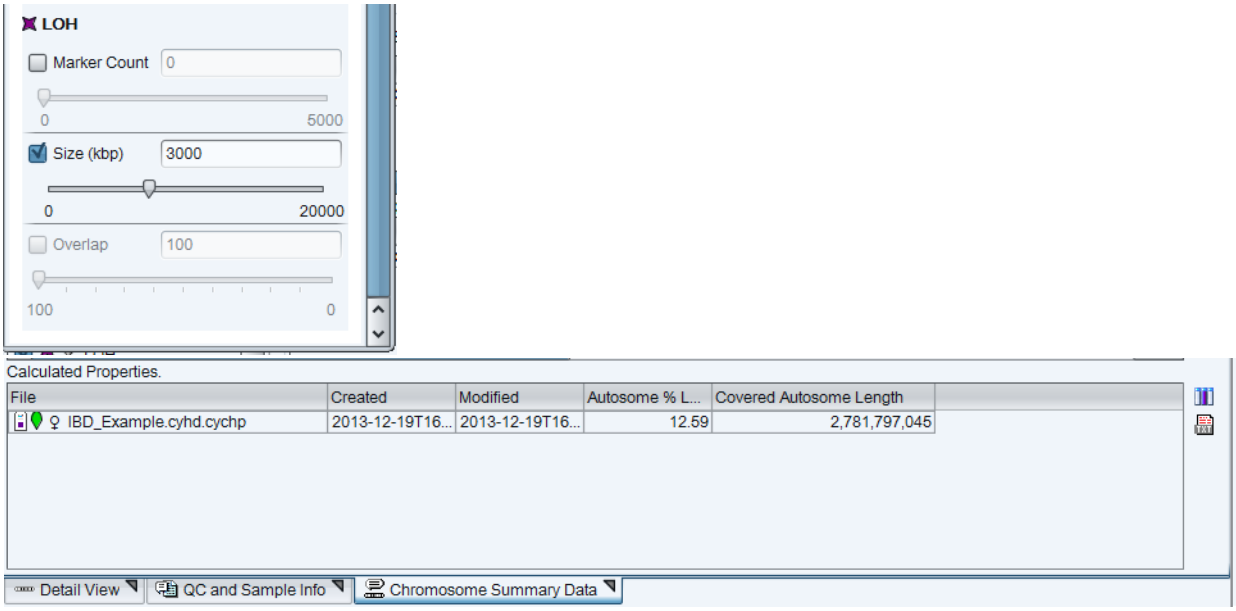


Figure 389 Custom Autosomal Genome LOH Percentage.

IMPORTANT! You must check the LOH Segment Data type to view the sample's percent LOH.

Search results

The Search function enables you to search:

- Detected Segments
- Reference Annotations
- Loaded Region Information Files

The search can find:

- Names of Reference Annotations
- BED and AED file elements, including those in files designated as CytoRegions or Overlap Maps
- Loaded and displayed segments

You can search by:

- File (select the files to be searched)
- ID Label
- Type

1. From the **View** menu, select **Search** or click the upper toolbar's  icon.
   The Search window opens. (Figure 390)

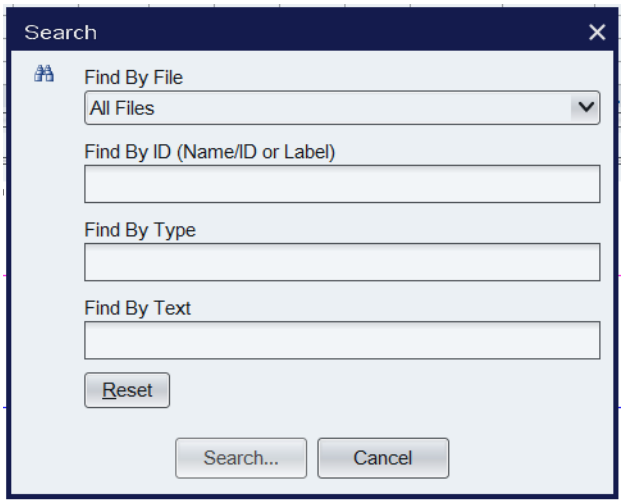


Figure 390 Search window.

2. Search all files in the File List.

   Alternatively, click the **All Files** dropdown to select the file you want to search.

3. In the **Find By ID** text field, enter the ID/Name you want to search.

   A wildcard (*) is not required when performing searches, however the "*" can be used to narrow searches. For example, performing the search *HOX* returns SHOX and RHOXF1 findings, while using a "*" (**HOX*), returns SHOX.

4. Optional: Use the **Find By Type** and/or **Find By Text** fields to enter a type name (not case sensitive) for the search.

You can enter:

- Names for types of reference annotation features (Genes, DGV, etc.)
- Names for types of segments (Loss, Gain, etc.)

5. Click **Search…** to begin the search.

   If no results are found, the following notice appears. (Figure 391)

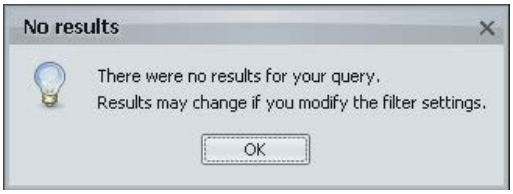


Figure 391 No results notice.

If the search takes more than a few seconds, a **Searching...** window appears. (Figure 392)



Figure 392 Searching notice.

If results are found, the Search Results table opens. (Figure 393)

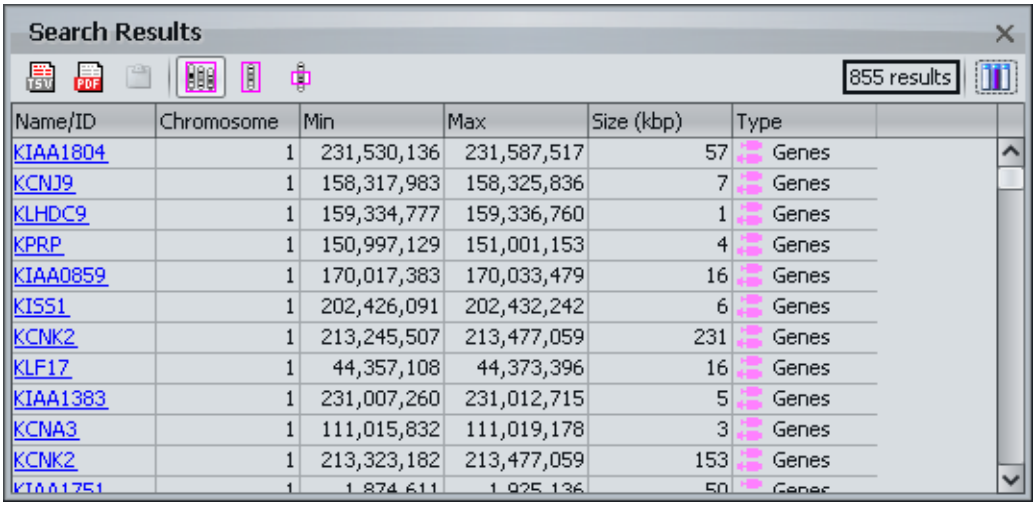


Figure 393 Search Results.

Search within a selected file

1. Right-click a selected (checked) file inside the **Files** list pane.

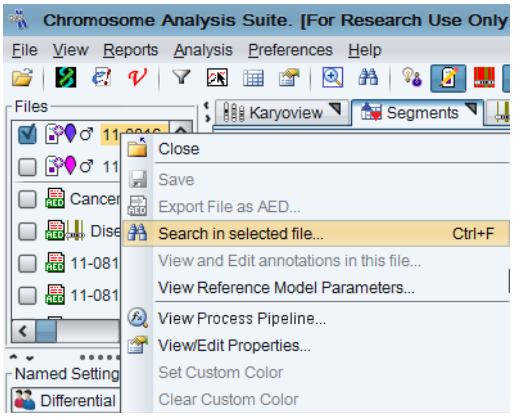


Figure 394 Searching notice.

2. Click to select **Search in selected file...** (Figure 394)
   The Search window opens. (Figure 395)

3. Click the appropriate radio button(s), enter your search criteria, then click **Search**.

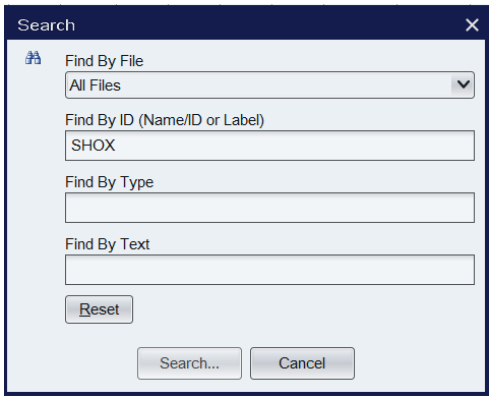


Figure 395 Search window.

If no results are found, the following notice appears. (Figure 396)

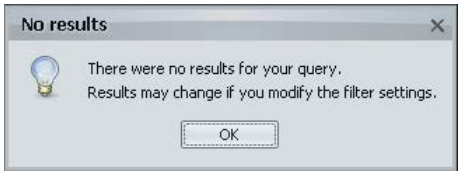


Figure 396 No results notice.

If the search takes more than a few seconds, an In Progress notice appears. (Figure 397)



Figure 397 Searching notice.

If results are found, the **Search Results** window/table opens. (Figure 398)

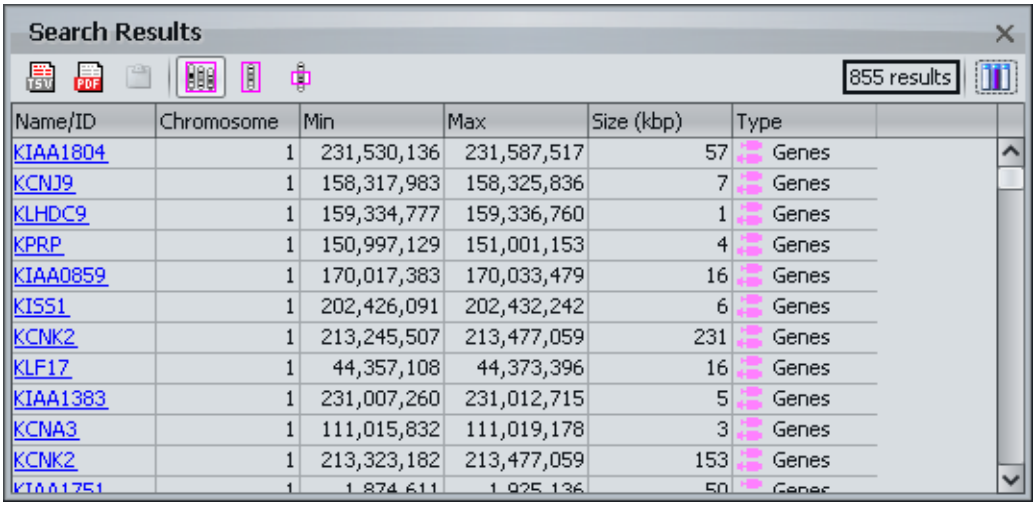


Figure 398 Search Results window/table.

To highlight features in the views or the table:

- Double-click in a row of the table to zoom to the feature in the Selected Chromosome and Detail Views.
- Click a feature in the Selected Chromosome or Detail View to highlight the feature in the Search Results table (the feature must be listed in the table to be highlighted).

You can perform the common table operations in the Search Results table (see "Common table operations" on page 305).

The Search Results table displays the following information:

Table 47 Search Results table columns.

Column	Description
Chromosome	Chromosome the items are located in.
Label	Name or ID of the item.
Max	Ending position of the item. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).
Min	Starting position of the item.
Size (kbp)	Size of the item.
Type	Type of item.

Find intersections

The **Find Intersection** feature enables you to find segments and regions that overlap for different:

- Detected Segments
- Reference Annotations
- Loaded Region Information Files

1. From the **View** menu, select **Find Intersections**…
   The **Find Intersection** window opens. (Figure 399)

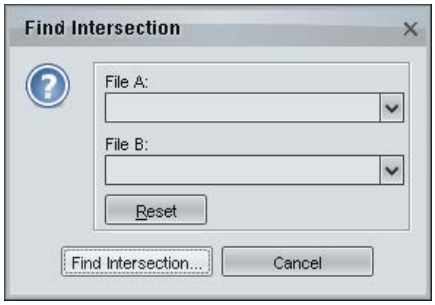


Figure 399 Find Intersection window.

2. Select the first file for the comparison from the File A dropdown list. (Figure 400)

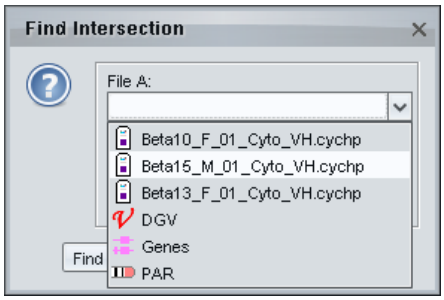


Figure 400 Dropdown list.

The list shows the available Sample files, Region Information Files, and Reference Annotations.

Note: Only files that are check marked in the Files List appear in the Match File dropdown list.

3. Select the second file from the File B dropdown list.
4. Click **Find Intersection…**
   The Finding Intersection notice opens. (Figure 401)

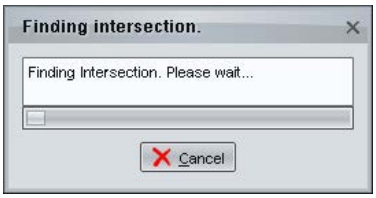
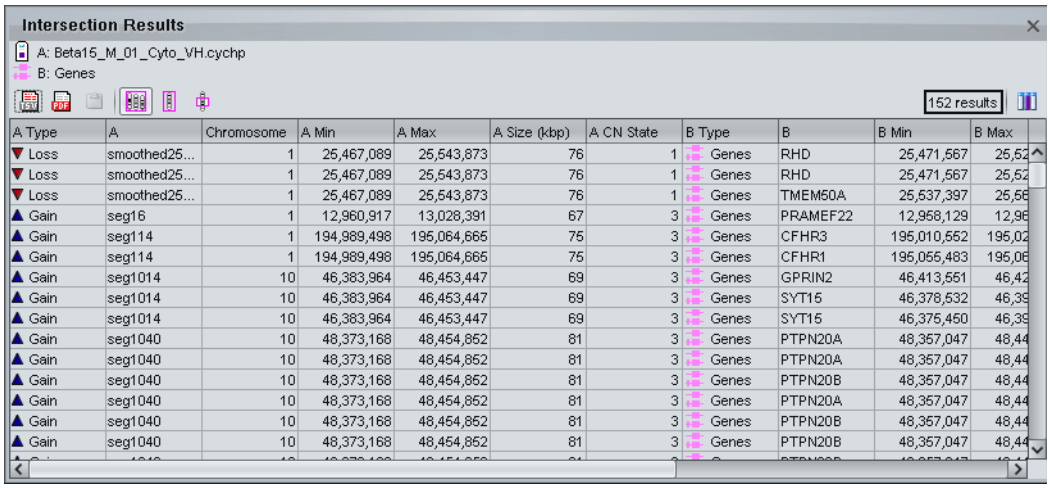


Figure 401 Finding Intersection notice.

When the comparison is finished, the Intersection Results table opens. (Figure 402)



A Type	A	Chromosome	A Min	A Max	A Size (kbp)	A CN State	B Type	B	B Min	B Max
Loss	smoothed25...	1	25,467,089	25,543,873	76	1	Genes	RHD	25,471,567	25,52
Loss	smoothed25...	1	25,467,089	25,543,873	76	1	Genes	RHD	25,471,567	25,52
Loss	smoothed25...	1	25,467,089	25,543,873	76	1	Genes	TMEM50A	25,537,397	25,56
Gain	seg16	1	12,960,917	13,028,391	67	3	Genes	PRAMEF22	12,958,129	12,98
Gain	seg114	1	194,989,498	195,064,665	75	3	Genes	CFHR3	195,010,552	195,02
Gain	seg114	1	194,989,498	195,064,665	75	3	Genes	CFHR1	195,055,483	195,08
Gain	seg1014	10	46,383,964	46,453,447	69	3	Genes	GPRIN2	46,413,551	46,42
Gain	seg1014	10	46,383,964	46,453,447	69	3	Genes	SYT15	46,378,532	46,39
Gain	seg1014	10	46,383,964	46,453,447	69	3	Genes	SYT15	46,375,450	46,39
Gain	seg1040	10	48,373,168	48,454,852	81	3	Genes	PTPN20A	48,357,047	48,44
Gain	seg1040	10	48,373,168	48,454,852	81	3	Genes	PTPN20A	48,357,047	48,44
Gain	seg1040	10	48,373,168	48,454,852	81	3	Genes	PTPN20B	48,357,047	48,44
Gain	seg1040	10	48,373,168	48,454,852	81	3	Genes	PTPN20A	48,357,047	48,44
Gain	seg1040	10	48,373,168	48,454,852	81	3	Genes	PTPN20B	48,357,047	48,44
Gain	seg1040	10	48,373,168	48,454,852	81	3	Genes	PTPN20B	48,357,047	48,44

Figure 402 Intersection Results table.

The table displays the names of the A and B files above the toolbar.

To highlight features in the views or the table:

- Double-click in a row of the table to zoom to the feature for File A in the Selected Chromosome and Detail Views.
- Click a feature in the Selected Chromosome or Detail View to highlight the feature in the Intersection Results table (the feature must be listed in the table to be highlighted).

You can perform the common table operations in the Intersection Results table (see "Common table operations" on page 305).

The Intersection Results table displays the following information:

Table 48 Intersection Results table columns.

Column	Description
% A Touching B	How much of the A item is covered by the B item.
% B Touching A	How much of the B item is covered by the A item.
A	Identifier used for item in A file.
A CN State	Copy number of the segment in file A.
A Max	Ending position of the A item. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445).
A Min	Starting position of the A item.
A Size (kbp)	Size of the A item.
A Type	Type of item in A file with overlap.

Table 48 Intersection Results table columns. (Continued)

Column	Description
B	Identifier used for item in B file.
B CN State	Copy number of the segment in file B.
B Max	Ending position of the B item. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 445)
B Min	Starting position of the B item.
B Size (kpb)	Size of the B item.
B Type	Type of Item in B file with overlap.
Chromosome	Chromosome the items are located in.
Shared Size (kbp)	Size of the overlap.

17 Prioritize segments

Segment Prioritization enables the sorting of copy number segments based on user-defined relevance with overlapping annotations.

Thermo Fisher Scientific offers multiple ways to help quickly annotate segments to reduce interpretation time for each sample.

Segment prioritization options:

- "ChAS Professional version of Franklin by QIAGEN"
- "Tier-based prioritization in ChAS" on page 350
- "Score-based prioritization in ChAS" on page 355

Note: The following NetAffx Genomic Annotation Files are required for full use of all the parameter in Segment Prioritization:

- NetAffxGenomicAnnotations.Homo_sapiens.hg19.na20230601/(or more current)
- NetAffxGenomicAnnotations.Homo_sapiens.hg38.na20230601 (or more current) for optimal segment prioritization results.

IMPORTANT! All data results from the Segment Prioritization process should be manually reviewed.

ChAS Professional version of Franklin by QIAGEN

Sample data can be uploaded to your Franklin account automatically using ChAS AIR (Automated Interpretation and Report) tokens. Use these tokens to take advantage of Franklin's ACMG classification. phenotype to gene relationships, literation searching, and access community and historical data. For more information on ChAS AIR tokens, contact your local sales representative.

ChAS AIR tokens

- Contact your local sales representative to purchase AIR Tokens in quantities of 24, 96, or 384 samples.
- Your sales representative provides a link for setting up an account in Franklin (required for new accounts only).
- The ChAS AIR Tokens are deposited into your Franklin account.
- One token is deducted from your AIR token balance for each CHP file you upload to Franklin (1 CHP file = 1 AIR token).

Upload your sample(s) to Franklin

1. Load the CHP files into the ChAS Browser
2. Review the QC metrics to ensure the sample quality prior to interpretation.
3. Review the breakpoints of each segment using the Detail View to compare the segment and the probe level data. Make any necessary adjustments/edits, as described throughout Chapter 10, "Segment modification" on page 211.

Note: Segments in the mosaic segment track will not be published. For these segments to be included in the upload to Franklin, they must first be promoted to the Gain/Loss Copy Number track See "Promote copy number mosaic segments" on page 228. Promoting is done to mitigate redundancy of Whole Integer Copy Number segments and Mosaic copy number segments in the same region.

4. In the File window, right-click the sample filename(s) you want to upload to Franklin, then click **Send File(s) to Franklin...** (Figure 403)

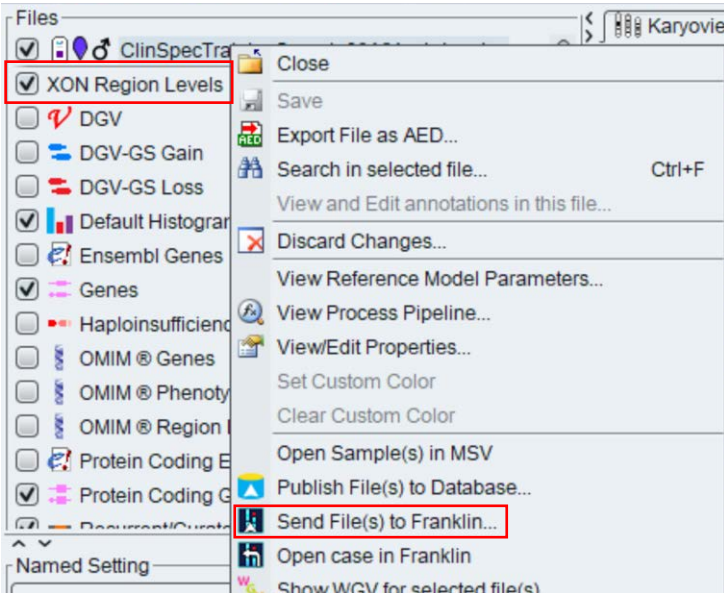


Figure 403 Send File(s) to Franklin.

A window appears confirming what data type and filters are going to be uploaded to Franklin. (Figure 404)

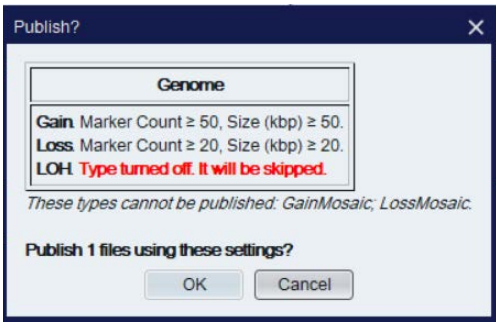


Figure 404 Publish to Franklin window.

- Click **Cancel** to make adjustments to the segment type and/or filter criteria.

- Click **OK** to keep the displayed settings.

  After clicking **OK**, an Enter credentials window appears prompting you to log into your Franklin account. If you do not have a Franklin account or do not know your account credentials, contact **support@genoox.com**.

5. After entering your Username and Password, click **OK**.

Note: Your Franklin credentials are saved until ChAS is closed. This eliminates the need to log in for each additional CHP file you want to upload.

A message appears confirming your files have been successfully sent. If ChAS was unable to connect to Franklin, an Error message appears. If it does, check your internet connection to the genoox.com website.

6. After Franklin processes your sample(s), go to genoox.com to view their analysis.

Note: If edits were made to a file already uploaded to Franklin, you will not be charged an additional AIR token for uploading and reanalyzing the file again.

IMPORTANT! Do not delete the open case in Franklin, simply re-upload the edited file(s) to Franklin to overwrite the existing data.

Return to an open case in Franklin from ChAS

1. To access a previously uploaded file in Franklin from ChAS, go to the File window, right-click the file(s), then click **Open Case in Franklin**. (Figure 405)

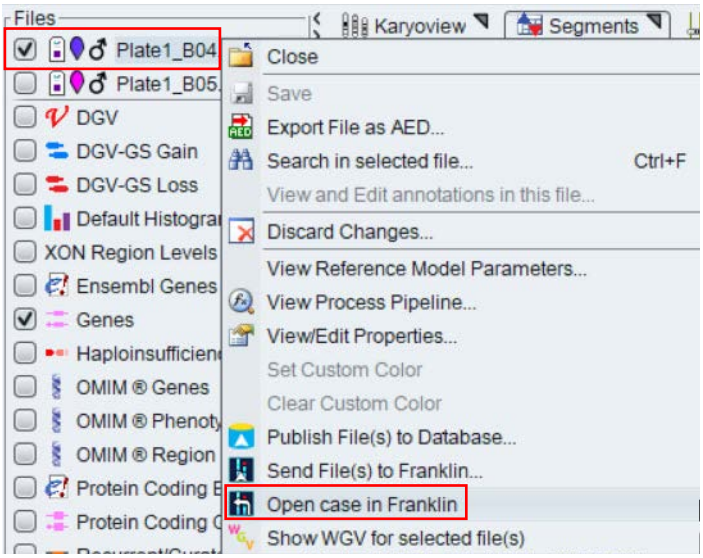


Figure 405 Open Case in Franklin.

The Franklin web page opens displaying the details of your open case.

Note: Before you can review a segment from the Franklin website in ChAS, the **HTTPS service** in ChAS must be enabled. From the ChAS Browser, click **Preferences → Configure HTTPS Service**. Confirm that the **Enable** box is selected and **Port 8348** is displayed. Also ensure that Port 8348 is not blocked by your firewall.

Tier-based prioritization in ChAS

The tier-based prioritization assigns Tiers 1–5 to copy number segments that overlap defined annotations. Copy number segments that overlap an annotation with an assigned tier will get have that tier assignment. If a copy number segment is assigned multiple tiers, the lowest tier number is assigned to the copy number segment. The exception to this general rule are annotations for DGV-GS and ChAS DB Count both. If a segment is meets the criteria for either of these annotation, the tier assignment will take priority over lower number tiers.

Configure the tier-based option

1. From the Segment Table toolbar, click the  button.

   The Segment Prioritization Options window appears. (Figure 406)

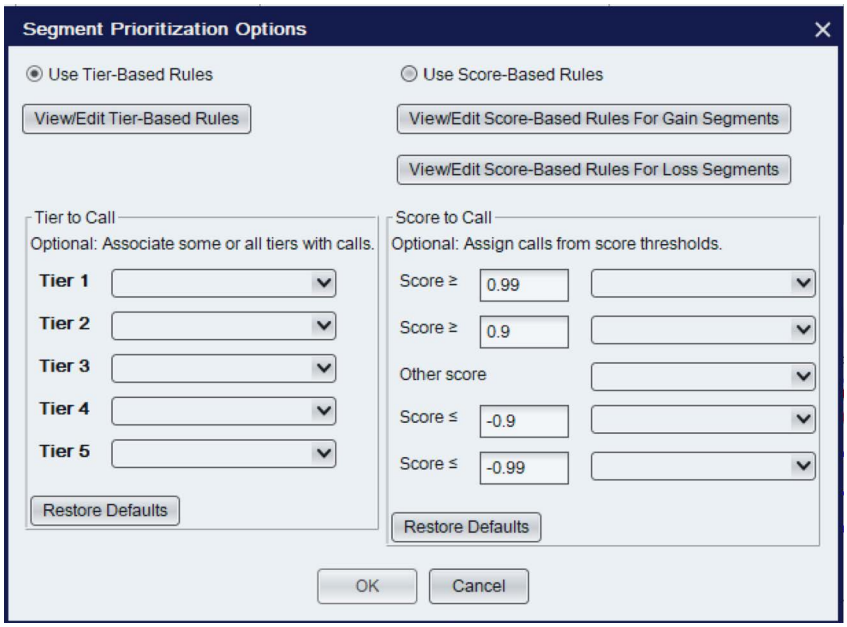


Figure 406 Segment Prioritization Options window.

2. Click the **View/Edit Tier-Based Rules** button.

   The **Select Tier Rules** window appears. (Figure 407)

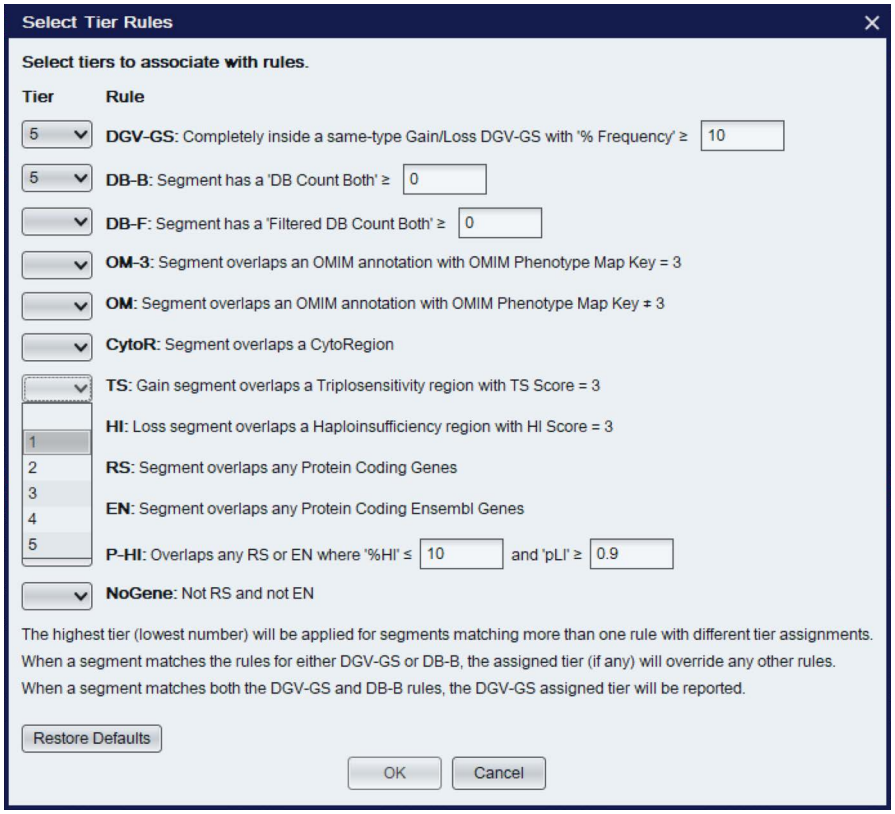


Figure 407 Select Tier Rules window.

3. To prioritize your copy number segments in a tier-based order, assign a tier value to the rule(s) you want to use. To do this, click the dropdown arrow adjacent to the rule, then click the tier value (1–5) you want to assign to it. A Tier 1 assignment denotes the highest rule priority, while Tier 5 is the lowest.

Note: Not every Rule requires an assigned tier. See the Table 49.

IMPORTANT! For copy number segments that meet rule(s) with different tier assignments, the highest tier (lowest number) is assigned to the segment. **Example:** A copy number segment overlaps a rule assigned as Tier 1 and also a rule that is Tier 3, the segment is assigned as a Tier 1 since that is the higher of the 2 tiers. Two Rules are exceptions: DGV-GS and DB-B, if either of these rules are met, the assigned tier overrides any other tiers. If both of these rules are met, the tier assignment for DGV-GS is assigned.

Table 49 Tier Rules.

Tier-based rule/evidence	Description
DGV-GS	Database of Genomic Variants–Gold Standard. The copy number segment is completely contained within an entry of like type (Gain or Loss) from the Gold Standard DGV track and meeting a defined frequency. Default frequency is ≥1%. This rule WILL override higher ranked tiers based on the tier selected in this rule.
DB-B	ChAS DB Both. Compare the copy number segment to the ChAS DB Both column data. If the number of entries in this column exceeds the defined threshold, then the copy number segment is assigned the tier associated with this rule (unless the DGV-GS rule is also met). This rule WILL override rules with higher ranked tiers (with the exception of DGV-GS).
DB-F	ChAS DB–Filtered. Compare the copy number segment to the Filtered ChAS DB Both column. Example: Set the Filtered ChAS DB query to filter on segments in the database with the Call 'unknown significance'. If the copy number segment overlaps enough segments in your ChAS DB called 'unknown significance', then the selected Tier is assigned.
OM-3	Any OMIM™ Genes annotation that is dark green in color. Dark Green is assigned for phenotype map key 3 OMIM™ records indicating the molecular basis is known; a mutation has been found in the gene.
OM	Any OMIM™ Genes annotation that is NOT dark green in color. See OM-3 (above).
Cyto-R	CytoRegions file. The segment overlaps any region in the assigned CytoRegion file. For more information on CytoRegions, see "Use CytoRegions" on page 251.
TS	Triplosensitivity. The copy number segment overlaps an entry in the Triplosensitivity track which has an assigned TS_score of 3.
HI	Haploinsufficiency. The copy number segment overlaps an entry in the Haploinsufficiency track which has an assigned HI_score of 3.
RS	RefSeq. The copy number segment overlaps an entry in the Protein Coding Genes Track.
EN	User-editable field populated by a user-configurable drop list of Calls
P-HI	The copy number segment overlaps a Protein Coding Gene or Protein Coding Ensembl Gene with predicted haploinsufficiency values meeting the defined thresholds. pLI derived from gnomAD (https://gnomad.broadinstitute.org/) and %HI derived from DECIPHER (https://decipher.sanger.ac.uk/).
NoGene	The copy number segment does not overlap any known Protein Coding Gene or Protein Coding Ensembl.

4. After all desired rules have an assigned tier, click **OK** to save the selections and return to the Segment Prioritization Options window.

   Click **Cancel** to return to the Segment Prioritization Options window without saving any tier assignment changes.

   Click **Restore Defaults** to return to the installation settings.

Tier to call settings

You can assign a Call to represent each tier. The contents of the dropdown list was generated from the Calls Vocabulary list. There are a set of default "Calls", but this list can be customized, as detailed in "Use the calls feature" on page 239.

1. Click the dropdown list adjacent to the Tier(s) you want to assign a Call to, then click a selection, as show in Figure 408.

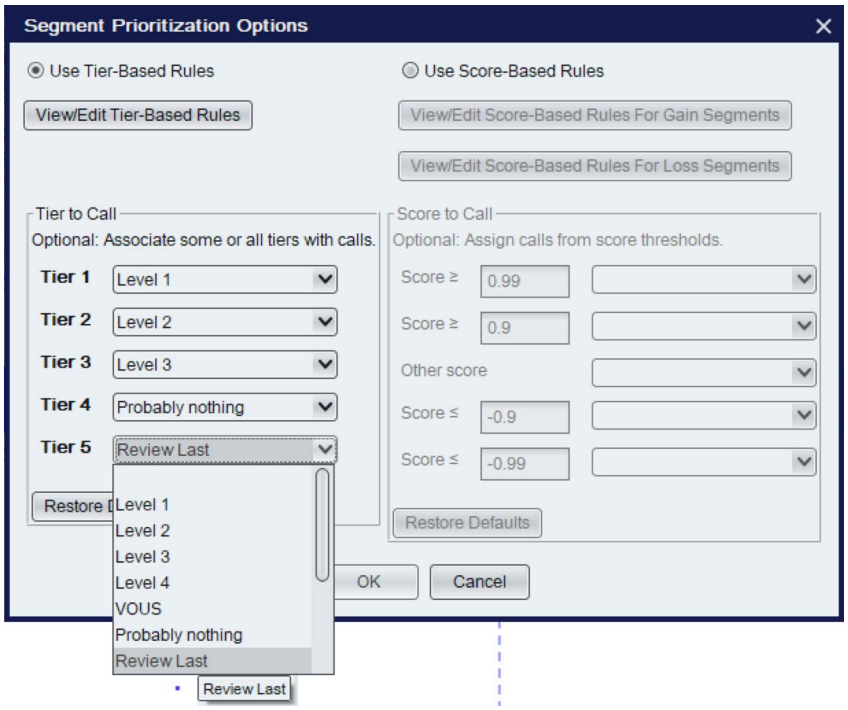


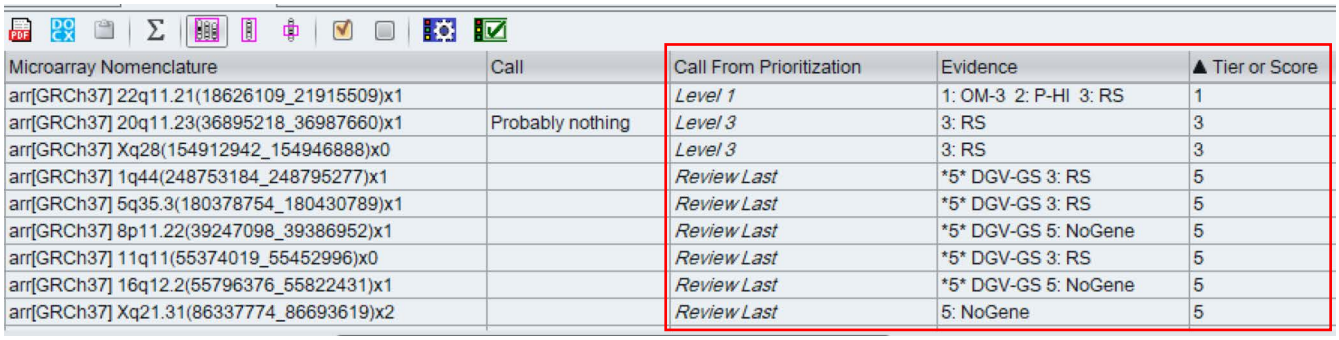
Figure 408 Tier to Call dropdown lists.

Note: Tiers are not required to have a Call assigned. Unassigned Tiers will appear blank in the Segment Table's **Calls From Prioritization** column.

2. After your Tier to Call assignments are complete, click **OK**.

View segment prioritization in the segments table

Three new segment prioritization columns now appear in the Segment Table, as shown in Figure 409.



Microarray Nomenclature	Call	Call From Prioritization	Evidence	▲ Tier or Score
arr[GRCh37] 22q11.21(18626109_21915509)x1		*Level 1*	1: OM-3 2: P-HI 3: RS	1
arr[GRCh37] 20q11.23(36895218_36987660)x1	Probably nothing	*Level 3*	3: RS	3
arr[GRCh37] Xq28(154912942_154946888)x0		*Level 3*	3: RS	3
arr[GRCh37] 1q44(248753184_248795277)x1		*Review Last*	*5* DGV-GS 3: RS	5
arr[GRCh37] 5q35.3(180378754_180430789)x1		*Review Last*	*5* DGV-GS 3: RS	5
arr[GRCh37] 8p11.22(39247098_39386952)x1		*Review Last*	*5* DGV-GS 5: NoGene	5
arr[GRCh37] 11q11(55374019_55452996)x0		*Review Last*	*5* DGV-GS 3: RS	5
arr[GRCh37] 16q12.2(55796376_55822431)x1		*Review Last*	*5* DGV-GS 5: NoGene	5
arr[GRCh37] Xq21.31(86337774_86693619)x2		*Review Last*	5: NoGene	5

Figure 409 New Segment Table columns.

- **Call From Prioritization**: Displays the Call associated with the Tier assigned to the copy number segment.
- **Evidence**: Displays the abbreviation representing the rules met based on which annotations the copy number segment overlaps. See the **Tier-based rule/evidence** table of definitions above for more details.

  Note: A copy number segment that does not overlap any rules with an assigned tier will display ***No rules match***.

- **Tier or Score**: This is a number from 1–5 representing the Tier that was assigned to the copy number segment based on the user-defined Tier-Based rules selected.

If the Call from Prioritization assignments are correct, they can be accepted as the Calls for each segment. To do this:

1. Click the  button.

   The Call from Prioritization assignments are copied into blank cells in the Call column.

   Note: Any Calls manually assigned will remain in the Call column and not be overwritten. Segments hidden by the filters will not have calls copied from the Calls from Prioritization column.

   A confirmation message appears (Figure 410) summarizing the Call from Prioritization assignments are to be copied into the Call column.

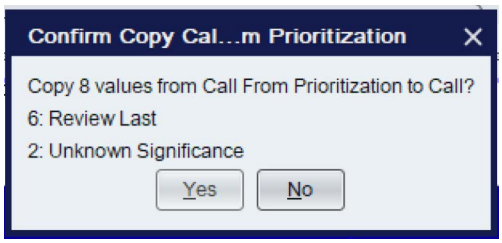


Figure 410 Confirm copy into Call column.

2. Click **Yes** to acknowledge the message.

   Note: Any Call can be manually adjusted by clicking the Call cell and selecting a new option from the dropdown list, as detailed in "Use the calls feature" on page 239.

Score-based prioritization in ChAS

The Score-Based prioritization is a research-based adaptation that is similar, but not identical to, the guidelines proposed in the Riggs et al. 2019 paper: *Genetics in MEDICINE*, Published online: 06 November 2019.

This segment prioritization method assigns numeric values (that is, scores) based on the overlap of a copy number segment with public and/or private annotations. Default score assignments are based on the aforementioned paper.

- A copy number segment's final score is summed based on the rules the segment matches.
- The score assigned to a copy number segment is then associated with a call based on user defined thresholds.
- Overlap between copy number segments and annotations are performed on all transcripts for a given gene that are ≥90% of the size of the gene coordinates in the Triplosensitivty and Haploinsufficiency tracks and have identical gene symbols.
- Segment prioritization applies to the following segment types: Gains, Mosaic Gains, XON Region Gains, Loss, Mosaic Loss, and XON Regions Loss.

Configure the score-based option

1. From the Segment Table toolbar, click the  icon.

   The Segment Prioritization Options window appears. (Figure 411)

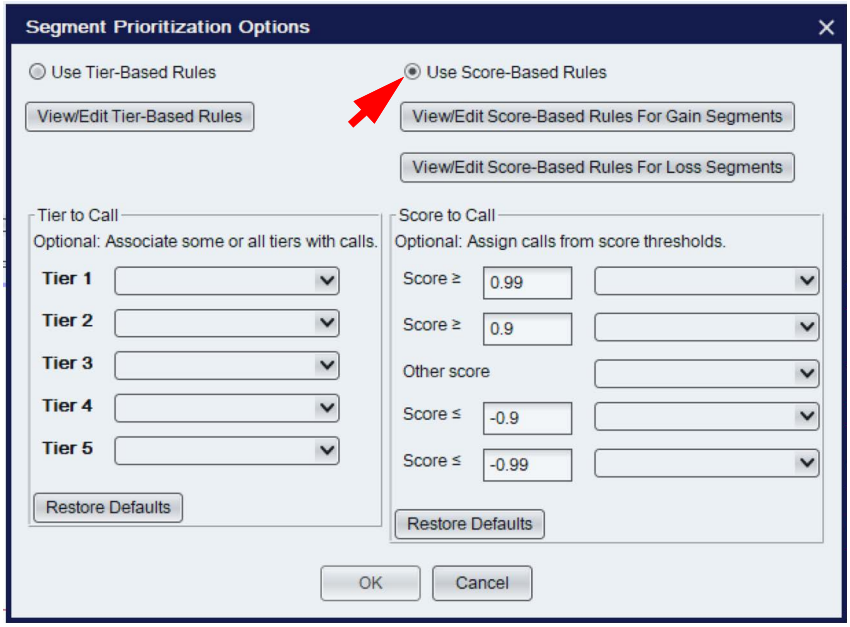


Figure 411 Segment Prioritization Options window.

2. Click the **Use Score-Based** radio button.

View/Edit Score-Based Rules for Gain Segments

1. Click the **View/Edit Score-Based Rules for Gain Segments** button.
   The Select Score Options for Gain window appears. (Figure 412)

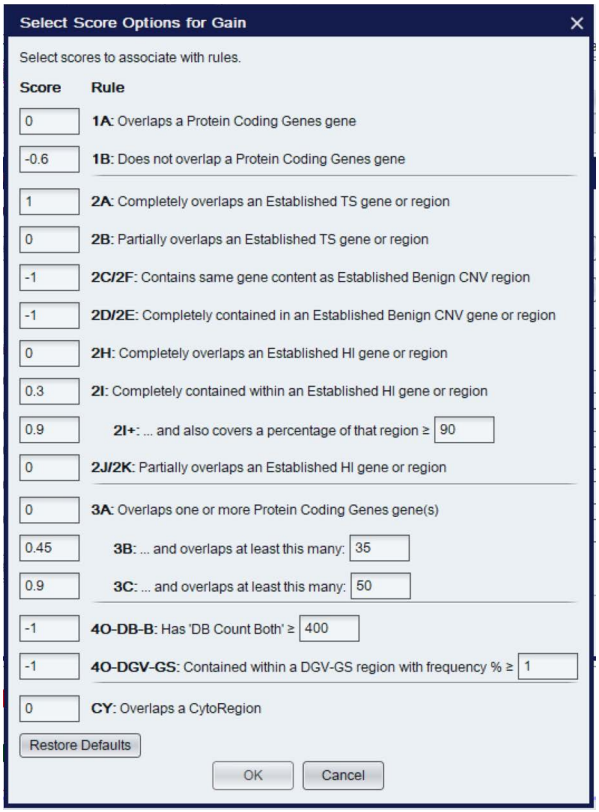


Figure 412 Select Score Options for Gain window.

2. Use the text field adjacent to the Rule to enter a new numerical. Click **Restore Defaults** to return to the factory values. See the following table for Score-based rule/evidence, descriptions, and default value information.

Table 50 Score-Based Rules for Gain.

Score-based rule/evidence	Description	Default value
1A	The Gain copy number segment fully or partially overlaps at least 1 annotation in the Protein Coding Genes track.	0
1B	The Gain copy number segment does not fully or partially overlap any annotation in the Protein Coding Genes track.	–0.6
2A	The Gain copy number segment completely overlaps an annotation in either the Triplosensitivity or Recurrent/Curated Regions track with a TS Score = 3.	1
2B	The Gain copy number segment partially overlaps an annotation in either the Triplosensitivity or Recurrent/Curated Regions track with a TS Score = 3. Partial overlap indicates one breakpoint of the Gain segment is located within the TS_Score = 3 gene/region.	0

Table 50 Score-Based Rules for Gain. (Continued)

Score-based rule/evidence	Description	Default value
2C/2F	The Gain copy number segment contains the same gene content as a Triplosensitivity or Recurrent/Curated Regions annotation with a TS Score = 40. The copy number Gain segment might be larger than the gene/region, but contains the same gene content as listed in the Triplosensitivity or Recurrent/Curated Regions tracks.	–1
2D/2E	Both breakpoints of the Gain copy number segment are contained within an annotation having a TS Score = 40 in either the Triplosensitivity or Recurrent/Curated Regions.	–1
2H	The Gain copy number segment completely overlaps an annotation in either the Haploinsufficiency or Recurrent/Curated Regions track with a HI Score = 3.	0
2I	Both breakpoints of the Gain copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency or Recurrent/Curated Regions. The copy number Gain segments is smaller than the user defined threshold (Default ≥90%).	0.3
2I+	Both breakpoints of the Gain copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency or Recurrent/Curated Regions. The copy number Gain segments is larger than the user defined threshold (Default ≥90%).	0.9
2J/2K	The Gain copy number segment partially overlaps an annotation in either the Haploinsufficiency or Recurrent/Curated Regions track with a HI Score = 3. Partial overlap indicates one breakpoint of the Gain segment is located within the HI_Score = 3 gene/region.	0
3A	The Gain copy number segment (partially or completely) overlaps at least 1 Protein Coding Gene annotation. Default is 1–34 genes.	0
3B	The Gain copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A. Default is 35–49.	0.45
3C	The Gain copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A or 3B. Default is ≥50.	0.9
4O-DB-B	The Gain copy number segment overlaps/covers a defined number of segments in your ChAS Database (DB Count Both column). Default is 400 segments. Configuration of DB Count Both parameters can be found in "Query a segment from the segment table" on page 365.	–1.0
4O-DGV-GS	Both breakpoints of the Gain copy number segment are contained within an annotation in the DGV-GS Gain (blue). The DGV-GS annotation must have an NR frequency greater than track defined. Default NR frequency is 1%.	–1.0
CY	The Gain copy number segment overlaps an annotation in the customer supplied CytoRegions File(s). For more information on CytoRegion files, see "Use CytoRegions" on page 251.	0

3. After your score assignments are complete, click **OK**.

View/Edit Score-Based Rules for Loss Segments

1. Click the **View/Edit Score-Based Rules for Loss Segments** button.
   The Select Score Options for Loss window appears. (Figure 412)

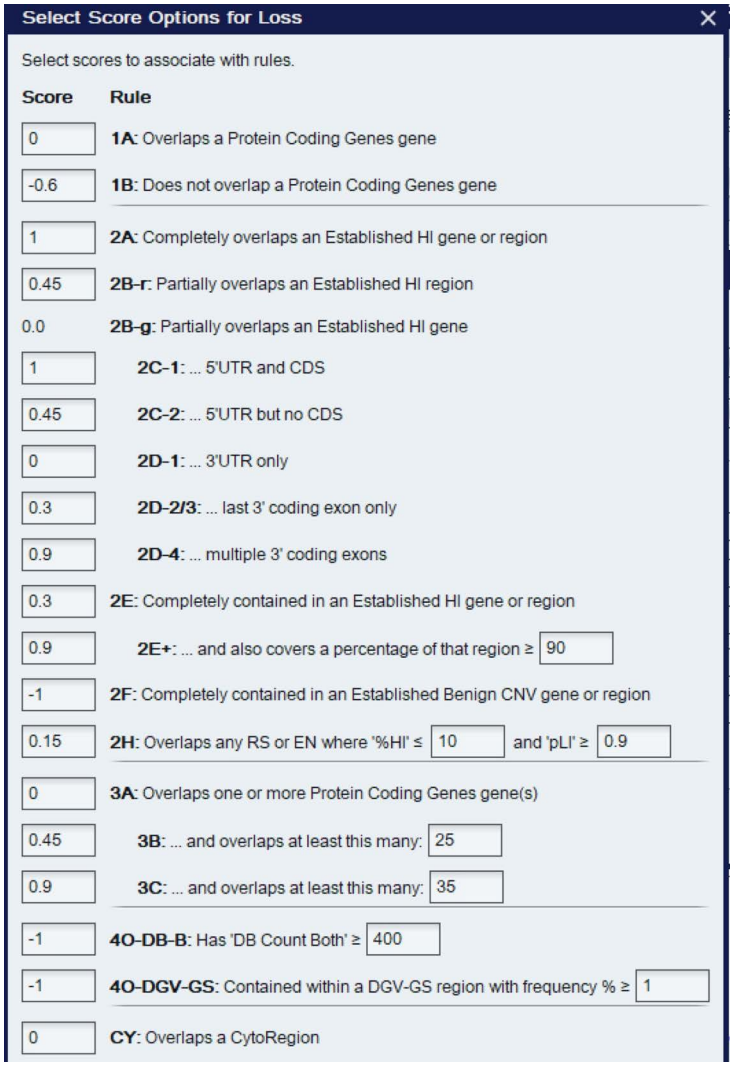


Figure 413 Select Score Options for Loss window.

2. Use the text field adjacent to the Rule to enter a new value. Click the **Restore Defaults** button to return to the factory values. See the following table for Score-based rule/evidence, descriptions, and default value information.

Table 51 Score-Based Rules for Loss.

Score-based rule/evidence	Description	Default value
1A	The Loss copy number segment fully or partially overlaps at least 1 annotation in the Protein Coding Genes track.	0
1B	The Loss copy number segment does not fully or partially overlap any annotation in the Protein Coding Genes track.	–0.6
2A	The Loss copy number segment completely overlaps an annotation in either the Triplosensitivity or Recurrent/Curated Regions track with a TS Score = 3.	1
2B-r	The Loss copy number segment partially overlaps an annotation in the Recurrent/ Curated Regions track with a HI Score = 3. Partial overlap indicates one breakpoint of the Loss segment is located within the HI Score = 3 region.	0
2B-g	The Loss copy number segment partially overlaps an annotation in the Haploinsufficiency track with a HI Score = 3. Partial overlap indicates one breakpoint of the Loss segment is located within the HI Score = 3 gene. If 2B-g is met, then move on to 2C–2E to assess a value based on location of the partial overlap.	0 (static value, further assessment required)
2C-1	The Loss copy number segment overlaps the 5'UTR and some CDS of a gene with HI score = 3 in the Haploinsufficiency track. **TIP:** Right-click the transcript, choose **View/Edit Annotation Properties**, then select the Structure tab to view the exons and CDS coordinates. **Note:** All transcripts for a gene are assessed as long as the transcript is ≤90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols.	0.9
2C-2	The Loss copy number segment overlaps the 5'UTR, but no CDS of a gene with HI score = 3 in the Haploinsufficiency track. **TIP:** Right-click the transcript, choose **View/Edit Annotation Properties**, then select the Structure tab to view the exons and CDS coordinates. **Note:** All transcripts for a gene are assessed as long as the transcript is ≤90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols.	0
2D-1	The Loss copy number segment overlaps the 3'UTR only, no CDS is involved for a gene with HI score = 3 in the Haploinsufficiency track. **TIP:** Right-click the transcript, choose **View/Edit Annotation Properties**, then select the Structure tab to view the exons and CDS coordinates. **Note:** All transcripts for a gene are assessed as long as the transcript is ≤90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols.	0
2D2/2D3	The Loss copy number segment overlaps the 3'UTR AND the last exon in the coding region for a gene with HI_score = 3 in the Haploinsufficiency track. **TIP:** Right-click the transcript, choose **View/Edit Annotation Properties**, then select the Structure tab to view the exons and CDS coordinates. **Note:** All transcripts for a gene are assessed as long as the transcript is ≤90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols.	0.3

Table 51 Score-Based Rules for Loss. (Continued)

Score-based rule/evidence	Description	Default value
2D-4	The Loss copy number segment overlaps the 3'UTR AND multiple exons in the coding region for a gene with HI_score = 3 in the Haploinsufficiency track. **TIP:** Right-click the transcript, choose **View/Edit Annotation Properties**, then select the Structure tab to view the exons and CDS coordinates. **Note:** All transcripts for a gene are assessed as long as the transcript is ≤90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols.	0.9
2E	Both breakpoints of the Loss copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency track or Recurrent/ Curated Regions track. The copy number Loss segment is smaller than the annotation in the track by less than the user defined threshold (Default ≥90%).	0.3
2E+	Both breakpoints of the Loss copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency track or Recurrent/ Curated Regions track. The copy number Loss segment is larger than the annotation in the track by less than the user defined threshold (Default ≥90%).	0.9
2F	Both breakpoints of the Loss copy number segment are contained within an annotation having a HI_ Score = 40 in either the Haploinsufficiency track or Recurrent/ Curated Regions track.	–1
2H	The Loss copy number segment overlaps a Protein Coding Gene or Protein Coding Ensembl Gene with predicted haploinsufficiency values meeting the defined thresholds. pLI derived from gnomAD (https://gnomad.broadinstitute.org/) and %HI derived from DECIPHER (https://decipher.sanger.ac.uk/).	0.15
3A	The Loss copy number segment (partially or completely) overlaps at least 1 Protein Coding Gene annotation. Default is 1–24 genes.	0
3B	The Loss copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A. Default is 25–34.	0.45
3C	The gain copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A or 3B. Default is ≥35.	0.9
4O-DB-B	The Loss copy number segment overlaps/covers a defined number of segments in your ChAS Database (DB Count Both column). Default is 400 segments. Configuration of DB Count Both parameters can be found in "Query a segment from the segment table" on page 365.	–0.9
40-DGV-GS	Both breakpoints of the Loss copy number segment are contained within an annotation in the DGV-GS gain (red). The DGV-GS annotation must have an NR frequency greater than track defined. Default NR frequency is 1%.	–0.9
CY	The Gain copy number segment overlaps an annotation in the customer supplied CytoRegions File(s). For more information on CytoRegion files, see "Use CytoRegions" on page 251.	0

3. After your score assignments are complete, click **OK**.

Configure the score-based option

1. From the Segment Prioritization Options window, go to the Score to Call pane. (Figure 414)

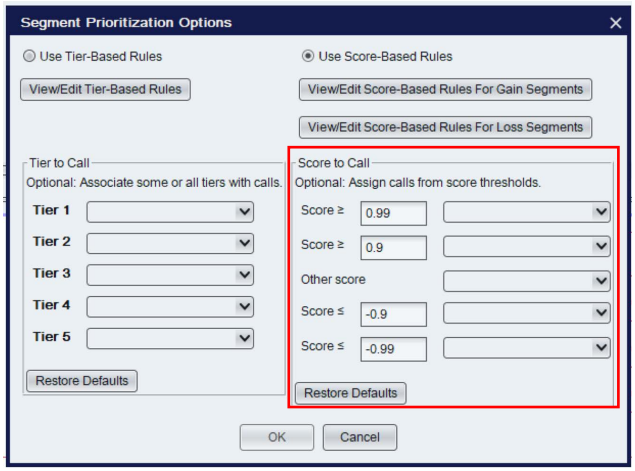


Figure 414 Score to Call pane.

- Define the Score Thresholds: In the appropriate text field, enter a Call based on the segment score as defined above. Your entered threshold values for each Call are populated in the Segment Table's **Call from Prioritization** column.
- Select Calls: Use the dropdown lists adjacent to each threshold to assign a Call that is associated with a range of scores.

Note: Calls in the dropdown lists can be customized by adding to the Calls Vocabulary window in the User Configuration.

In the following example (Figure 415), a copy number segment with a Score of 1.3 would have a Call from Prioritization assignment of "Level 1". A copy number segment with a score of –0.96 would have a Call from Prioritization assignment of "Probably nothing".

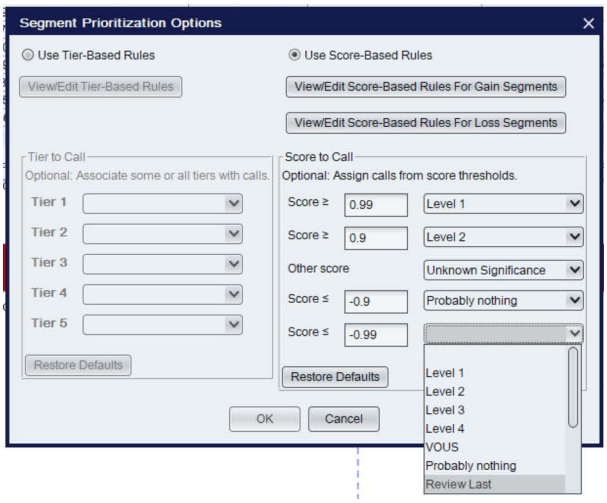


Figure 415 Score to Call dropdown lists.

2. Click **OK** to accept the Score thresholds and Calls or click Cancel to return to the ChAS Browser without saving any new assignments. Click the **Restore Defaults** button to return to the factory values.

View segment prioritization in the segments table

Three new segment prioritization columns now appear in the Segment Table. (Figure 409)

Microarray Nomenclature	Call	Call From Prioritization	Evidence	▼ Tier or Score	D
arr[GRCh37] 22q11.1q11.21(16888900_22141230)x1-2		*Level 1*	1A 2A ("22q11.2_recurrent_(DGS/VCFS)_re...	1.9	
arr[GRCh37] 22q11.21(18626109_21915509)x1		*Level 1*	1A 2A ("22q11.2_recurrent_(DGS/VCFS)_re...	1.9	
arr[GRCh37] 20q11.23(36895218_36987660)x1		*Unknown Significance*	1A 3A (2)	0	
arr[GRCh37] Xq28(154912942_154946888)x0		*Unknown Significance*	1A 3A (1)	0	
arr[GRCh37] Xq21.31(86337774_86693619)x2	Benign	*Unknown Significance*	1B	-0.6	
arr[GRCh37] 14q32.33(106160630_106736911)x3		*Review Last*	1B 4O-DB-B (3217)	-1.5	
arr[GRCh37] 1q44(248753184_248795277)x1		*Review Last*	1A 3A (2) 4O-DB-B (794) 4O-DGV-GS (gssv...	-1.8	
arr[GRCh37] 5q35.3(180378754_180430789)x1		*Review Last*	1A 3A (1) 4O-DB-B (531) 4O-DGV-GS (gssv...	-1.8	
arr[GRCh37] 11q11(55374019_55452996)x0		*Review Last*	1A 3A (3) 4O-DB-B (1366) 4O-DGV-GS (gss...	-1.8	
arr[GRCh37] 8p11.22(39247098_39386952)x1		*Review Last*	1B 4O-DB-B (2106) 4O-DGV-GS (gssvL124...	-2.4	
arr[GRCh37] 16q12.2(55796376_55822431)x1		*Review Last*	1B 4O-DB-B (557) 4O-DGV-GS (gssvL4460...	-2.4	

Figure 416 New Segment Table columns.

- **Call From Prioritization**: Displays the "Call" associated with the score threshold ranges.
- **Evidence**: Displays the rules met based on which annotations the copy number segment overlaps.
- **Tier or Score**: This is a numeric value representing the score generated and assigned to the copy number segment based on the user-defined Score-Based rules selected.

If the Call from Prioritization assignments are correct, they can be accepted as the Calls for each segment. To do this:

1. Click the  button.

   The Call from Prioritization assignments are copied into blank cells in the Call column.

   Note: Any Calls manually assigned will remain in the Call column and not be overwritten. Segments hidden by the filters will not have calls copied from the Calls from Prioritization column.

   A confirmation message appears (Figure 417) summarizing the Call from Prioritization assignments are to be copied into the Call column.

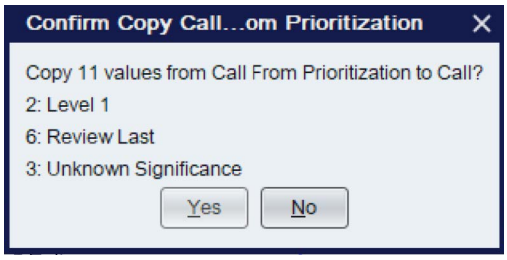


Figure 417 Confirm copy into Call column.

2. Click **Yes** to acknowledge the message.

18 Interact with the ChAS Database

A segment can be queried against the ChAS Database for intersecting segments from previously published samples. Using both the Overlap Threshold and Coverage Threshold can focus the query results to segments that are of approximately the same size as the segment in the current sample.

Note: ReproSeq™ Aneuploidy data can not be published into the ChAS DB.

Set up a ChAS DB query

1. From the ChAS Browser, click **Preferences → Edit User Configuration**.
2. Click the **DB Query** window tab. (Figure 418)

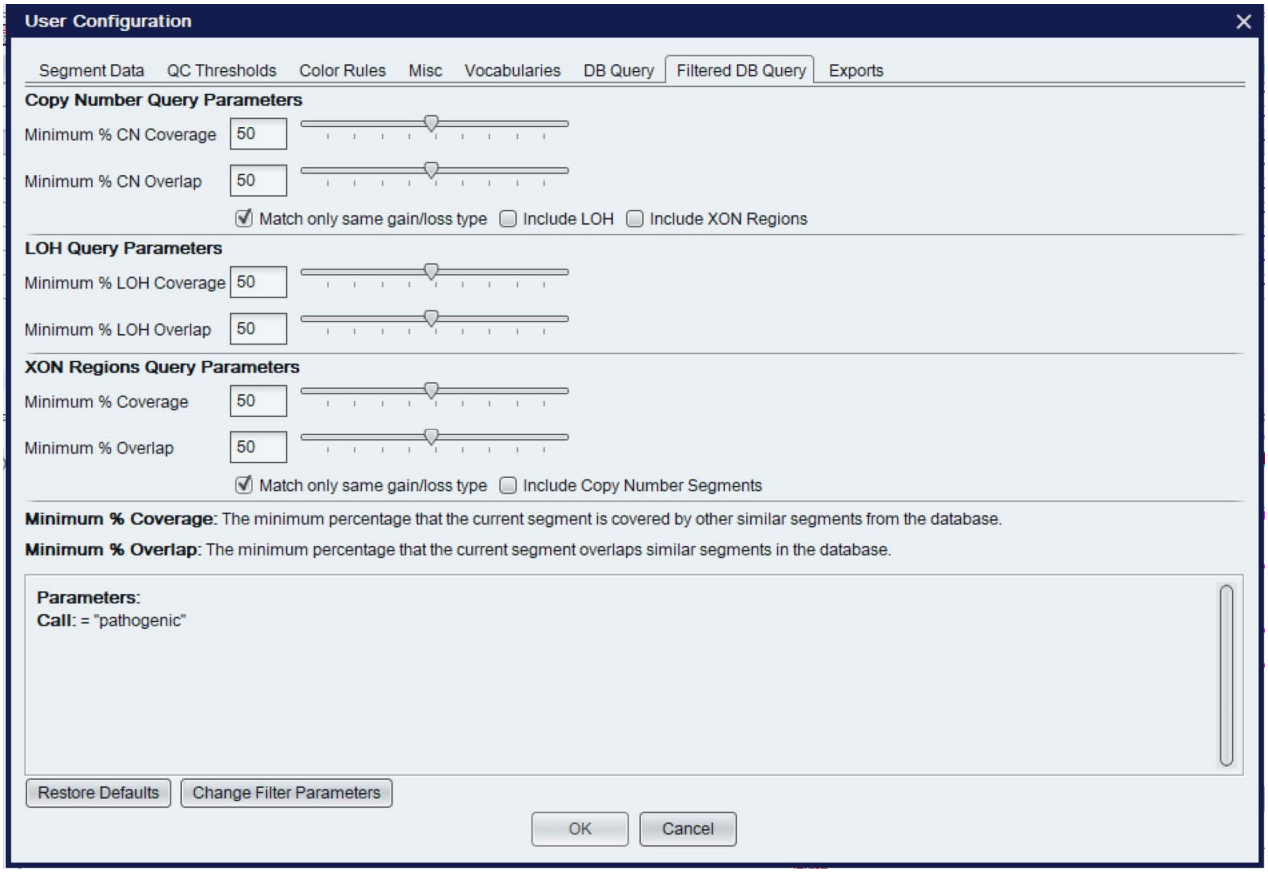


Figure 418 User Configuration - DB Query window/tab.

Set up query parameters for a copy number search

Note: When querying on a copy number segment in the Browser, the values set in the Copy Number Query Parameters section are used.

1. Enter minimum percentage values for both Overlap and Coverage using the text boxes or click and drag the appropriate slider bar. (Figure 419)

   Note: The default values are set to 50%.

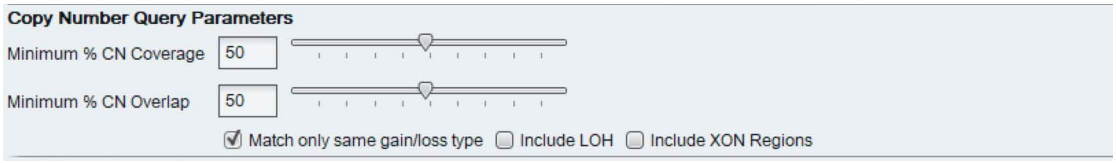


Figure 419 Copy Number Query Parameters.

2. Select the **Match only same gain/loss type** checkbox if you want to query the database for only similar copy number types (gains only or losses only). Deselect this checkbox if you want to query all copy number segment types.
3. Select the **Include Exon Regions** checkbox if you want to include Exon Region Segments in your query.
4. Select the **Include LOH** box to include LOH segments in your query.
5. Click **OK** to save your changes or click **Restore Defaults** to return the parameter settings back to their default settings.

Set up query parameters for an LOH segment search

Note: When querying on an LOH segment in the Browser, the values set in the LOH Query Parameters section are used.

1. Enter minimum percentage values for both Overlap and Coverage using the text boxes or click and drag the appropriate slider bar. (Figure 420)

   Note: The default values are set to 50%.

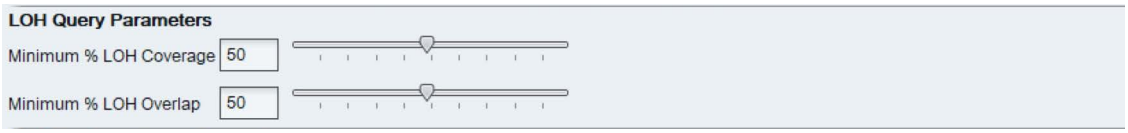


Figure 420 LOH Query Parameters.

2. Click **OK** to save your changes or click **Restore Defaults** to return the parameter settings back to their default settings.

Set up query parameters for an XON region segment search

Note: When querying on an XON Region segment in the Browser, the values set in the Exon Region Query Parameters section are used.

1. Enter minimum percentage values for both Overlap and Coverage using the text boxes or click and drag the appropriate slider bar. (Figure 421) The default values are set to 50%.

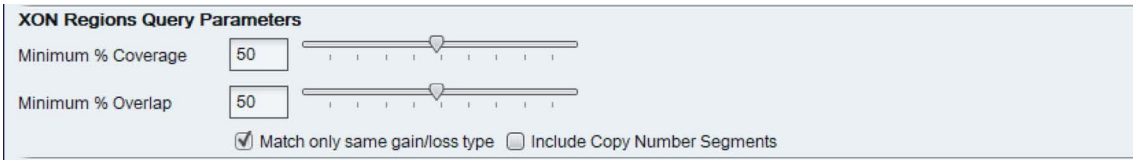


Figure 421 XON Regions Query Parameters.

2. Select the **Match only same gain/loss type** checkbox if you want to query the database for only similar XON Region segment types (gains only or losses only). Deselect this checkbox if you want to query all XON Region segment types.
3. Select the **Include Copy Number Segments** checkbox to include Copy Number Segments in your query.
4. Click **OK** to save your changes or click **Restore Defaults** to return the parameter settings back to their default settings.

Query a segment from the segment table

To view what segments in the database intersect with the currently loaded segment, you must first make the **DB Count Both** column visible. The DB Count Both column displays the number of segments in the database that meet the criteria set in the DB Query tab.

1. Right-click in the DB Count Both cell for the segment(s) you want to view, then click **Query ChAS DB for "DB Count Both"**. (Figure 422)

Note: Segments matching ONLY the Coverage OR Overlap thresholds can also be returned in the Segment Intersections window by right-clicking in either the DB Coverage Count or DB Overlap Count columns respectively. You can also right-click the segment in the Detail View and choose Query ChAS DB to return segments meeting either the Coverage OR the Overlap thresholds. See "Filter DB count columns" on page 374. If you want to add column(s) to your Segment table, see "Select columns to display or hide" on page 307.

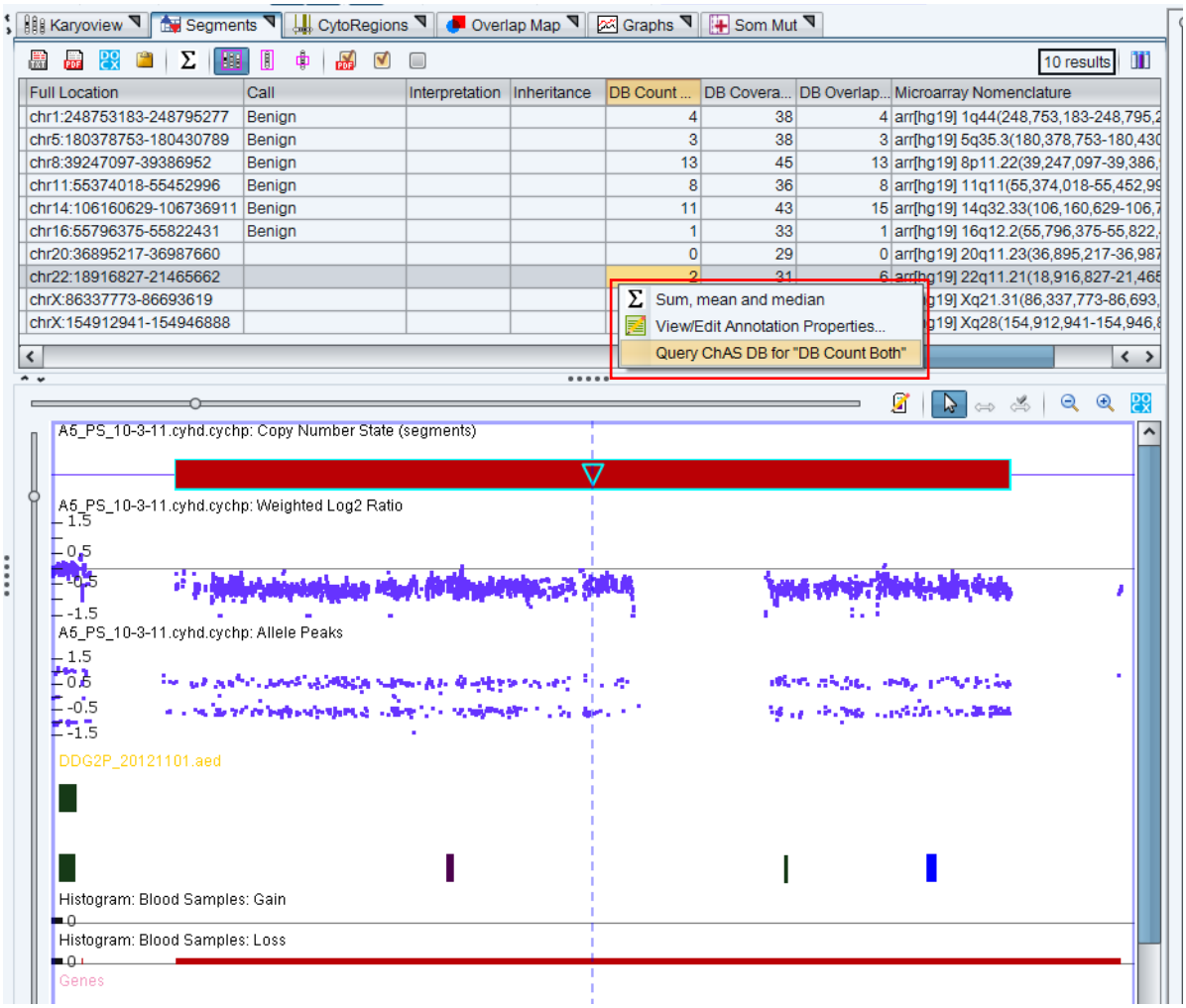


Figure 422 Query ChAS DB for “DB Count Both”.

Segment intersections

The **Segment Intersections** view appears with the results from the query. (Figure 423)

The Segment Intersections view shows samples in the database that contain segments that meet the criteria set in the DB Query.

The middle portion of this view contains table information about the samples in the database including any Call, Interpretation or Inheritance information assigned to the segments for the samples shown in the example above. To display or hide columns within this table, click  (upper right corner).

The lower portion of the view provides the same external annotations available in the Detail View. To display an annotation track, go to the ChAS Browser’s Files Menu and check the box. The annotation track will then be displayed in both the Detail View and the Segment Intersections View. (Figure 423)

You can return segments from the database based on either DB Overlap or DB Coverage. These segments meet either one of the overlap or coverage threshold criteria.

Note: Segment Intersection search results are limited to 1000 intersecting segments. When querying on a copy number segment in the Browser, the Copy Number Query Parameter Thresholds are used for all segment types. When querying on an LOH segment in the Browser, the LOH Query Parameter Thresholds are used for all segment types.

Example: You have a gain segment in the ChAS Browser and run a query to retrieve intersecting segments. Both Gain and LOH segments counts appear in DB Count Both, the LOH segment being returned are using the Copy Number Query Parameter Thresholds since the original segment is a GAIN.

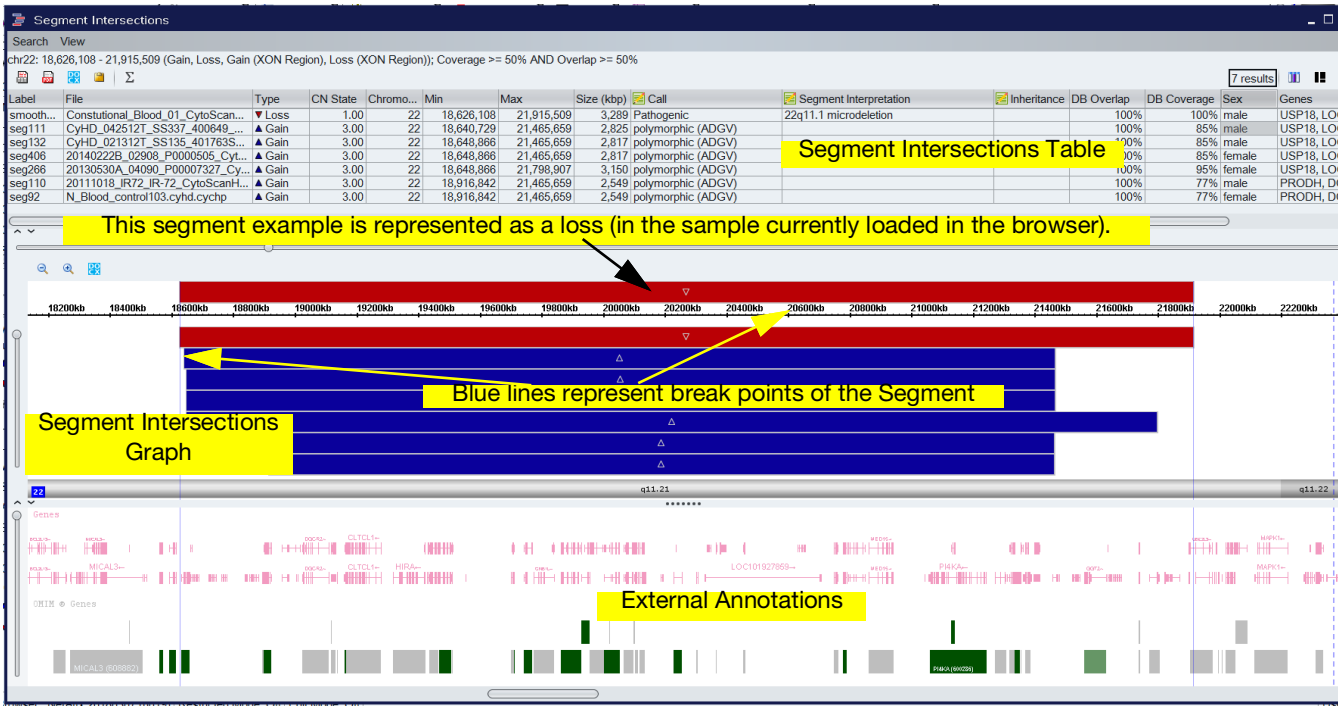


Figure 423 Query ChAS DB - Segment Intersections window.

Click the Side-by-Side icon (upper right) to split the Segment Intersections window, as shown in Figure 424 on page 368.

Note: Columns with a pad and pencil icon represent a segment field that can be edited. All edits are stored directly to the database.

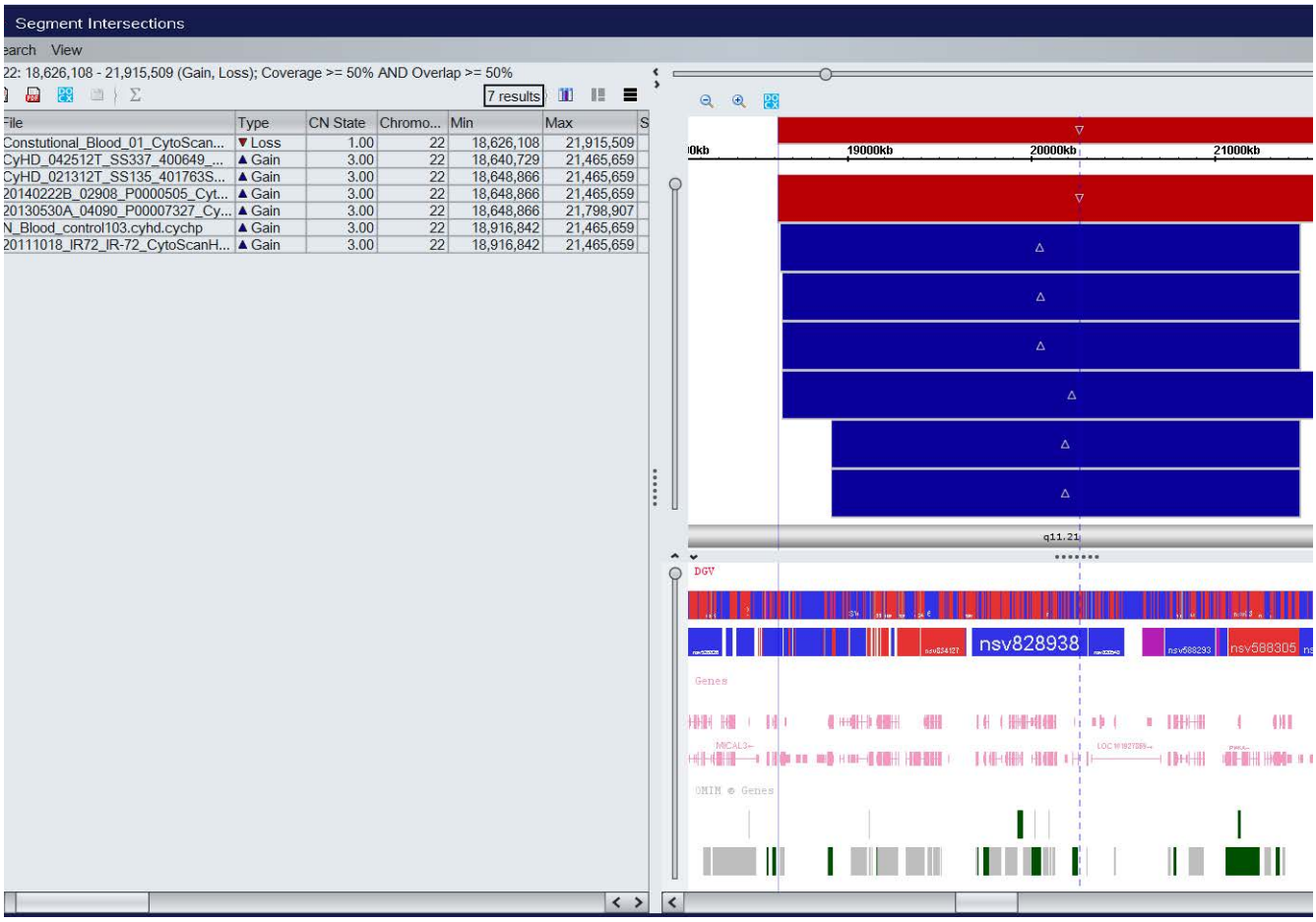


Figure 424 Query ChAS DB - Segment Intersections window (Side-by-Side).

Note: Columns listed with an adjacent icon denote the column is user-editable.

Table 52 Segment Intersection View table columns.

Column	Description
Call	User-editable field populated by a user-configurable dropdown list of Calls.
Inheritance	User-editable field populated by user-configurable dropdown list of Inheritance.
Oncomine Reporter	Displays the Oncomine™ Reporter terminology assigned to the segment.
Segment Interpretation	Displays Segment Interpretation assigned to this sample.
% of Overlap Item covered by Segment	The percentage of the Overlap Map Item covered by the segment.
Call from Prioritization (Stored)	The Call term assigned based on Tier or Score Classification at the time the sample was published to ChAS DB.

Table 52 Segment Intersection View table columns. (Continued)

Column	Description
Chromosome	Chromosome on which the item is located.
CN State	Copy Number State.
CytoRegions	Names of the CytoRegions with which the segment shares coordinates.
DB Coverage	Number of segments in the database meeting the minimum Percent Coverage Count.
DB Overlap	Number of segments in the database meeting the minimum Percent Overlap Count.
DGV	List of DGV variations that share coordinates with the segment.
Evidence (Stored)	Provides information on which annotations the segment overlapped at the time the sample was published to ChAS DB.
Genes	List of RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Interpretation	User-editable field for free-text interpretation on the segment.
Label	Identifier for the item.
Max	Zero-based index position of the last base pair in the sequence, plus one. Adding one ensures that the length of any (hypothetical) segment containing a single marker would be one, and ensures that the coordinates match the coordinate system used in BED files. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table
Min	Zero-based index position of the first base pair in the sequence.
OMIM Genes	List of OMIM™ Genes that share coordinates with the segment.
Overlap Map	Item(s) in the Overlap Map which overlap the segment.
Overlap Map Items	The percentage of the segment that is overlapped by the Overlap Map Item.
Phenotype	Displays Phenotype annotation assigned to this sample.
Published	Displays the date and time of your query.
Publisher	Displays Publisher's name.
Sample DB ID	A xxCHP file ID automatically assigned when the xxCHP file is published to the database.
Sample Type	Displays the Sample Type assigned to this xxCHP file.
Sample UUID	Unique identifier for the CHP file.
Segment DB ID	An ID automatically assigned to each segment when the xxCHP file is published to the database
Segment ID	The unique identifier for the copy number segment.
Segmental Duplications	List of Segmental Duplications that share coordinates with the segment.
Sex	Displays Male or Female.
Size (kpb)	Size of the item.

Table 52 Segment Intersection View table columns. (Continued)

Column	Description
Tier or Score (Stored)	The assigned Tier or Score value based on the Segment Prioritization method selected at the time the sample was published to ChAS DB. When using Tier based, the column will display the assigned Tier. When using Score based, the column will display the score value based on the annotations the segment overlaps.
Type	Type of segment (Gain, Loss, Gain Mosaic, LossMosaic, LOH and LOHMosaic) or annotation.

Additional segment intersection information

Additional Segment Intersection information becomes available after querying a ChAS DB that has been remapped from a previous genome build that includes additional columns that are populated in the Segment Intersection Table. These remapped segments, are also represented in different graphical patterns, as shown in Figure 425 and detailed in Figure 426.

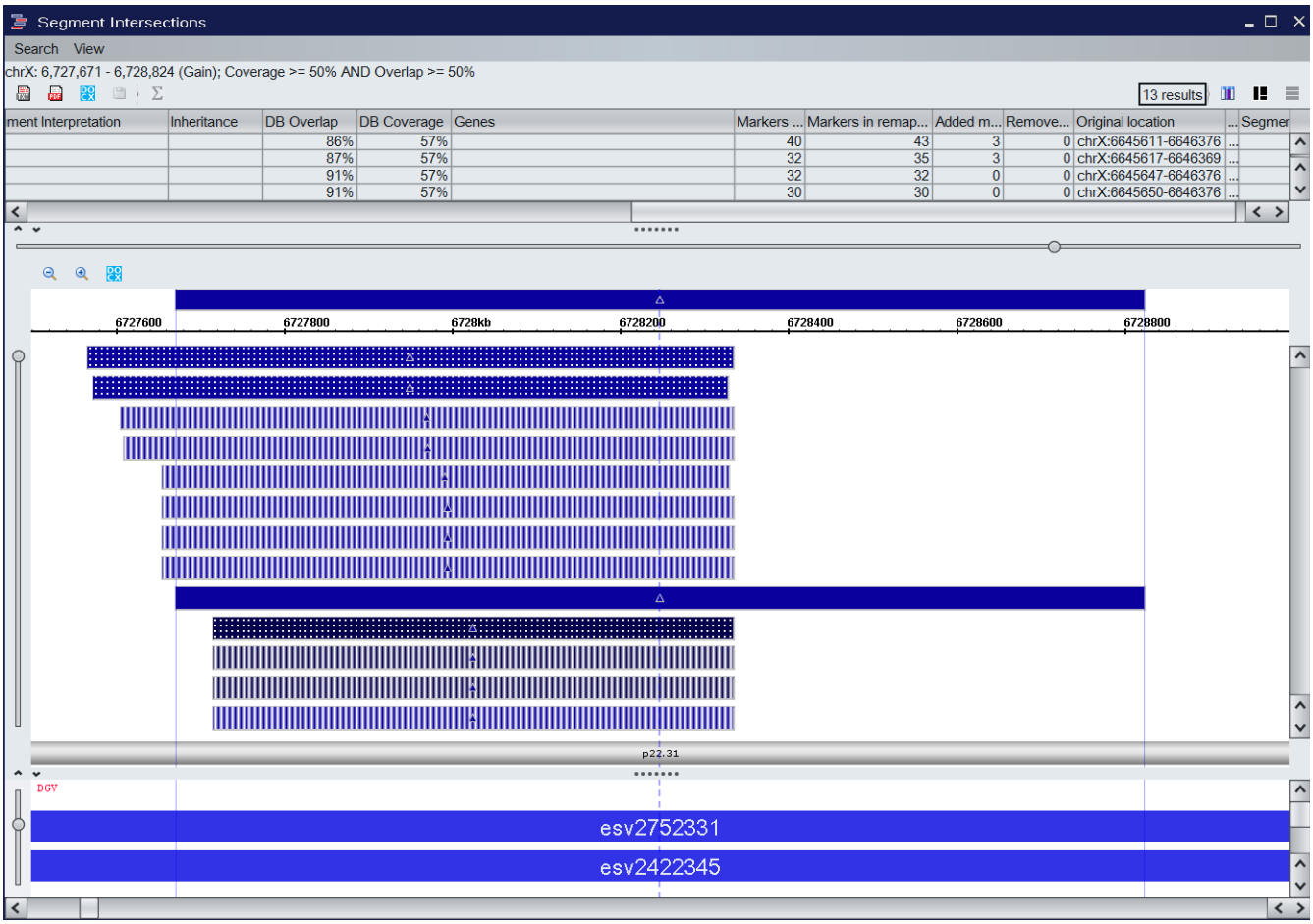


Figure 425 Additional Segment Interaction Information example.

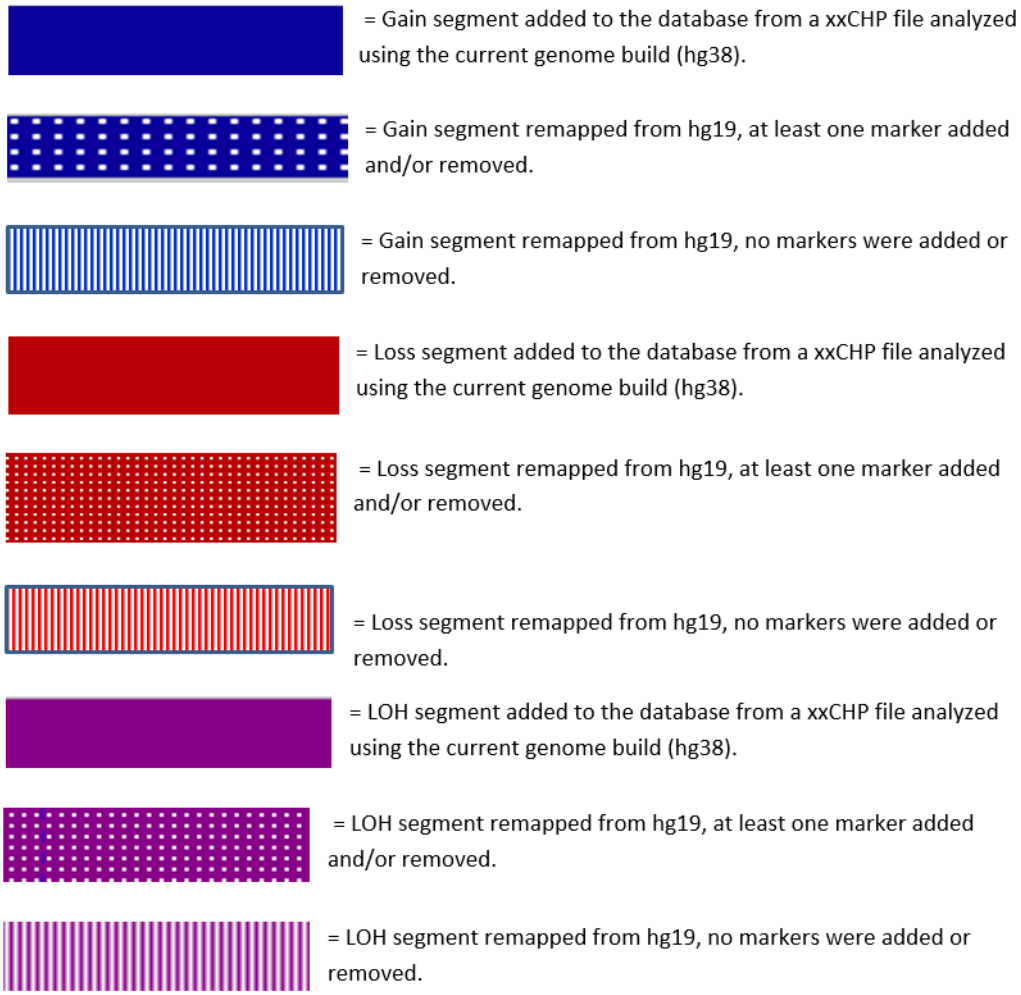


Figure 426 Additional Segment Interaction Legend.

Table 53 Segment Intersection table columns.

Column	Description
Original location	Chr:start-stop genome location of the original genome build for the segment.
Markers in original segment	Total number of markers in the original segment.
Added markers	Number of markers to the segment added by remapping.
Removed markers	Number of original markers removed from the segment from remapping.
Segment length difference	Size difference in the segment (Original - Remapped).
Original Genome Build	Genome Build from the original analysis prior to remapping.
Markers in remapped segment	Total number of markers in the remapped segment.

Download segments from a sample file in ChAS DB

From the Segment Intersection window, all the segments for a selected sample can be downloaded and viewed in the Segments Table and Detail View. Only the segment data and annotations from the sample are displayed.

Note: Files downloaded from ChAS DB can not be opened in the MSV.

Download and view Segments from a sample(s) stored in ChAS DB

1. From the Segment Intersections window, right-click the sample(s) in either the table or the graphical view.
2. Select **Download file(s) from ChAS DB**. (Figure 427)

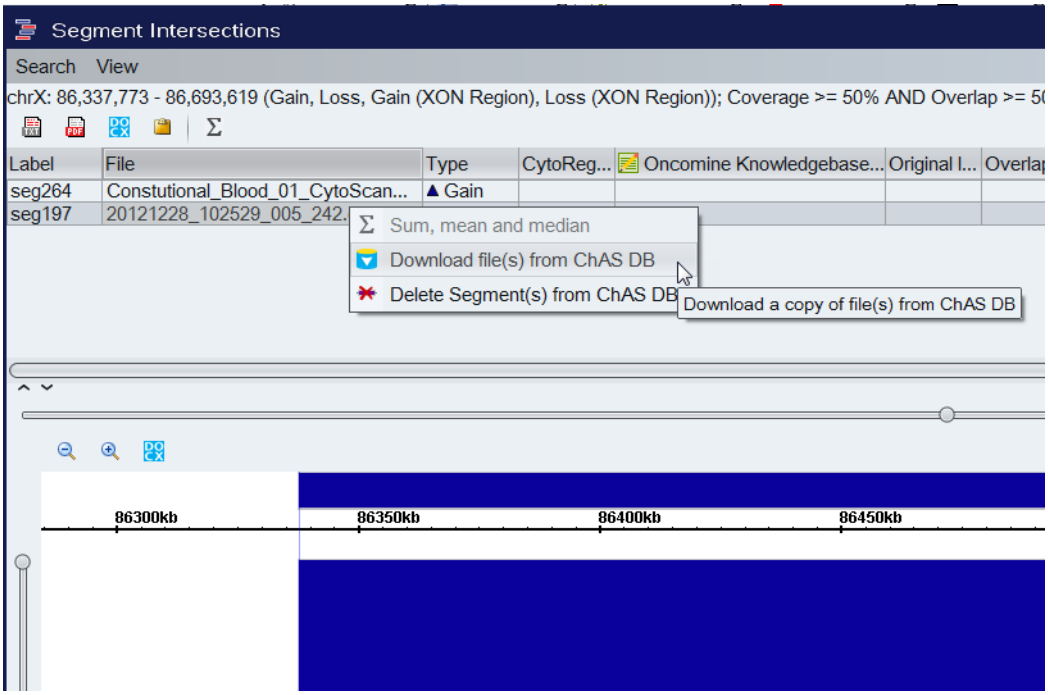


Figure 427 Segment Intersections - Download file(s) from ChAS DB.

Note: As shown in Figure 428, sample files downloaded from ChAS DB are listed in the Files Tree with a database symbol. Segments from samples files downloaded from ChAS DB are listed in the Segments Table with a database symbol in the File column.

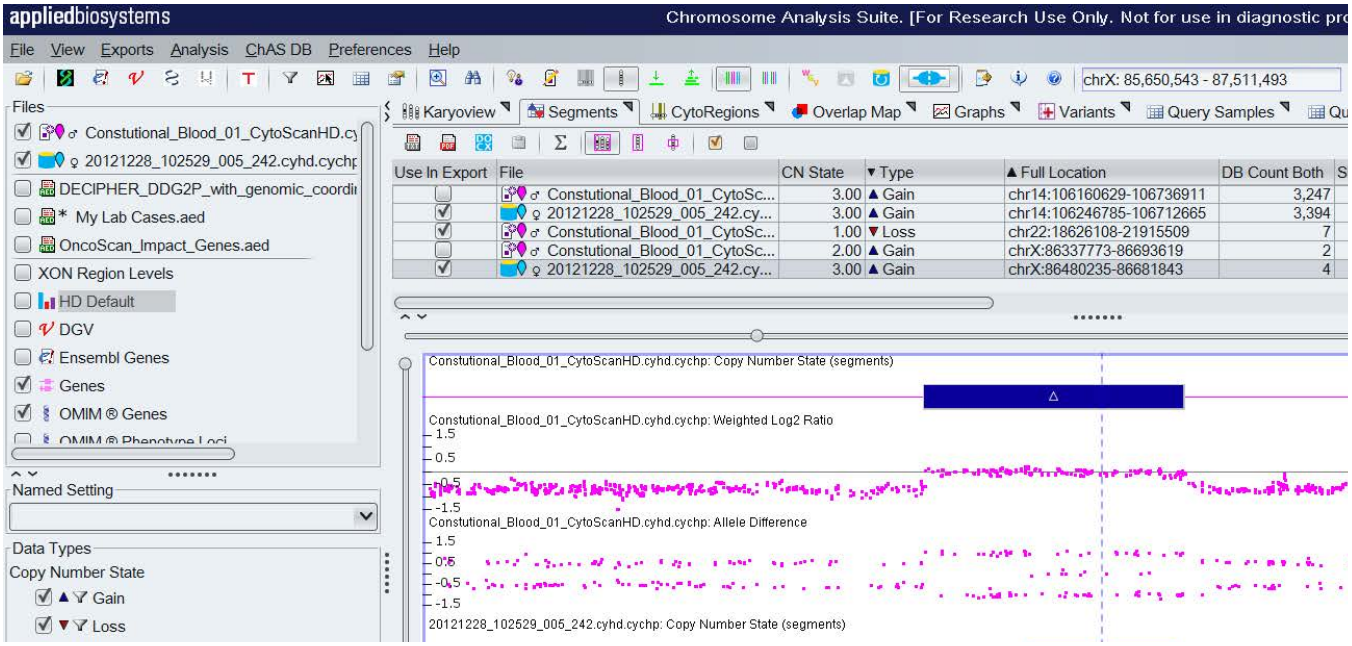


Figure 428 Segment Intersections - Download file(s) from ChAS DB.

Note: Downloaded segments can be deleted when in Edit Mode, but no other segment modifications are enabled as the underlying data is unavailable.

Update downloaded segment annotations

Segment annotations in the ChAS DB can be updated using the following methods:

- Right-clicking the segment and selecting **View/Edit Annotation Properties → Curation Tab**.
- Clicking a column/field pad and pencil  icon to edit the segment annotation(s).
- Right-clicking the Filename in the Files tree, select **View/Edit Properties → Sample Properties**.

Filter DB count columns

Filtered DB count columns are available in the Segment Table and reflect the number of segments in the database matching the filtered criteria.

Note: The DB count columns reflect the number of segments in the database meeting the Minimum Percent Overlap/Coverage criteria only. The Filtered DB count columns allow you to display segments that not only meet the Minimum Percent Overlap/Coverage criteria, but also additional filters such as Call or Gender.

1. Click **Edit User Configuration**, then click the **Filtered DB Query** tab. (Figure 429)

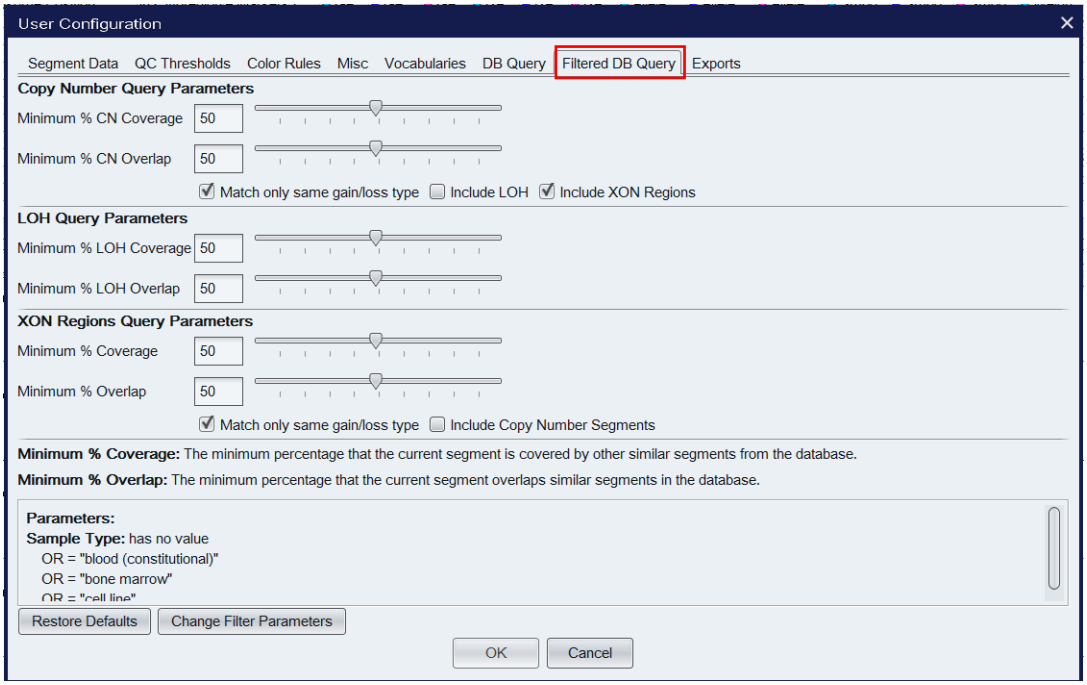


Figure 429 User Configuration - Filtered DB Query window/tab.

2. See "Set up a ChAS DB query" on page 363 for how to set the Percent Minimum Overlap/Coverage Thresholds.
3. Return to the **Filtered DB Query** tab.
4. The **Filtered DB Query** tab displays the current filter setting. To change it, click **Change Filter Parameters**.

   A Set Filter Parameters window appears. (Figure 430)

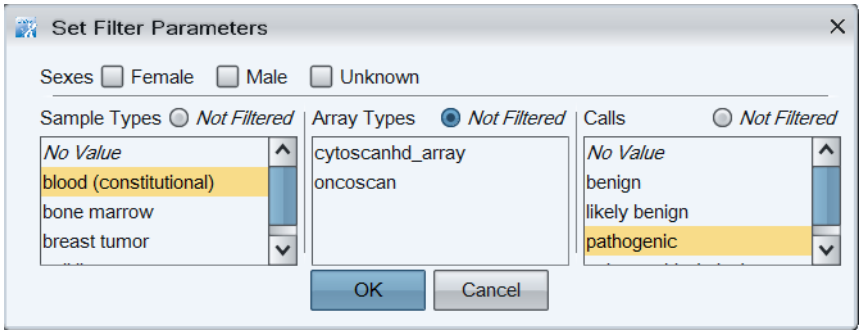


Figure 430 Set Filter Parameters window.

5. Use the windows checkboxes, radio buttons, and selections to change your filter parameters.
6. Click **OK**.

   Your new query parameters are saved and displayed at the bottom of the Filtered DB Query window tab, as shown in Figure 431.

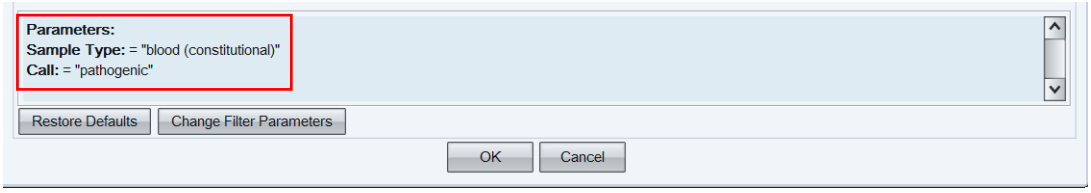


Figure 431 Displayed Filter Parameters example.

To reset the Filtered DB Query Parameters back to default settings, click **Restore Defaults**. For more information see, "Filter DB count columns" on page 374.

Figure 432 shows the **DB Count Both** and **Filtered DB Count Both** columns. It illustrates that DB Count Both queries the database for all segments matching the Minimum Percent Overlap/Coverage and gain/loss/LOH parameters. The Filter DB column reflects the additional Filter Criteria.

Microarray Nomenclature (ISCN 2016)	Full Location	▼ DB Count Both	Filtered DB Count Both	Size (kbp)	Ma
arr[GRCh37] 14q32.33(105864870_107283202)x2-3	chr14:105864870-107283202	163	27	1,418	
arr[GRCh37] Xq28 or Yq12(154941868_155233731 or 59044874...	chrX:154941868-155233731	7	3	292	
arr[GRCh37] 8p11.22q24.3(38556627_146295771)x2-3	chr8:38556627-146295771	7	0	107,739	
arr[GRCh37] 5p15.33p13.2(113576_38205477)x2-3	chr5:113576-38205477	5	0	38,092	
arr[GRCh37] Xp22.33(168546_2703986)x3-4	chrX:168546-2703986	3	0	2,535	
arr[GRCh37] 21q21.1q22.12(20605960_37047544)x2-3	chr21:20605960-37047544	3	0	16,442	
arr[GRCh37] 22q11.1q13.33(16888899_51197838)x2-3	chr22:16888899-51197838	3	0	34,309	
arr[GRCh37] 17q21.32q22(45010182_57572273)x2-3	chr17:45010182-57572273	3	0	12,562	
arr[GRCh37] 17q24.1q25.1(63940809_72905641)x2-3	chr17:63940809-72905641	3	0	8,965	

Figure 432 Displayed Filter Parameters example.

Query overlapped segments

1. Right-click a Segment of interest within the Detail View. (Figure 433)
2. Click **Query ChAS DB**.

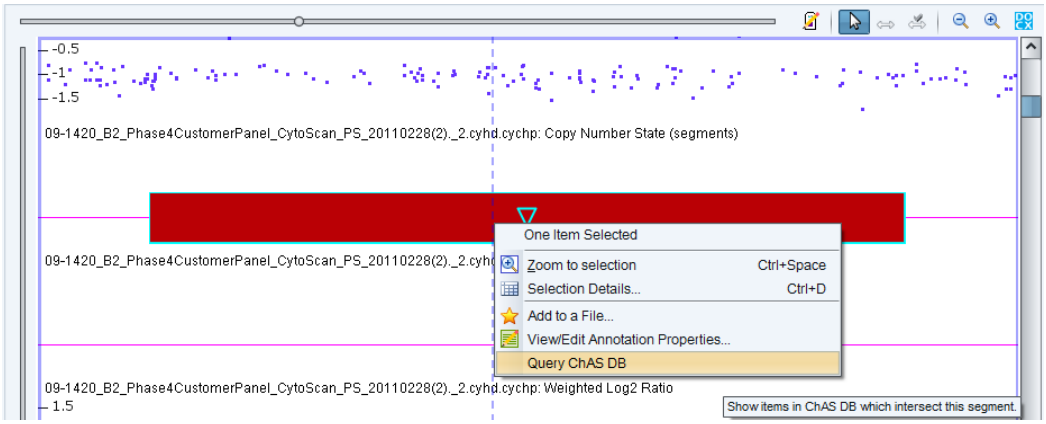


Figure 433 Query ChAS DB from Detail View.

The results returned when querying a segment in the detail view will contain segments that meet the DB Coverage filter or the DB Overlap filter set up previously. (Figure 418 on page 363)

Change or refine the DB query criteria

1. From the upper left corner, click **Search → Search Again...** (Figure 434)

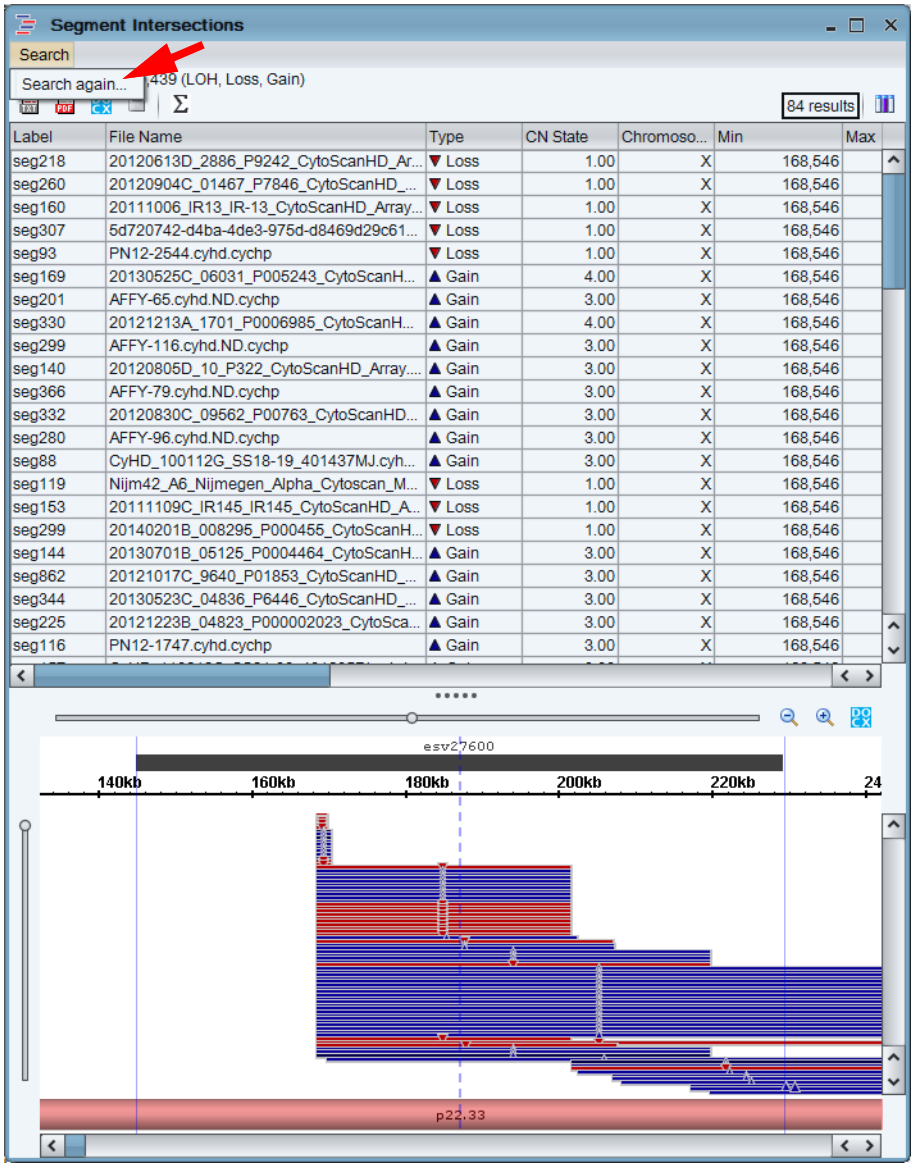


Figure 434 Segments Intersections window.

The **Search parameters** window appears. (Figure 435)

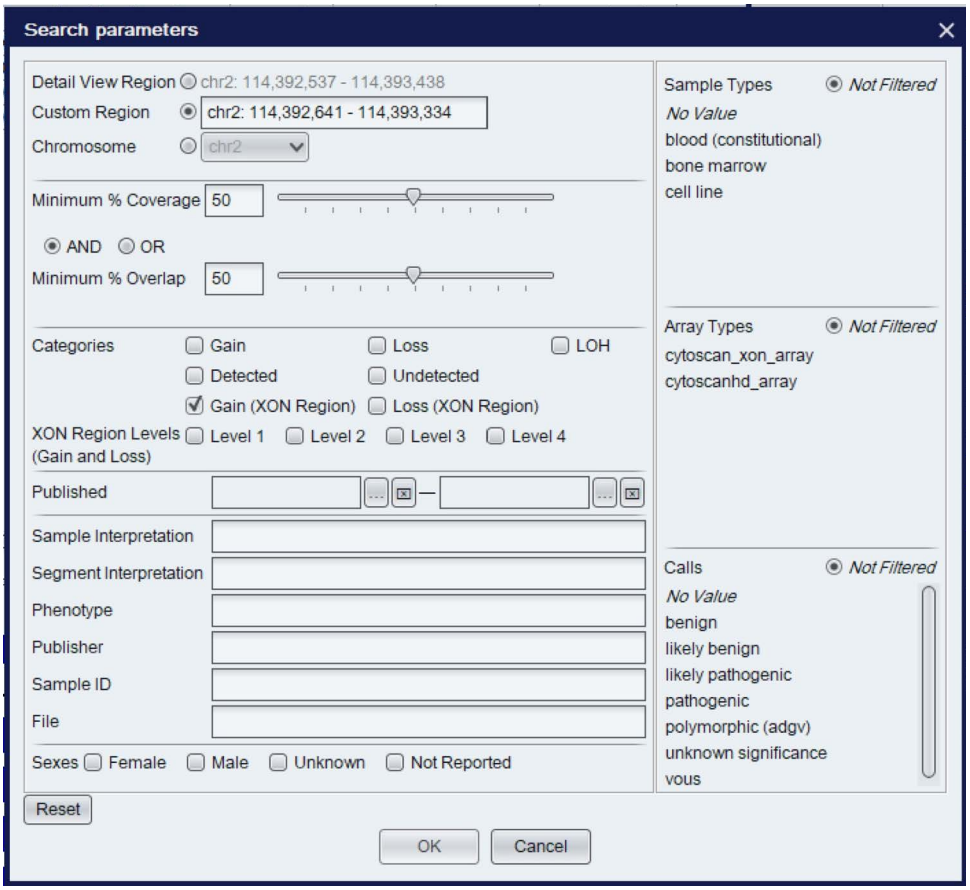


Figure 435 Search Parameter window.

2. Use the provided radio buttons, checkboxes, and text fields to customize your search, then click **OK**.

Note: Altering these parameters only affects the current segment query. The following fields, **Sample Types**, **Array Types** and **Calls** are populated based on what has been published by the user into the ChAS Database If the ChAS Browser is unable to contact the database, these fields are populated based on the library file and vocabularies entries.

Publish data to the database

After sample and its segments have been analyzed, curated and annotated, it can be added to a database by a process called publishing.

IMPORTANT! You *must* have Manager or Admin Role permissions to publish data to the database. For details, see "Administration" on page 423.

Publish data or multiple data to the database

Method 1

1. In the File tree, right-click a file name, then click **Publish File(s) to Database...** (Figure 436)

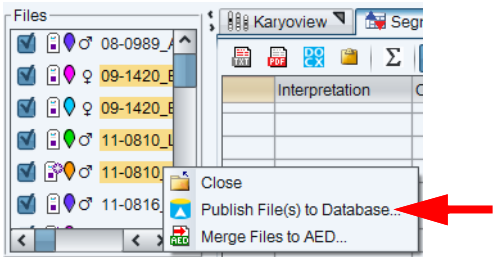


Figure 436 Example: Multiple files selected for publishing to the database.

Method 2

1. Click to highlight the sample name(s), then click the toolbar's Publish to Database icon .
   A summary of uploaded segments/Publish? appears. (Figure 437)

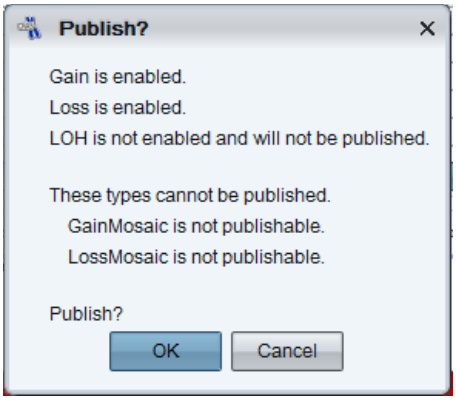


Figure 437 Publish? window.

2. Acknowledge the message, then click **OK**.

Note:

- Only segments in the copy number, XON region and LOH segment tracks can be uploaded. Segments uploaded are reflective of the current filters and settings applied.
- Only segments listed as enabled and filtered are published. If a segment category is listed as not enabled, click Cancel and select the checkbox in the Data Types File tree, then right-click the sample filename(s) to start the publishing again.
- Publishing time is dependent on the number of segments in the sample.
- Segments in the Mosaic track are not uploaded to the database. Mosaic segment must be promoted to the copy number state track to be published to the database. See "Promote copy number mosaic segments" on page 228.
- If a xxCHP file has been previously Published to the database, you will receive a warning indicating this sample already exists in the database. You can choose to overwrite the existing information or cancel to keep the existing information.
- The Segment Table Columns DB Count and Filtered DB Count are automatically updated.
- If ChAS is set to manual mode, the histogram need to be manually updated to include recently published samples. To update the histograms, click **ChAS DB → Refresh ChAS DB data**.

Publish to database

Important rules and restrictions

- A sample cannot be published if it is in Edit Mode. See "Use edit mode" on page 212.
- Only samples that are highlighted (not checked) are published to the database.
- Samples published using hg18 cannot be published to the database.
- Multiple filenames can be highlighted and published at the same time.
- Segments from xxCHP files analyzed using hg19 analysis files cannot be loaded into a ChAS Database genome version hg38. The opposite is also true.
- Mosaic Segments can not be published.
  - To promote mosaicism in the database, the Mosaic Segment should be assigned a copy number value and promoted to Copy Number segments using the edit function. For more details, see "Promote copy number mosaic segments" on page 228.
- You must be logged in as an administrator or manager (with manager role permissions) to publish data.
- Previously published samples can be edited and published again, however publishing a second time overwrites the original database entry.

Manual or automatic connection mode

By default, ChAS starts in automatic connection mode.

While in automatic connection mode, the DB count columns and histograms are refreshed/updated whenever a file is published.

If you do not want to wait for the data to be refreshed/updated each time a file is published, choose manual connection mode. In manual mode, the DB count columns and histograms are only be updated when you click **ChAS DB → Refresh ChAS DB data** or click the  icon.

There are 2 ways to switch from automatic to manual connection mode.

- At start up, select a user (as you normally would), select the **Manual connection** checkbox, then click **OK**. (Figure 438)

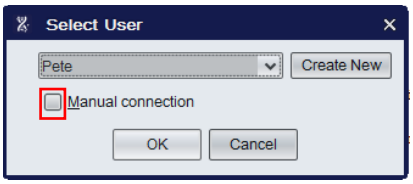


Figure 438 Select User window.

- During a ChAS session, go to the upper bar of icons and click  for automatic connection or click  for manual connection.

  Or

- Click **ChAS DB → Auto-Connect**, then select the checkbox to toggle between connection modes, as shown in Figure 439.

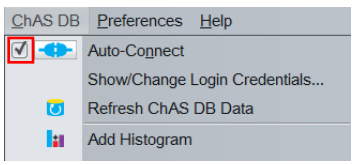


Figure 439 ChAS DB dropdown list.

Query samples in the ChAS Database

1. Click **ChAS DB → Query Samples** or click the **Query Samples** tab, then click the **Query Samples** button.
   The **Query Samples** window appears. (Figure 440)

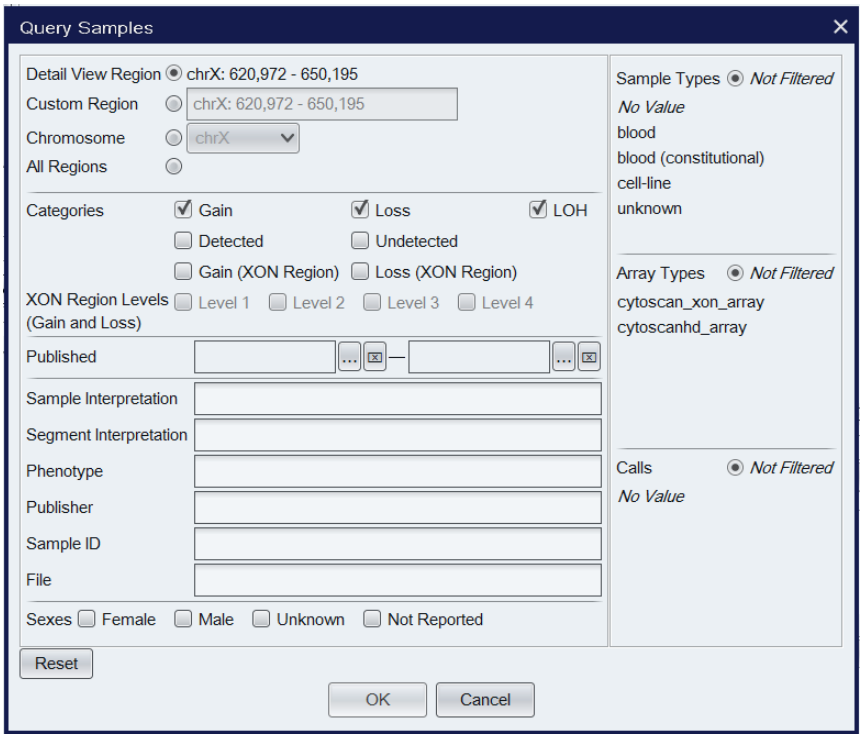


Figure 440 Query Samples window.

2. Use the provided radio buttons, checkboxes, and text fields to customize your query, then click **OK**.
   The Query Samples table populates with your filtered search results. (Figure 441)
   The contents for columns in which the headers have an Edit Icon can be modified. Changes will apply directly to the ChAS Database.
   Columns with a chip/pencil icon represent sample properties that can be edited.

Edit column contents

1. Double-click a cell to be edited.
   An Edit Value window appears. (Figure 441)
2. Enter or change the value in the window.
3. Click **OK**.
   Your changes are now saved to the ChAS Database.

Edit multiple cells with the same value

1. Highlight the cells you want to edit.
2. Right-click, then select **Edit Property Values**.

   An Edit Value window appears. (Figure 441)
3. Enter a new value or edit the existing displayed value.
4. Click **OK**.

   Your changes (for the multiple cells you selected) are now saved to the ChAS Database.

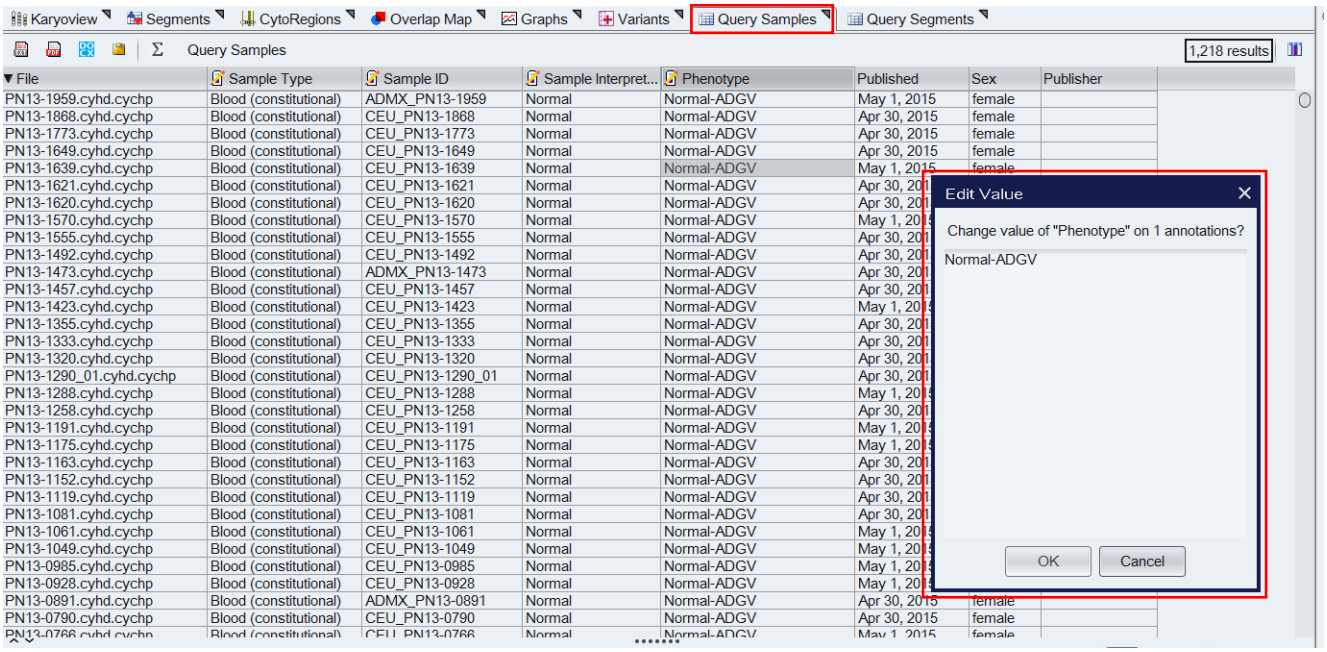


Figure 441 Query Samples window tab table - Filtered search results.

For instructions on how to use the table's features, see "Common table operations" on page 305.

Note: The following fields, **Sample Types**, **Array Types** and **Calls** are populated based on what has been published by the user into the ChAS Database. If the ChAS Browser is unable to contact the database, these fields are populated based on the library file and vocabularies entries. Queries are not automatically refreshed when publishing to or deleting from the ChAS DB. Queries must be re-run to reflect changes to the database made after the initial query.

Remove a sample from the query window

You can remove a sample from the query window, however this action does NOT remove the sample from the database.

1. Highlight the sample(s) you would like to remove from the results display.
2. Right-click, then click **Remove Query Results**. (Figure 442)

   A warning message appears asking you to confirm the removal of the file(s) from the results window.
3. Click **OK** to remove the files from the results. Click **Cancel** to return to the main screen.

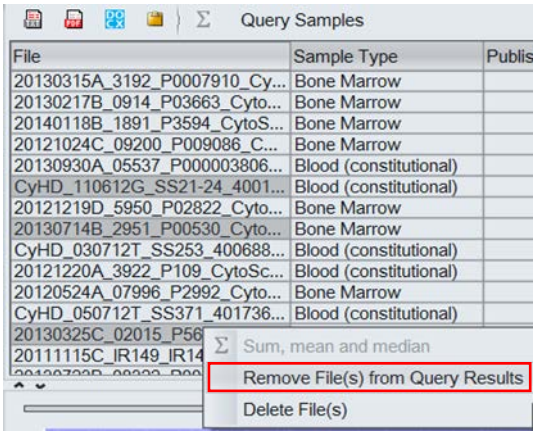


Figure 442 Remove Query Results.

Delete sample(s) from the ChAS Database

> ⚠ **WARNING!** You must have manager or admin permissions for the ChAS Database to delete samples.
>
> Sample(s) deleted from the ChAS Database are permanently deleted and cannot be retrieved. There is no undo delete feature.

Delete a single sample

To remove a single sample in a database, use the Query Samples window to locate the file to be deleted.

1. In the Query Samples window (Figure 440 on page 380), enter the file's **Filename** or **Sample ID**, then click **OK**.

   The sample appears in the table. (Figure 443)

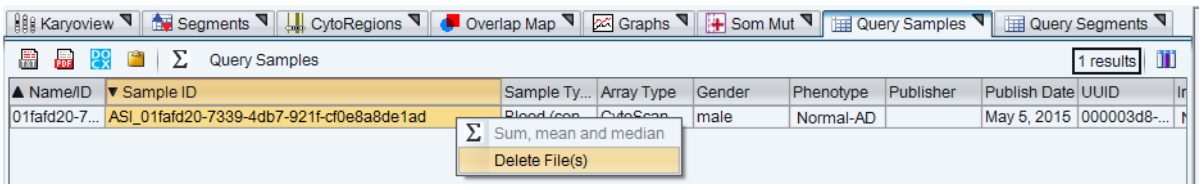


Figure 443 Query Samples window tab table - Deleting a sample.

2. Right-click the sample, then click **Delete**.

When a sample is deleted from a database, the reason for the deletion is required. Enter the reason in the **Enter delete reason** window. (Figure 444)

Note: This reasoning you enter is captured in the ChAS DB and it can be exported. For details, see "Export a deletion log" on page 407.

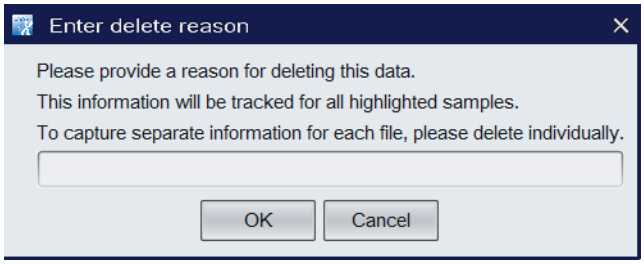


Figure 444 Enter deletion reason window.

3. Click **OK** to delete. Click **Cancel** to return to the query window.

   The sample is removed.

Delete multiple samples

1. Multiple samples can be highlighted to delete. They can be selected using the following keyboard and mouse combinations: Ctrl+click, Shift+click or Ctrl+a. (Figure 445)

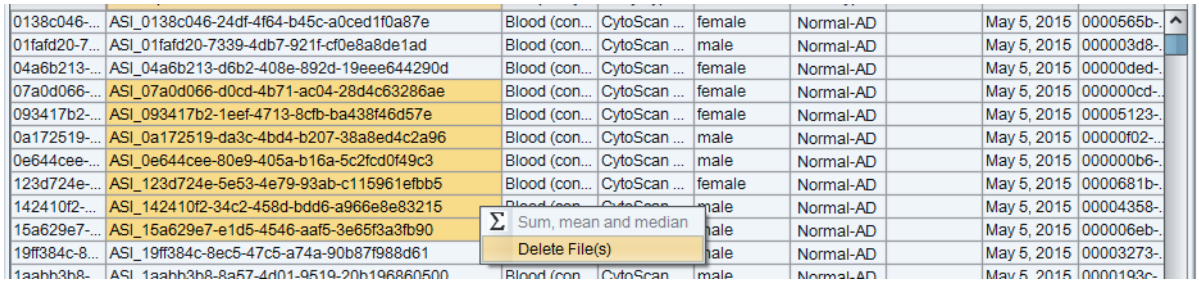


Figure 445 Query Samples window tab table - Deleting multiple samples.

2. Right-click the highlighted area, then click **Delete**.

   The samples are removed.

Query segments to the ChAS Database

1. Click **ChAS DB → Query Segments** or click the **Query Segments** tab, then click the **Query Segments** button.

   The **Query Segments** window appears. (Figure 446)

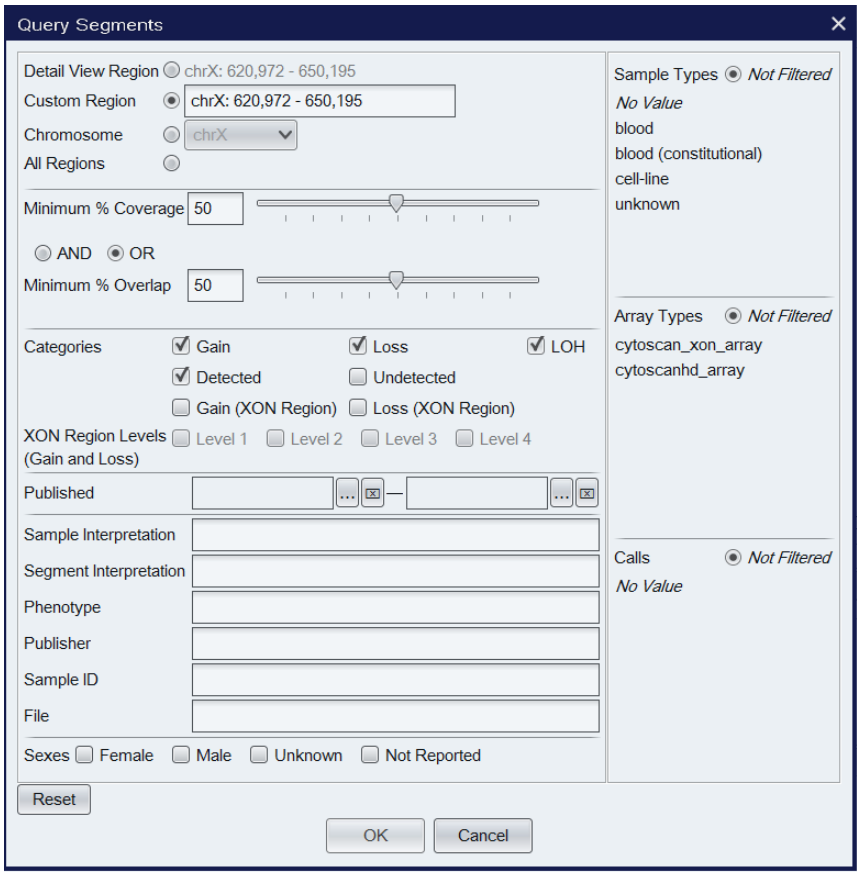


Figure 446 Query Segments window.

2. Use the provided radio buttons, checkboxes, and text fields to customize your query, then click **OK**.

   The Query Segments table populates with your filtered search results. (Figure 447)

   The contents for columns in which the headers have an Edit Icon can be modified. Changes will apply directly to the ChAS Database. Editable columns are: Call, Segment Interpretation, Inheritance, Oncomine Reporter.

Edit column contents

1. Double-click a cell to be edited.

   An Edit Value window appears. (Figure 447)

2. Enter or change the value in the window.
3. Click **OK**.

   Your changes are now saved to the ChAS Database.

Edit multiple cells with the same value

1. Highlight the cells you want to edit.
2. Right-click, then select **Edit Property Values**.

   An Edit Value window appears. (Figure 447)
3. Enter a new value or edit the existing displayed value.
4. Click **OK**.

   Your changes (for the multiple cells selected) are now saved to the ChAS Database.

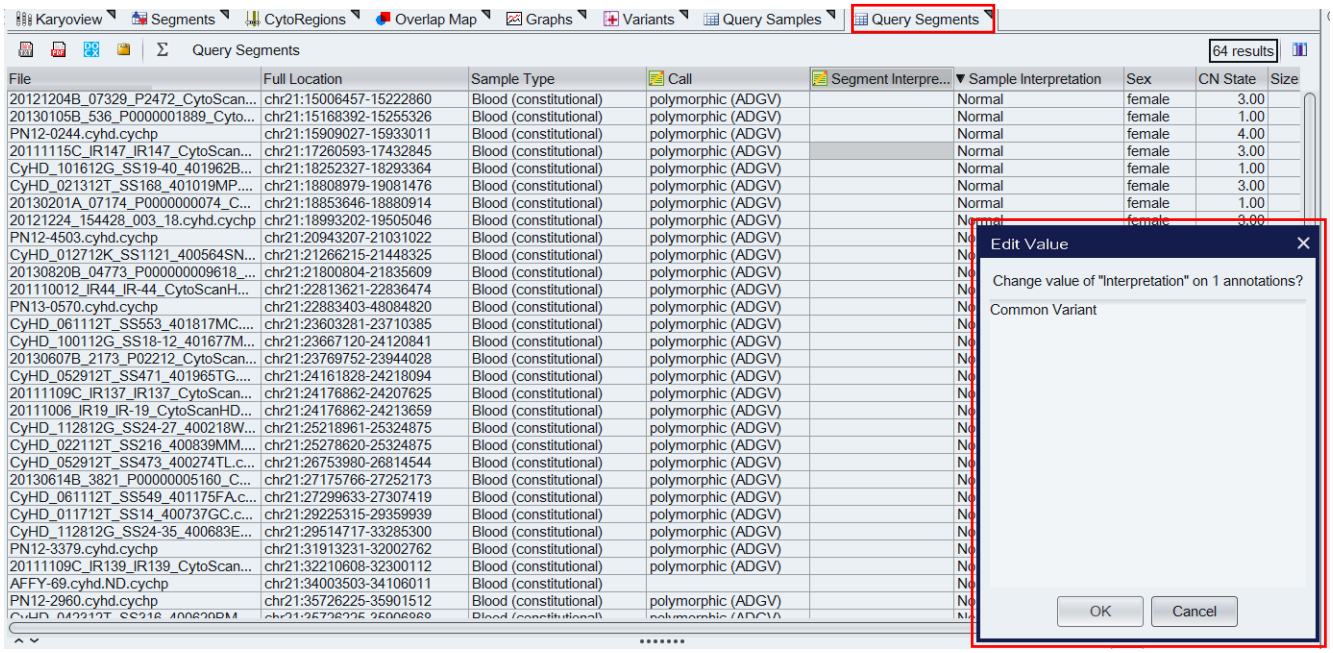


Figure 447 Query Segments window tab table - Filtered search results.

For instructions on how to use the table's features, see "Common table operations" on page 305.

Note: To delete sample files from the Query Segments tab, follow the same instructions outlined in "Delete sample(s) from the ChAS Database" on page 382. This procedure deletes the entire sample file and all file information associated with it.

19 Export results

IMPORTANT! Edit Mode must be **OFF** before exporting from ChAS.

Chromosome Analysis Suite includes the following tools for reporting results:

- Export the Karyoview, Selected Chromosome View, and Detail View as a DOCX, PDF or PNG file. See "Export graphic views".
- Export to a DOCX file. See "Create signature and background profiles".
- Export table data as a DOCX, PDF, TXT file, or copy selected data onto your clipboard. See "Export table data".
- Combine PDF reports. See "Combine PDFs into a single PDF"
- Use a ClinVar export template. See "Export with ClinVar".
- Export copy number and variant data as VCF. See "Export VCF files".

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

Note: If you have trouble displaying non-English characters on screen or in exported PDF files, ensure that the font "Arial Unicode MS" is installed on the machine. Open the "C:\Windows\Fonts" folder and search for a **ARIALUNI.TTF** file.

Export graphic views

You can export the Karyoview, Selected Chromosome View, and Detail View in the following formats:

- PDF: "Export as a PDF".
- Microsoft™ Word (DOCX): "Export as Word™ (DOCX) format" on page 391.
- PNG graphic file: "Export as PNG" on page 393.

Export as a PDF

ChAS provides a variety of options for exporting graphic views as PDFs. The PDF Report displays the graphic with basic information about data files and settings.

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1. From the **Exports** menu, select the PDF option you want to use.
   - Export application window PDF: Creates PDF with entire software screen and information about data files
   - Export Karyoview PDF: Creates PDF with Karyoview and information about data files.
     Optional: Select the checkbox for either or both "Hide the X Chromosome in the Karyoview" and/or "Hide the Y Chromosome in the Karyoview" to export the Karyoview with out the sex chromosome ideograms.
   - Export Selected Chromosome PDF: Creates PDF with Selected Chromosome View and information about data files.
   - Export Detail View PDF: Creates PDF with selected Detail View and information about data files.
   - Export Segments Table PDF: Creates PDF with Segment Table. For normal samples, the Segments Table export contains the nomenclature for normal sex call when applicable.
     - Male Sex Call: arr (X,Y)×1,(1–22)×2
     - Female Sex Call: arr (X,1–22)×2
     - Unknown Sex Call: arr (1–22)×2
   - Export QC and Sample Info PDF: Creates PDF with the QC and Sample Information.
   - Export Chromosome Summary Data PDF: Creates PDF with the Chromosome Summary Data.

   An **Export Details** window appears. (Figure 448)

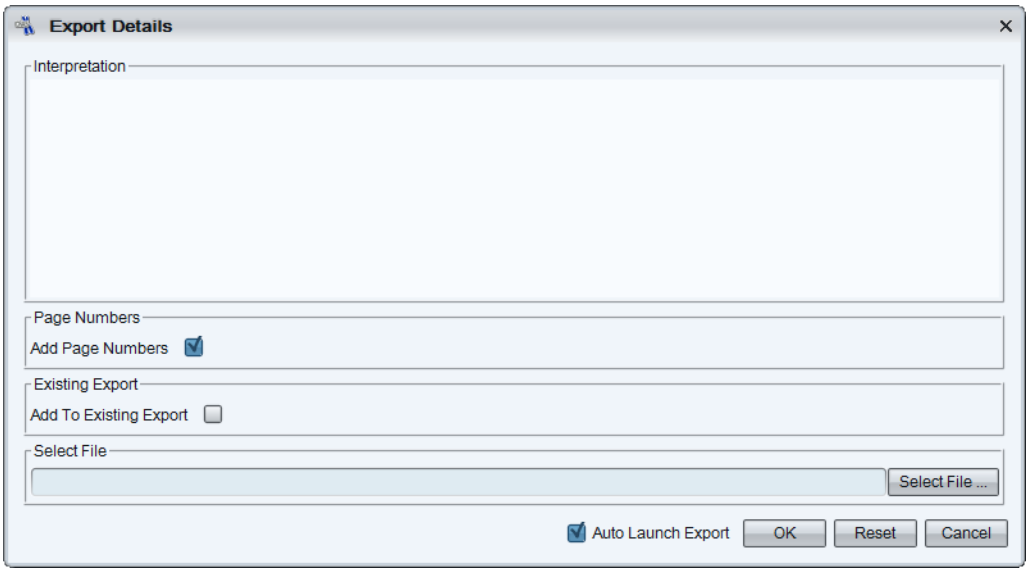


Figure 448 Export Details window.

2. Optional: If you have added a Sample Interpretation in the View/Edit Sample Properties window, the information is populated into the Interpretation dialog box. You can also type free text into the Interpretation box.
3. Optional: Select the option for adding page numbers.

4. Optional: Select the option for adding to an existing report, if desired. See "Combine PDFs into a single PDF" on page 400.
5. Click **Select File**.

   The **Save** window opens.
6. Select a folder location for the PDF using the navigation tools.

   This folder location is automatically selected when exporting other PDFs during a session.
7. Enter a name for your PDF file.

   If you are adding the graphic to an existing PDF, select the PDF file.
8. Click **Save**.

   You are returned to the **Export Details** window.
9. Optional: Leave the Auto Launch Export checkbox selected, if you want your newly saved PDF to open automatically after clicking **OK**.
10. Click **OK**.

    A PDF is created with your selected export details saved.

Create signature and background profiles

Create signature profiles

Signature Profiles, including a logo, address, reviewer name and credentials, can be added to a DOCX export. Use saved signature profiles for quick recalls with any DOCX export

1. Click the Preference Menu, then select **Edit User Configuration**.
2. Click the **Exports** Tab.

3. Click the **Summary Exports** tab, then click the **Summary Export** tab. (Figure 449)

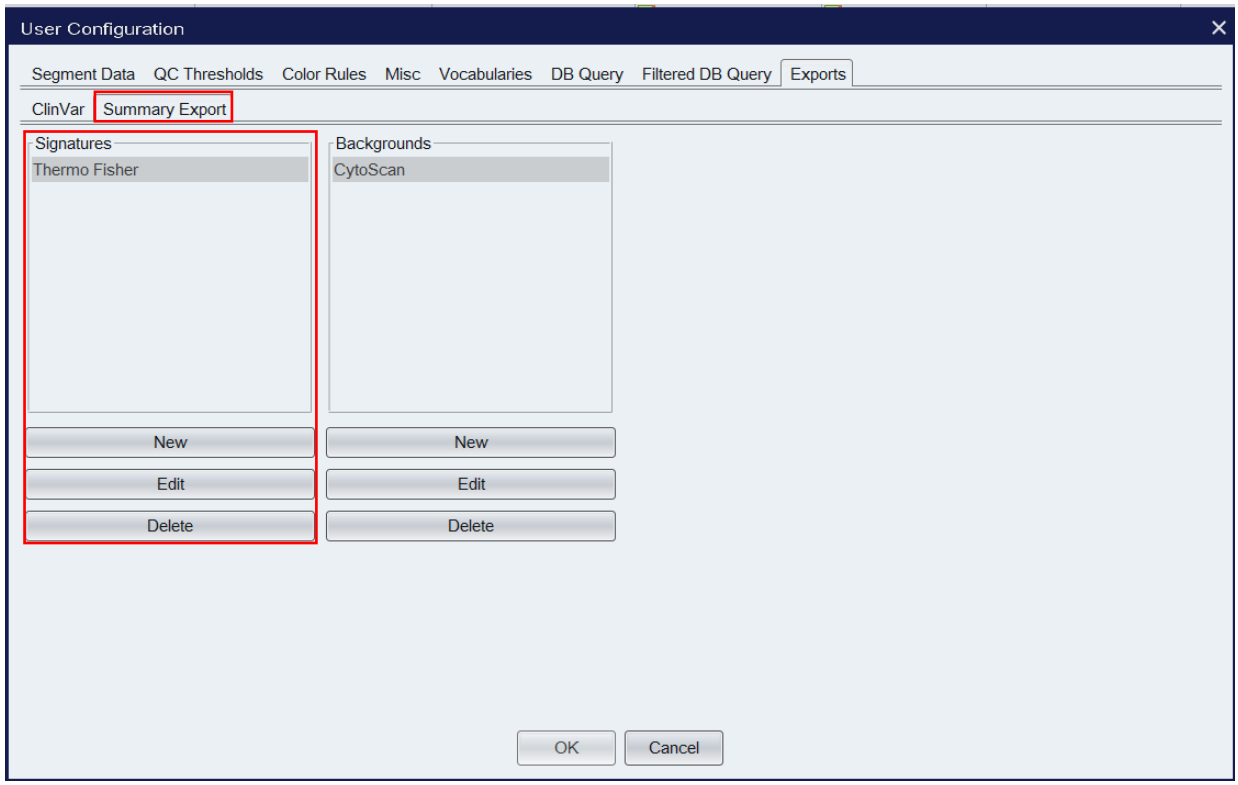


Figure 449 Summary Export window - Signatures.

Create a new signature

1. Click the **New** button.
2. Complete the text fields.
3. Optional: Click the **Upload Logo** button to add your organization's logo.
4. Click **OK** to save.

   Your saved signature name is the same as your organization name.
5. Optional: Repeat steps 1–4 to create additional signatures.

Edit a saved Signature

1. Highlight the Signature name you would like to modify.
2. Click the **Edit** button.
3. Modify the appropriate fields.
4. Click **OK** to save your updated signature.

Delete a signature

1. Highlight the Signature name you would like to delete.
2. Click the **Delete** button.
3. Click **OK** to permanently remove the signature.

Background profiles

Background profiles (Figure 450) provide saved text(s) that can be added to each DOCX export. For example, a noteworthy background about the assay profiles you want to save.

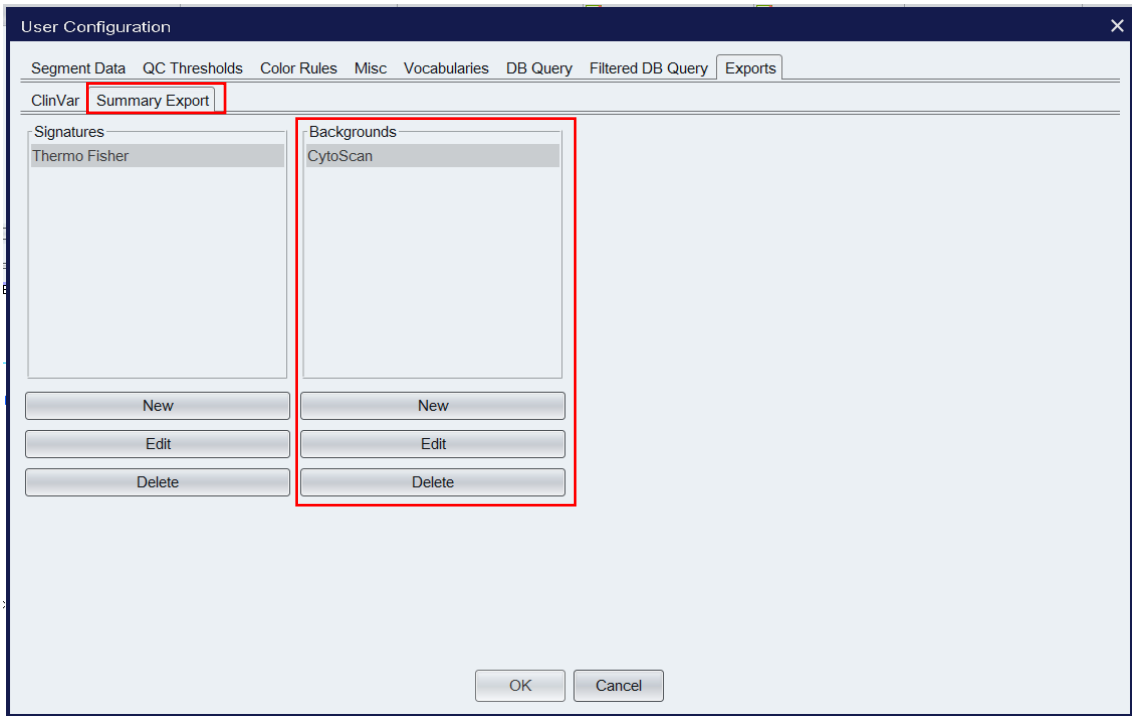


Figure 450 Summary Export window - Backgrounds.

Create a new background

1. Click the **New** button.
2. Name the Background using the Title field.
3. Enter the text for the background information that you want to appear on the export.
4. Click **OK** to save.
5. Optional: Repeat steps 1–4 to create additional Backgrounds.

Edit a saved background

1. Highlight the Background name you want to modify.
2. Click the **Edit** button.
3. Modify the text.
4. Click **OK** to save your updated background.

Delete a background

1. Highlight the Background name you would like to delete.
2. Click the **Delete** button.
3. Click **OK** to permanently remove the Background.

Export as Word™ (DOCX) format

1. From the Exports menu, select **Export - Word (docx) Format**.

   The **Export Details** window opens. (Figure 451)

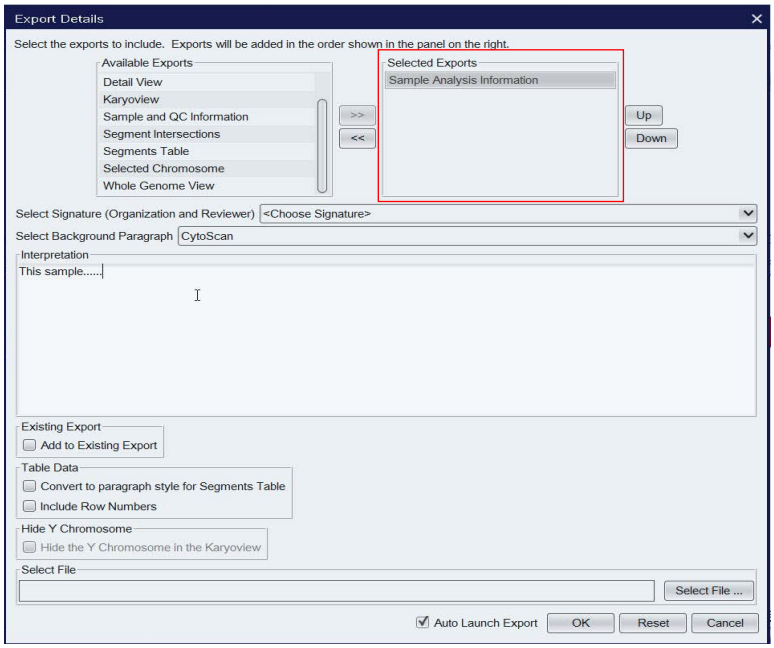


Figure 451 Export Details window.

Note: To export a graph (Karyoview, Whole Genome View, Detail View, Selected Chromosome View), the graph must be visible in the ChAS Browser before export.

2. Click, Ctrl+click, or Shift+click the **Available Exports** (left pane), then click the right double arrow button (Figure 451) to add them to the **Selected Exports** (right pane). Use the **Up** and **Down** buttons to define each report's order within the master report.
3. Optional: Select a saved Signature and/or Background from the dropdown lists to add them to your DOCX export.

   If you previously entered Sample Level Interpretations, they will appear in the Interpretation field.

Note: Before adding an entry in the Interpretation field, the Selected Exports pane must first display **Sample Analysis Information**, as shown in Figure 451.

4. Optional: Select the **Add to Existing Export** checkbox to add this new report to an existing one. After selecting this checkbox, click the **Select File** button to navigate to and select an existing DOCX file.
5. Optional: Select the **Convert to paragraph style for Segments Table** checkbox if you want your Segments Table data translated into a paragraph-style format, as show in Figure 452.

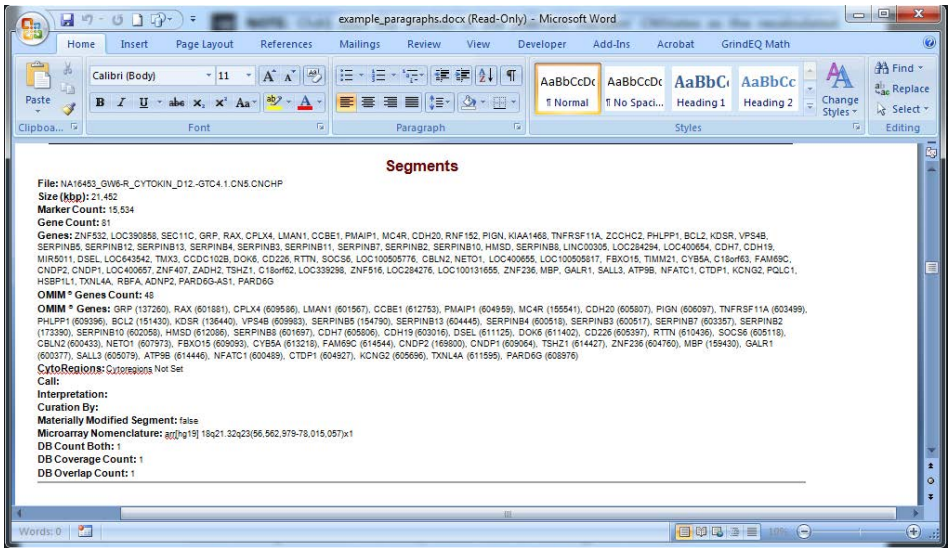


Figure 452 Segments Table to paragraph-style format example.

6. Optional: Select the **Include Row Number** checkbox to add row numbers to the Segment Table.
7. Optional: Optional: Select the **Hide the Y Chromosome** in the Karyoview checkbox to export the Karyoview without the Y Chromosome ideogram.
8. Optional: Select the **Hide the X Chromosome** in the Karyoview checkbox to export the Karyoview without the X Chromosome ideogram.
9. Click the **Select File** button to navigate to a saved report location, enter a filename, then click **Save**.

   The name of the file defaults to the name of the filename.
10. By default, the report automatically opens in Microsoft™ Word after it is generated. Deselect the **Auto Launch Export** checkbox to disable this feature.
11. Click **OK** to generate the DOCX report. Click the **Reset** button to return the Report Details window back to its factory defaults.

Note: For normal samples, the when exporting Segments Table, the export contains the nomenclature for normal sex call, when applicable.

- Male Sex Call: arr (X,Y)×1,(1–22)×2
- Female Sex Call: arr (X,1–22)×2
- Unknown Sex Call: arr (1–22)×2

Export as PNG

You can also create a PNG screen shot of the entire ChAS Window, Karyoview or Selected Chromosome View.

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

1. Click **Exports** → Export- PNG Format.
2. Select the graph you would like to export.
   - Export Window PNG to export the entire ChAS Window.
   - Export Karyoview PNG to export the Karyoview in PNG format.
   - Export Selected Chromosome View PNG to export the Selected Chromosome View as a PNG.

   A **Save As** window opens.
3. Select a folder location for the file using the navigation tools.

   This folder location is automatically selected when exporting other screen shots during a session.
4. Enter a name for the PNG file in the File Name box.
5. Click the **Save** button.

   The PNG file screen shot is saved in the selected location.

   The PNG file can be cropped in a graphics program like Paint and inserted into a word processing document if desired.

Export table data

ChAS provides several options for exporting table data:

- "Export table data into a PDF"
- "Export views and tables as a DOCX file"
- "Export tables as TXT file"
- "Export a segments table with modified segments to a TXT file"

Export table data into a PDF

For information on how to choose and export preset column content (from previously saved table states). See "Saved table states" on page 310.

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Note: You can export data from the Segment Table by selecting Export Segment Table PDF from the Exports menu, but you cannot export graph table data in a PDF format.

To track which of your segments have been modified (merged, created de novo, deleted, had their start or end coordinates edited, or had their type or state changed), you *must* perform the following *before* exporting the Segments Table to PDF. The only PDF format capable of tracking which segments have been modified is the Segments Table PDF, all other PDFs show NO visual or textual distinction between modified and non-modified segments.

BEFORE exporting table data into a PDF file:

1. Click  (top right of Segments table).

   The Select Columns window appears.
2. Scroll down, locate, then select the **Modified Segment** checkbox.
3. Click **OK**.

   The Modified Segment column is now added to your Segments table.

Export table data into a PDF file

1. At the Segments table upper toolbar, click the PDF button  .

   The Select Columns and Files window appears. (Figure 453)

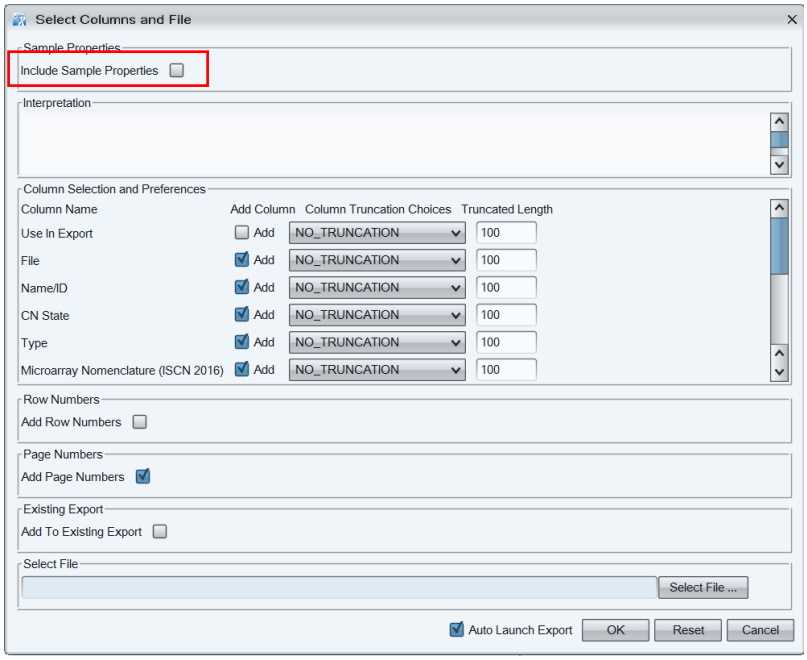


Figure 453 Select Columns and File window for Segment Table PDF.

If you have added a Sample Interpretation in the View/Edit Sample Properties window, the information is populated into the Interpretation dialog box. You can also type free text into the Interpretation box. You must select the **Include Sample Properties** checkbox to enable the Interpretation field.

2. Enter the appropriate text in the Interpretation field.

   To add the other Sample Properties, select the **Include Sample Properties** checkbox. If these fields have been populated, they are exported in the PDF.

3. Select the columns to be displayed and the columns truncation rules. The Column Selection and Preferences window (Figure 454), include:

   - **Column Name**: Header of the column in the table.
   - **Add Column**: Select the checkbox to display the column in the PDF report.
   - **Column Truncation Options**:
     - **No_Truncation**: Field is exported as is, using wrap-around if necessary.
     - **Truncate_Beginning**: Truncates content at the beginning of the field, leaving as many characters as specified in Truncated Length box.
     - **Truncate_Middle**: Truncates content in the middle of the field, leaving characters at the beginning and end, with ellipses (…) to mark the truncated characters.
     - **Truncate_End**: Truncates content at the end of the field, leaving as many characters as specified in Truncated Length box.
   - **Truncated Length**: Number of characters displayed after truncating the data.

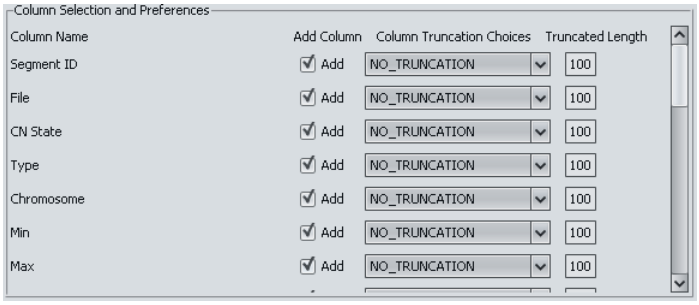


Figure 454 Column Selection and Preferences.

4. Select the option for adding page and row numbers, if desired.
5. Select the option for adding to an existing report, if desired. See "Combine PDFs into a single PDF" on page 400.
6. Click the **Select File** button.

   The **Save As** window opens. (Figure 455)

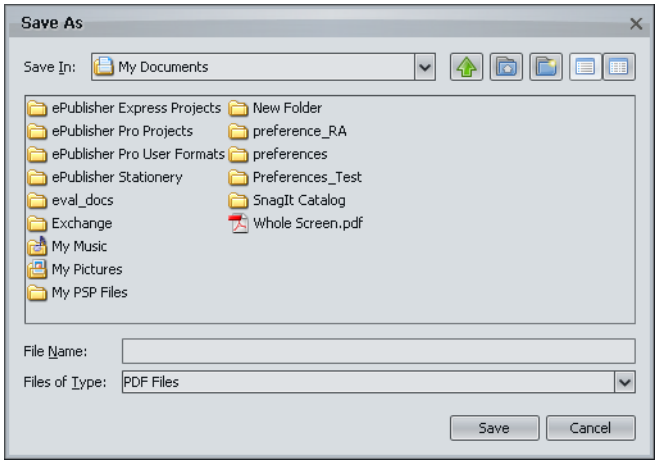


Figure 455 Save As for Segment Table PDF.

7. Select a folder location for the file using the navigation tools.
8. Enter a name for the PDF file, or select a file for the information to be appended to.
9. Click **Save** in the Save As window.
10. Click **OK** in the **Select Columns and File** window.

    A PDF file is created with the selected data type saved.

    The PDF report displays:

    - Table type
    - Information on chromosome and genome region
    - Interpretation
    - Data files
    - Genome or CytoRegion Segment Filters used
    - Settings for Data Processing
    - Microarray Nomenclature
    - Details of the table data

Export tables tips

Follow these tips to improve the export of table data in a PDF file:

- Use truncation.
- Select only columns you need or select columns from a saved table state. See "Saved table states" on page 310.
- Use filtering options (Segment filters, displaying only results for a chromosome or area in the detail view, etc.) to limit the number of values being exported.

Export views and tables as a DOCX file

You can save the currently viewed table in a new or existing DOCX report.

1. While in a desired View or Table, click the DOCX button  .

   A Save window appears.

Do one of the following:

- **New Reports:** Enter a filename to create a new DOCX file, then click **Save**.
- **Adding to an existing report:** Click to select an existing DOCX file. Click **Save**. The message window, You will be adding contents to an existing file. Continue? appears. Click **Yes** to append the currently displayed table contents onto an existing file.

IMPORTANT! Before adding a file, it must be closed.

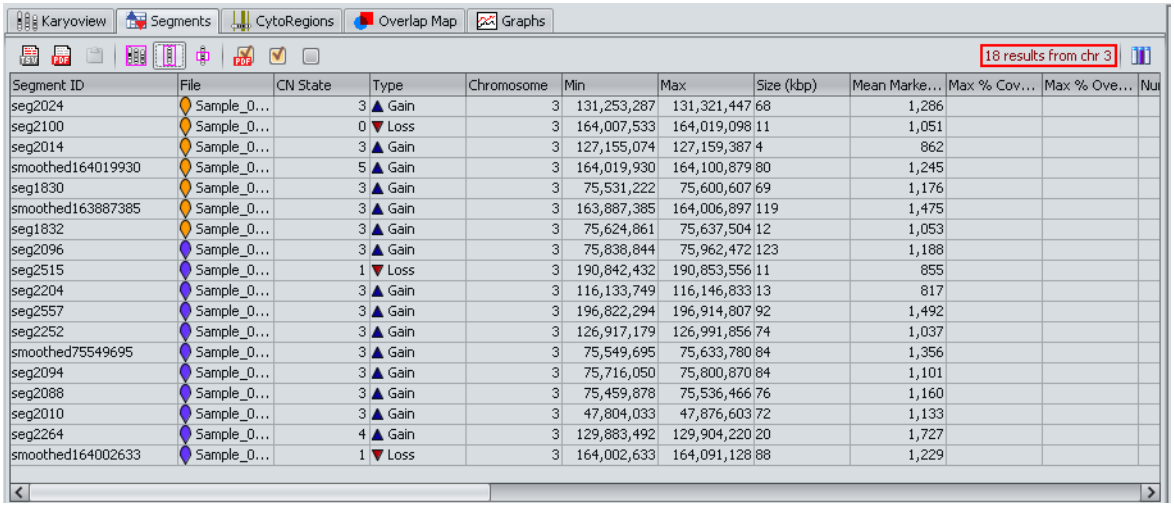
Export tables as TXT file

The TXT file format enables you to transfer data to other software for analysis.

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

Export table information as a text file

1. Perform pre-filtering on the data in the table. (Figure 456)



Segment ID	File	CN State	Type	Chromosome	Min	Max	Size (kbp)	Mean Marke...	Max % Cov...	Max % Ove...	Nu
seg2024	Sample_0...	3	Gain	3	131,253,287	131,321,447	68	1,286			
seg2100	Sample_0...	0	Loss	3	164,007,533	164,019,098	11	1,051			
seg2014	Sample_0...	3	Gain	3	127,155,074	127,159,387	4	862			
smoothed164019930	Sample_0...	5	Gain	3	164,019,930	164,100,879	80	1,245			
seg1830	Sample_0...	3	Gain	3	75,531,222	75,600,607	69	1,176			
smoothed163887385	Sample_0...	3	Gain	3	163,887,385	164,006,897	119	1,475			
seg1832	Sample_0...	3	Gain	3	75,624,861	75,637,504	12	1,053			
seg2096	Sample_0...	3	Gain	3	75,838,844	75,962,472	123	1,188			
seg2515	Sample_0...	1	Loss	3	190,842,432	190,853,556	11	855			
seg2204	Sample_0...	3	Gain	3	116,133,749	116,146,833	13	817			
seg2557	Sample_0...	3	Gain	3	196,822,294	196,914,807	92	1,492			
seg2252	Sample_0...	3	Gain	3	126,917,179	126,991,856	74	1,037			
smoothed75549695	Sample_0...	3	Gain	3	75,549,695	75,633,780	84	1,356			
seg2094	Sample_0...	3	Gain	3	75,716,050	75,800,870	84	1,101			
seg2088	Sample_0...	3	Gain	3	75,459,878	75,536,466	76	1,160			
seg2010	Sample_0...	3	Gain	3	47,804,033	47,876,603	72	1,133			
seg2264	Sample_0...	4	Gain	3	129,883,492	129,904,220	20	1,727			
smoothed164002633	Sample_0...	1	Loss	3	164,002,633	164,091,128	88	1,229			

Figure 456 Segments table with data filtered.

2. In the Table toolbar, click the Export **TXT** button.
   The Save as TXT window opens. (Figure 457)

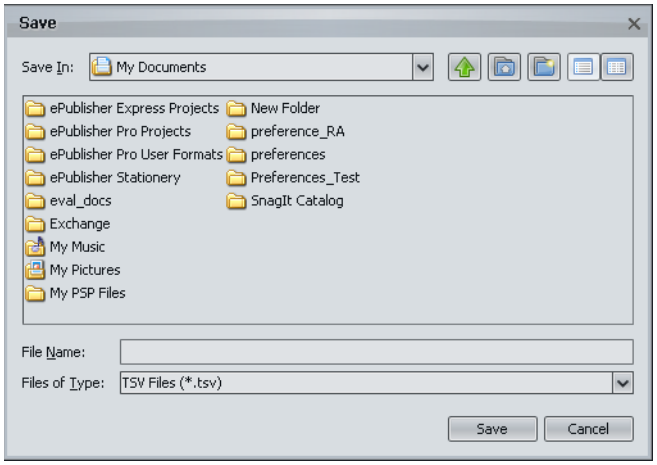
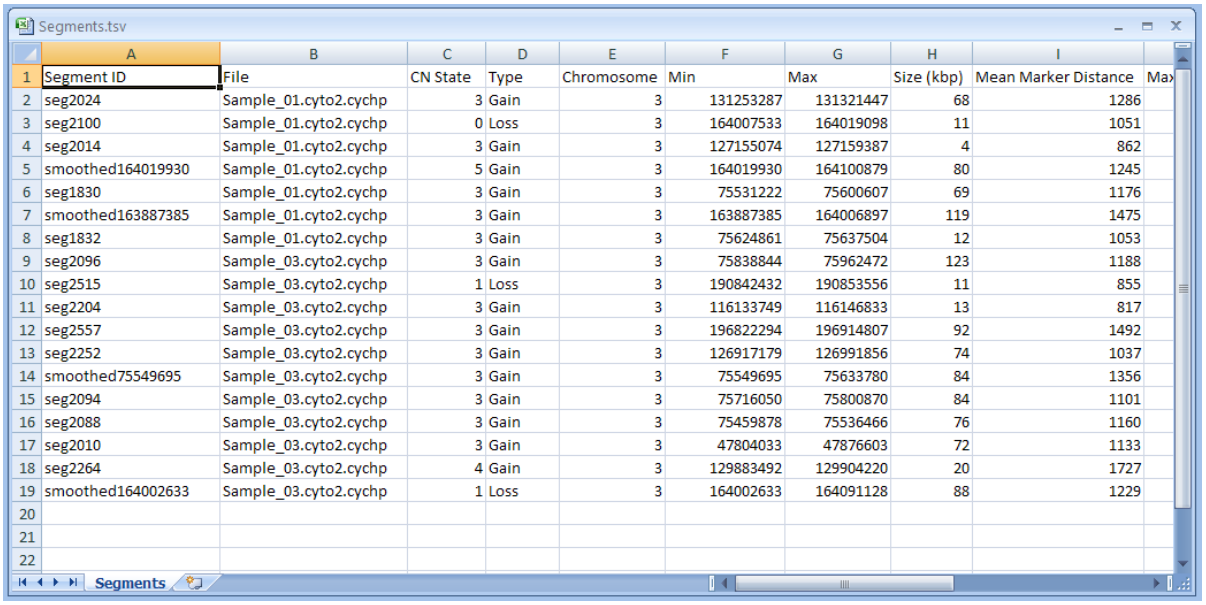


Figure 457 Save as TXT window.

3. Select a folder location for the file using the navigation tools.
4. Enter a name for the TXT file.
5. Click **Save**.
   The TXT file is saved in the selected location (Figure 458).

It can be opened using a text editing or spreadsheet program, or in other software designed to use Tab Separated Value TXT format.



Segments.tsv

	A	B	C	D	E	F	G	H	I
1	Segment ID	File	CN State	Type	Chromosome	Min	Max	Size (kbp)	Mean Marker Distance
2	seg2024	Sample_01.cyto2.cychp	3	Gain	3	131253287	131321447	68	1286
3	seg2100	Sample_01.cyto2.cychp	0	Loss	3	164007533	164019098	11	1051
4	seg2014	Sample_01.cyto2.cychp	3	Gain	3	127155074	127159387	4	862
5	smoothed164019930	Sample_01.cyto2.cychp	5	Gain	3	164019930	164100879	80	1245
6	seg1830	Sample_01.cyto2.cychp	3	Gain	3	75531222	75600607	69	1176
7	smoothed163887385	Sample_01.cyto2.cychp	3	Gain	3	163887385	164006897	119	1475
8	seg1832	Sample_01.cyto2.cychp	3	Gain	3	75624861	75637504	12	1053
9	seg2096	Sample_03.cyto2.cychp	3	Gain	3	75838844	75962472	123	1188
10	seg2515	Sample_03.cyto2.cychp	1	Loss	3	190842432	190853556	11	855
11	seg2204	Sample_03.cyto2.cychp	3	Gain	3	116133749	116146833	13	817
12	seg2557	Sample_03.cyto2.cychp	3	Gain	3	196822294	196914807	92	1492
13	seg2252	Sample_03.cyto2.cychp	3	Gain	3	126917179	126991856	74	1037
14	smoothed75549695	Sample_03.cyto2.cychp	3	Gain	3	75549695	75633780	84	1356
15	seg2094	Sample_03.cyto2.cychp	3	Gain	3	75716050	75800870	84	1101
16	seg2088	Sample_03.cyto2.cychp	3	Gain	3	75459878	75536466	76	1160
17	seg2010	Sample_03.cyto2.cychp	3	Gain	3	47804033	47876603	72	1133
18	seg2264	Sample_03.cyto2.cychp	4	Gain	3	129883492	129904220	20	1727
19	smoothed164002633	Sample_03.cyto2.cychp	1	Loss	3	164002633	164091128	88	1229
20									
21									
22									

Segments

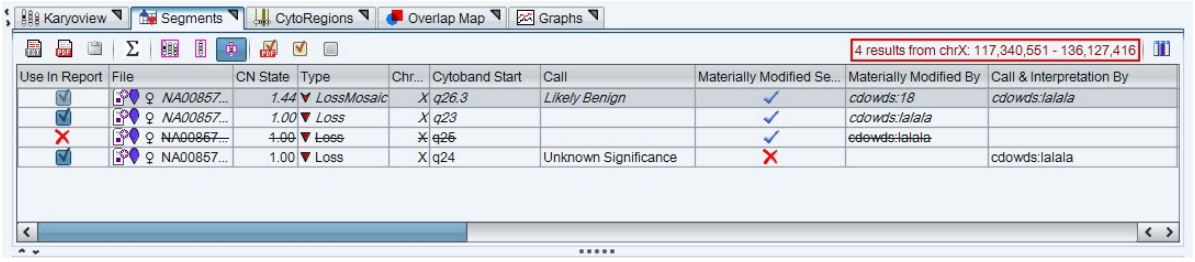
Figure 458 TXT file opened in an Excel™ spreadsheet.

Export a segments table with modified segments to a TXT file

In the Segments Table:

- Materially Modified segments are shown in italic text when Edit Mode is ON, but not italicized when Edit Mode is OFF.
- Calls and Interpretations don't cause a segment's row text to be italicized in Edit Mode.
- Deleted segments are shown in strike-through text when Edit Mode is ON, and are not present in the table when Edit Mode is OFF.
- In the TXT Exports of the Segments Table table, note that Deleted segments are part of the export when Edit Mode is ON, and will not be part of the export if Edit Mode is OFF

Figure 459 is an example of a Segment Table with Edit Mode **ON**. Note its italicized text representing materially modified segments and the strike-through text showing deletions.



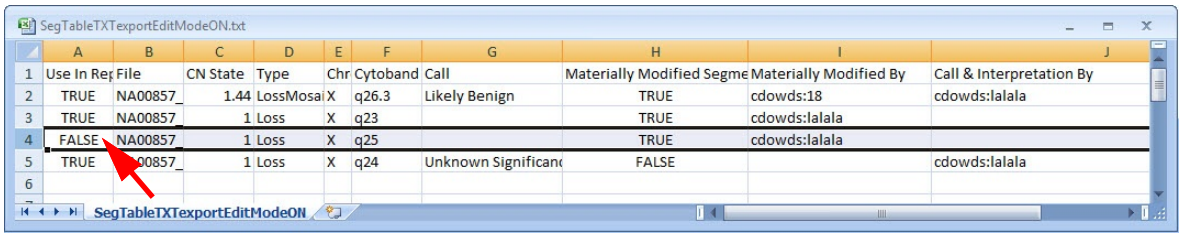
Karyoview | Segments | CytoRegions | Overlap Map | Graphs

4 results from chrX: 117,340,551 - 136,127,416

Use In Report	File	CN State	Type	Chr...	Cytoband Start	Call	Materially Modified Se...	Materially Modified By	Call & Interpretation By
☑	♀ *NA00857...*	*1.44*	*LossMosaic*	*X*	*q26.3*	*Likely Benign*	✓	*cdowds:18*	*cdowds:lalala*
☑	♀ *NA00857...*	*1.00*	*Loss*	*X*	*q23*		✓	*cdowds:lalala*	
✗	♀ ~~NA00857...~~	~~1.00~~	~~Loss~~	~~X~~	~~q25~~		✓	~~cdowds:lalala~~	
☑	♀ NA00857...	1.00	Loss	X	q24	Unknown Significance	✗		cdowds:lalala

Figure 459 Segment table - Edit Mode ON.

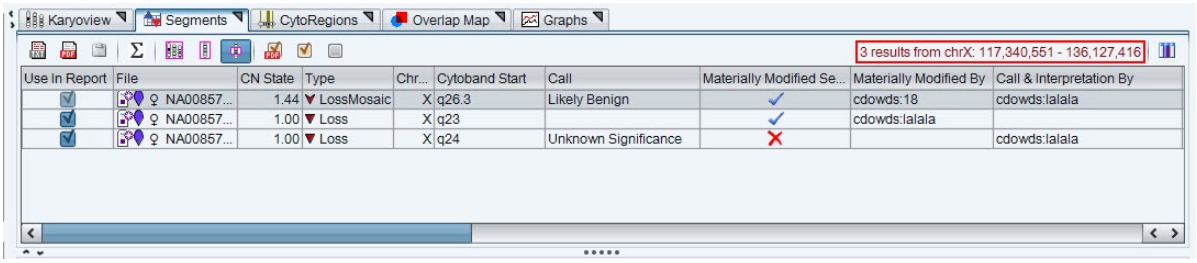
Figure 460 is an example of a Segment table that has been exported with the Edit Mode **ON**. Note the 4th row of the Use in Report column. In the case of this segment, it reads FALSE, because this segment was deleted.



	A	B	C	D	E	F	G	H	I	J
1	Use In Rep	File	CN State	Type	Chr	Cytoband	Call	Materially Modified Segme	Materially Modified By	Call & Interpretation By
2	TRUE	NA00857_	1.44	LossMosai	X	q26.3	Likely Benign	TRUE	cdowds:18	cdowds:lalala
3	TRUE	NA00857_	1	Loss	X	q23		TRUE	cdowds:lalala	
4	FALSE	NA00857_	1	Loss	X	q25		TRUE	cdowds:lalala	
5	TRUE	00857_	1	Loss	X	q24	Unknown Significanc	FALSE		cdowds:lalala
6										

Figure 460 Segment table TXT EXPORT - Edit Mode ON.

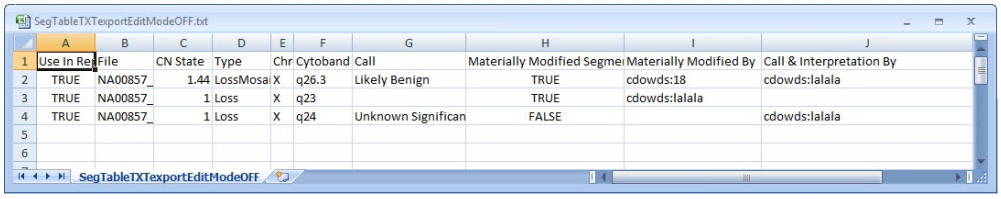
Figure 461 is an example of a Segment Table with Edit Mode **OFF**. The italicized text representing materially modified segments is no longer present. The deleted segment and strike-through text showing a deletion (shown in Figure 459 and Figure 460) also do not appear.



Use In Report	File	CN State	Type	Chr...	Cytoband Start	Call	Materially Modified Se...	Materially Modified By	Call & Interpretation By
☑	♀ NA00857...	1.44	LossMosaic	X	q26.3	Likely Benign	✓	cdowds:18	cdowds:lalala
☑	♀ NA00857...	1.00	Loss	X	q23		✓	cdowds:lalala	
☑	♀ NA00857...	1.00	Loss	X	q24	Unknown Significance	✗		cdowds:lalala

Figure 461 Segment table - Edit Mode OFF.

Figure 462 is an example of how an exported TXT table appears with Edit Mode **OFF**. Note the deleted segment shown in Figure 459 and Figure 460 is not present.



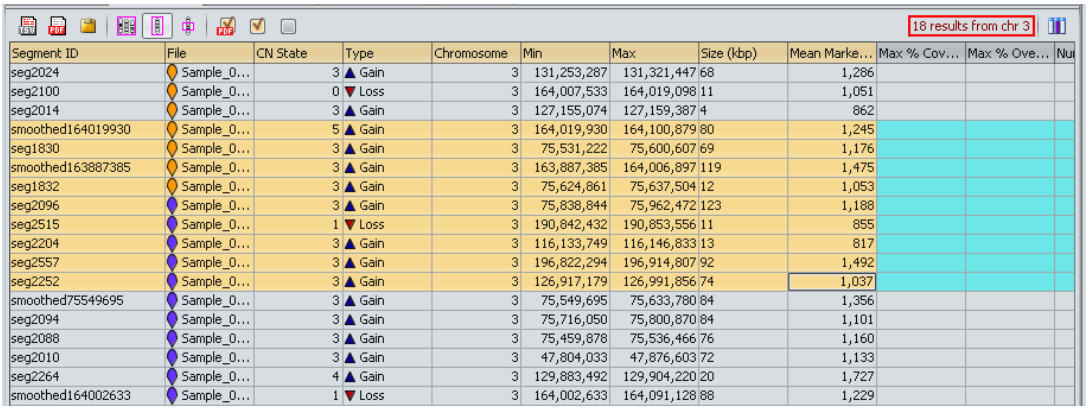
	A	B	C	D	E	F	G	H	I	J
1	Use In Rep	File	CN State	Type	Chr	Cytoband	Call	Materially Modified Segme	Materially Modified By	Call & Interpretation By
2	TRUE	NA00857_	1.44	LossMosai	X	q26.3	Likely Benign	TRUE	cdowds:18	cdowds:lalala
3	TRUE	NA00857_	1	Loss	X	q23		TRUE	cdowds:lalala	
4	TRUE	NA00857_	1	Loss	X	q24	Unknown Significan	FALSE		cdowds:lalala
5										
6										

Figure 462 Segment table TXT EXPORT - Edit Mode OFF.

Transfer to clipboard

You can copy data from selected cells to the clipboard for pasting into a text or spreadsheet file.

1. Select the cells you want to copy in the table. (Figure 463)



18 results from chr 3

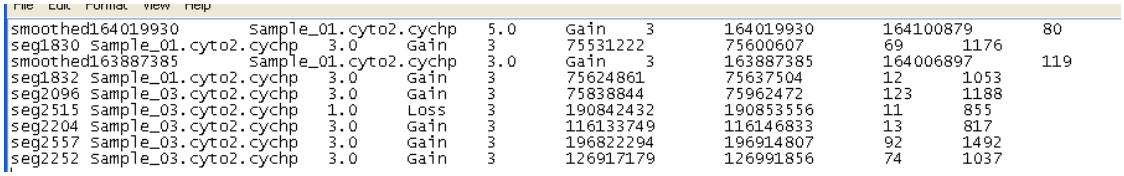
Segment ID	File	CN State	Type	Chromosome	Min	Max	Size (kbp)	Mean Marke...	Max % Cov...	Max % Ove...	Nu
seg2024	Sample_0...	3	Gain	3	131,253,287	131,321,447	68	1,286			
seg2100	Sample_0...	0	Loss	3	164,007,533	164,019,098	11	1,051			
seg2014	Sample_0...	3	Gain	3	127,155,074	127,159,387	4	862			
smoothed164019930	Sample_0...	5	Gain	3	164,019,930	164,100,879	80	1,245			
seg1830	Sample_0...	3	Gain	3	75,531,222	75,600,607	69	1,176			
smoothed163887385	Sample_0...	3	Gain	3	163,887,385	164,006,897	119	1,475			
seg1832	Sample_0...	3	Gain	3	75,624,861	75,637,504	12	1,053			
seg2096	Sample_0...	3	Gain	3	75,838,844	75,962,472	123	1,188			
seg2515	Sample_0...	1	Loss	3	190,842,432	190,853,556	11	855			
seg2204	Sample_0...	3	Gain	3	116,133,749	116,146,833	13	817			
seg2557	Sample_0...	3	Gain	3	196,822,294	196,914,807	92	1,492			
seg2252	Sample_0...	3	Gain	3	126,917,179	126,991,856	74	1,037			
smoothed75549695	Sample_0...	3	Gain	3	75,549,695	75,633,780	84	1,356			
seg2094	Sample_0...	3	Gain	3	75,716,050	75,800,870	84	1,101			
seg2088	Sample_0...	3	Gain	3	75,459,878	75,536,466	76	1,160			
seg2010	Sample_0...	3	Gain	3	47,804,033	47,876,603	72	1,133			
seg2264	Sample_0...	4	Gain	3	129,883,492	129,904,220	20	1,727			
smoothed164002633	Sample_0...	1	Loss	3	164,002,633	164,091,128	88	1,229			

Figure 463 Segment table with cells selected.

2. Click the **Copy to Clipboard** button  in the table toolbar.

   The selected data is copied to the clipboard.

   You can paste the data on the clipboard into a text or spreadsheet file (Figure 464).



```
File  Edit  Format  View  Help
smoothed164019930       Sample_01.cyto2.cychp   5.0     Gain    3       164019930       164100879       80
seg1830 Sample_01.cyto2.cychp   3.0     Gain    3       75531222        75600607        69      1176
smoothed163887385       Sample_01.cyto2.cychp   3.0     Gain    3       163887385       164006897       119
seg1832 Sample_01.cyto2.cychp   3.0     Gain    3       75624861        75637504        12      1053
seg2096 Sample_03.cyto2.cychp   3.0     Gain    3       75838844        75962472        123     1188
seg2515 Sample_03.cyto2.cychp   1.0     Loss    3       190842432       190853556       11      855
seg2204 Sample_03.cyto2.cychp   3.0     Gain    3       116133749       116146833       13      817
seg2557 Sample_03.cyto2.cychp   3.0     Gain    3       196822294       196914807       92      1492
seg2252 Sample_03.cyto2.cychp   3.0     Gain    3       126917179       126991856       74      1037
```

Figure 464 Data pasted into text file.

Combine PDFs into a single PDF

You can combine different PDFs into a single PDF with multiple pages and content.

You can do this by:

- Adding new data to an existing PDF file.
- Merging 2 or more existing PDF files.

IMPORTANT! The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

Add a new PDF export to an existing PDF file

1. When exporting a table or graph as a PDF, select the **Add to Existing Export** checkbox in the **Export Details** window. (Figure 465)

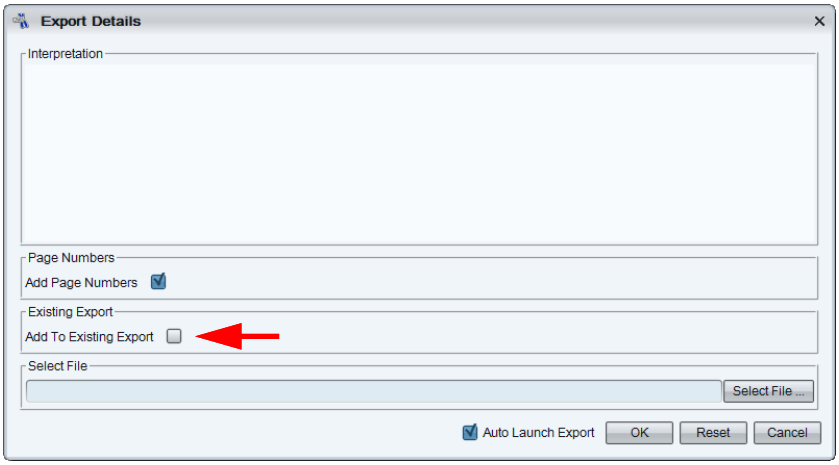


Figure 465 Export Details window with Add to Existing Export selected.

2. Click **Select File** and select an existing PDF file for the data to be added to.

   The **Save As** window opens. (Figure 466)

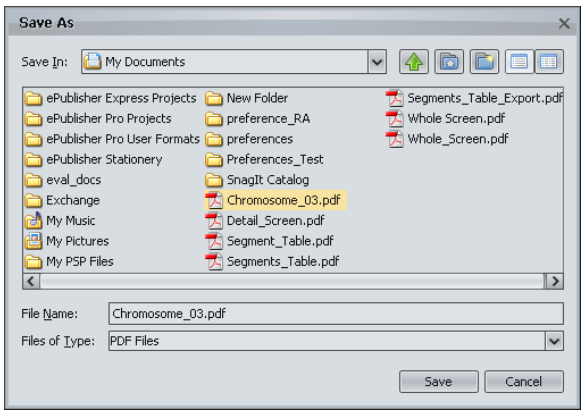


Figure 466 Save As window.

3. Select a PDF file, then click **Save** in the Save As window.

   A confirmation message appears, asking if you want to overwrite or add to the data.
4. Click Yes in the Confirm Rewrite notice to append the data in the selected file.
5. Click **OK** in the **Select Columns and File** or **Export Details** window.

   The new report data is combined with the existing report.

Combine 2 existing PDF files

1. Click **Exports → Combine PDFs**.

   The **Combine PDF Exports** window opens. (Figure 467)

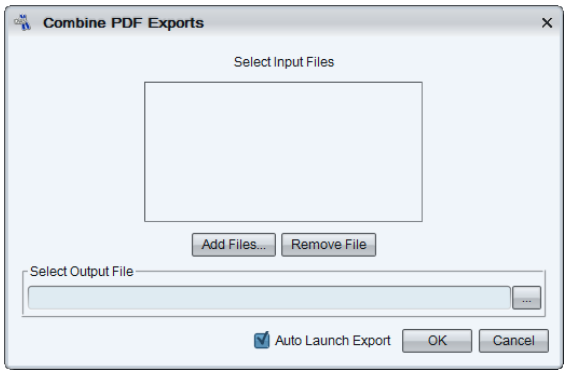


Figure 467 Combine PDF Exports window.

2. Click **Add Files…**.

   The **Select PDF Files to combine** window opens. (Figure 468)

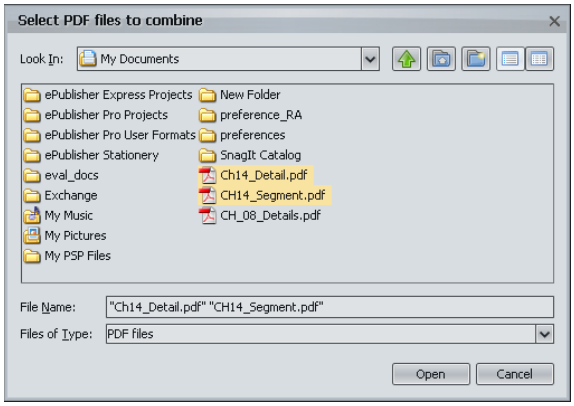


Figure 468 Select PDF Files to Combine window.

3. Select the PDF files to combine, then click **Open** in the Select PDF Files window.

   The selected files are displayed in the Select Input Files list.

   You can use the Remove File button to remove a selected input file.

   Click and drag on a file in the list to change the order of data in the Combined PDF.

4. Click the **Select Output File** button.

   The **Save As** window opens. (Figure 469)

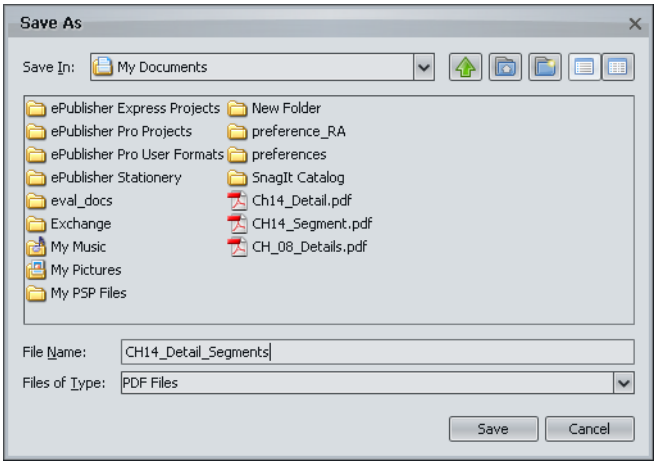


Figure 469 Save As window.

5. Enter a file name for the combined PDF file, then click **Save** in the **Save As** window.

   You are returned to the **Combine PDF Exports** window.

6. Click **OK**.

   Your selected PDFs are combined.

Export with ClinVar

The ClinVar export enables exporting of copy number data using the ClinVar Full Template for easy submission directly to ClinVar. A ClinVar Submission Profile must be created to use this Export. For details on the template, go to: **www.ncbi.nlm.nih.gov/clinvar/docs/submit/**

IMPORTANT! ChAS is a research use only application and any submission to ClinVar is the responsibility of the submitter.

All fields exported into the ClinVar submission template are selected and defined by the user. Standard ClinVar nomenclature is provided for required submission fields and can be customized as shown in "Add vocabulary content" on page 405.

Create a ClinVar submission profile

1. Click **Preferences** → **Edit User Configuration**.
   The User Configuration window appears.
2. Select the **Exports** tab, then select the **ClinVar** tab. (Figure 470)

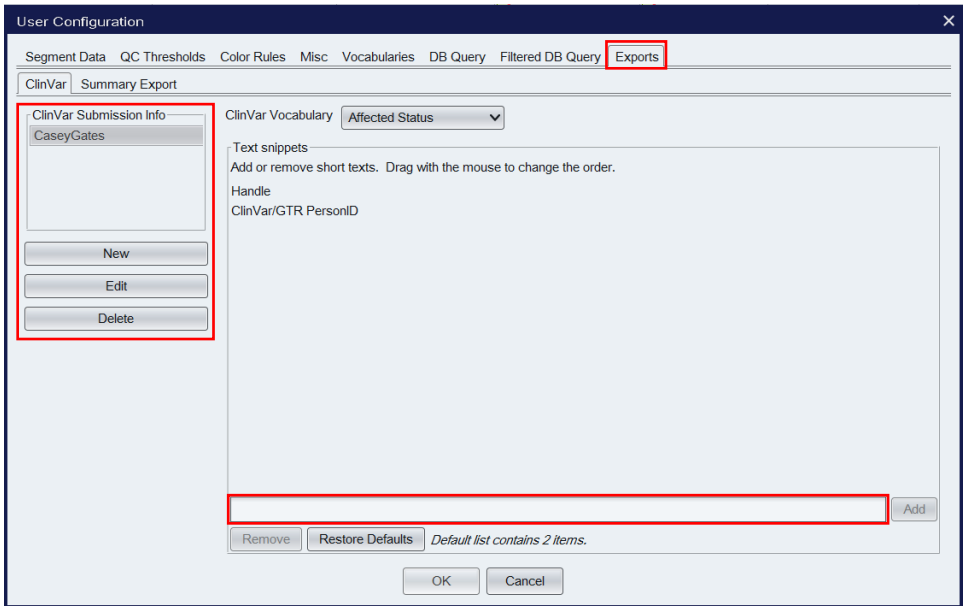


Figure 470 ClinVar window tab.

3. In ClinVar Submission Info pane, click the **New** button to create a new submission profile.
   An Edit Profile window appears.
4. Complete all the appropriate fields. Fields with an * are required by ClinVar.
5. Click **OK** to save the profile.
6. Optional: To create additional ClinVar profiles, repeat steps 3–5

Edit an existing ClinVar profile

1. Highlight the profile name in the ClinVar Submission Info list box that is to be modified.
2. In ClinVar Submission Info pane, click the **Edit** button.
3. Edit the appropriate fields.
4. Click **OK** to save your changes.

Delete a profile

1. Highlight the profile name in the ClinVar Submission Info list box you want to delete.
2. Click the **Delete** button.
3. Click **OK** to confirm the profile deletion, or click **Cancel** to keep the profile.

Add vocabulary content

By default, recommended ClinVar vocabularies are stored for certain required fields, but additional terms can be added to any field.

1. Click **Preferences** → **Edit User Configuration**.

   The User Configuration window appears.

2. Click the **Exports** tab, then click the **ClinVar** tab.
3. Select a category you want to add a term(s) to, then use the text field (Figure 470) to enter the additional term(s).
4. From the ClinVar vocabularies dropdown, select the category that you want to add a term(s) to.
5. Use the text field (at the bottom) to enter the additional term(s).
6. Click the **Add** button to add the term to the category's list.

Remove vocabulary content

1. From the ClinVar vocabularies dropdown list, select a category that contains the term you want to remove.
2. Highlight the term, then click **Remove**.

Export in ClinVar format

There are certain fields that are required before uploading to ClinVar. It is recommended you use the **ClinVar Table State** in the Segments Table to expose all required fields. To use the ClinVar Table State, see "Saved table states" on page 310.

1. In the Segments Table, apply the ClinVar Table State.
2. Select the segments to be exported using the **Use in Export** checkbox.
3. Fill in all columns for the selected segments, as all columns in the ClinVar Table State are required before you can upload to Clinvar.
4. In the Exports Menu, select ClinVar Export.

   A browse window appears.

5. Select a location to save the export, then use the File Name text box to name the export, then click **OK**.

   A Submission Info/Segment Data window appears.

6. Select the ClinVar Profile you want to use for this export.
7. Optional: If you want to add any additional comments to the export, click the Segment Data tab to enter them within this tab.
8. Click **OK** to export.

Note: You can open the ClinVar export in an Excel™ spreadsheet to add information to the optional fields. Opening the ClinVar export from ChAS, auto-populates all currently required ClinVar fields.

Table 54 Variant tab: Auto-populated columns into the ClinVar submission template.
All other optional columns are blank upon export.

Auto-populated column	Description
Chromosome	Populated from Segment Table (Chromosome)
Variant type	Populated from Segment Table (Gain/Loss)
Variant length	Populated by ClinVar upon submission
Copy Number	Populated from Segment Table (Gain/Loss)
Variation identifiers	Populated using OMIM™ track
Condition category	Populated from ClinVar Vocabulary
Clinical significance	Populated from Segment Table (Call)
Date last evaluated	Uses date of export unless otherwise specified on the Submission Info window before exporting.
Comment on clinical significance	Populated from Segment Table (Segment Interpretation)
Collection method	Populated from ClinVar Vocabulary
Allele origin	Populated from ClinVar Vocabulary
Affected status	Populated from ClinVar Vocabulary
Structural variant method/analysis type	SNP Array
Platform type	Microarray
Platform name	Populated based on the Microarray used
Software name and version	Chromosome Analysis Suite 4.6

Table 55 Case Data tab: Auto-populated columns into the ClinVar submission template.
All other optional columns are blank upon export.

Auto-populated column	Description
##Linking ID	Populated from Segment Table (Full Location)
Collection method	Populated from ClinVar Vocabulary
Allele origin	Populated from ClinVar Vocabulary
Affected status	Populated from ClinVar Vocabulary
Structural variant method/analysis type	SNP Array
Clinical Features	Populated from Sample Properties (Phenotype)
Tissue	Populated from Sample Properties (Sample Type)
Sex	Populated from Gender determination
Platform type	Microarray
Platform name	Populated based on the Microarray used
Software name and version	Chromosome Analysis Suite 4.6

Export a deletion log

The user, time, filename and reason why a file is deleted or republished to a ChAS Database can be exported.

1. Click **ChAS DB → Download Deletion Log** (Figure 471)

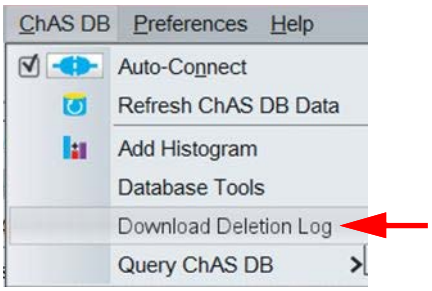


Figure 471 Download Deletion Log.

Your default internet browser opens.

2. Enter your ChAS Database credentials, as you normally would.
3. Depending on your internet browser, you may be prompted to either download the file or save it. To save it, click **File → Save As.**
4. To view your exported log file, open it in an Excel™ spreadsheet. (Figure 472)

C12

	A	B	C	D
1	Filename	User	Timestamp	Comment
2	20120622_111026_005_	cgates	2015-10-15T05:56:10.851-07:00	Added Calls to Segments no calls were made in original file

Figure 472 File viewed in an Excel™ spreadsheet example.

Deletion Logs can also be exported from the ChAS DB Tools Maintenance Page. See "Download deletion logs" on page 422.

20 User profiles and named settings

ChAS provides many options for customizing the display of data and annotations.

The User Profiles and Named Settings functions enable you to save your analysis and display settings.

Types of settings

ChAS provides 2 ways to store setup information. User profiles and Named settings. Each way works differently and performs different functions.

User profiles

A ChAS Browser User Profile stores your selections for various display settings as they were when the software was last shut down while using that user profile.

A new user profile can be created or selected only when starting the software.

The user profile saves the following display settings:

- Screen size, displayed tabs, and sizing of display areas
- The views displayed in ChAS, and the size of the display panes
- Available Named Settings: Different users can have different lists of named settings to choose from
- Name of the currently selected named setting
- Copies of the user's custom (not shared) named settings
- Data Display Configurations
- Region information files selected for CytoRegions and Overlap Map
- Which types of graph and segment data are turned on or off
- Display options for graph data (height, grid, values, etc.)
- Chromosome and area displayed.
- Selected Reference Annotation database (ChAS Browser NetAffx Genomic Annotations File)
- Loaded AED and BED files
- The files and Reference Annotations (Genes, DGV, etc.) that are checked or unchecked
- Custom color rules

Named settings

A Named Setting stores the user's choices for:

- Which types of graph and segment data are turned on or off
- Segment Filter Settings
- Restricted Mode on/off

  The Named Setting doesn't save a particular CytoRegion file, but does inform you if no file is selected when you select a setting with restricted mode on.

  It is possible to apply a Named Setting with restricted mode using a different CytoRegion file than was selected for the initial creation of the setting.

You can switch between different Named Settings in the same user profile to look at different types of data.

ChAS provides pre-configured (shared) Named Settings indicated by the  icon as described in the following table. These named settings cannot be deleted.

Table 56 Named settings.

Name	Genome segment filters	CytoRegion segment filters	Data types	
Standard	**Gain:** Marker Count = 50 Size (kbp) = 400; **Loss:** Marker Count = 50 Size (kbp) = 400	**Gain:** Marker Count = 50 Size (kbp) = 400 **Loss:** Marker Count = 50 Size (kbp) = 400	Gain Loss GainMosaic LossMosaic	Copy Number State Weighted Log2 Ratio Allele Peaks Allele Difference
High Resolution	**Gain:** Marker Count = 50 Size (kbp) = 100; **Loss:** Marker Count = 50 Size (kbp) = 100	**Gain:** Marker Count = 25 Size (kbp) = 50 **Loss:** Marker Count = 25 Size (kbp) = 50	Gain Loss GainMosaic	LossMosaic Copy Number State Weighted Log2 Ratio
LOH only (3Mb and 50 SNPs)	LOH: Marker Count = 50 Size (kbp) = 3000	LOH: Marker Count = 50 Size (kbp) = 3000	LOH Genotype Calls	Allele Peaks Allele Difference
Differential Gains and Losses	**Gain:** Marker Count = 50 Size (kbp) = 400; **Loss:** Marker Count = 50 Size (kbp) = 100	**Gain:** Marker Count = 25 Size (kbp) = 50 **Loss:** Marker Count = 25 Size (kbp) = 50	Gain Loss GainMosaic LossMosaic	Copy Number State Weighted Log2 Ratio
OncoScan defaults	0	0	0	
XON-Level 1	XON Level: Level 1 = On Level 2–4 = Off XON Gain/Loss Marker: Count = 0 Size (kbp) = 0	0	XON Region Gain XON Region Loss LOH Segments	Log2 Ratio Smooth Signal Allelic Difference

Create and use user profiles

You can only select or create user profiles upon starting ChAS.

1. Double-click the ChAS icon on the desktop; or

   From the Windows™ Start Menu, select **Programs** → **Thermo Fisher Scientific** → **Chromosome Analysis Suite** → **Chromosome Analysis Suite**.

   The ChAS Splash Screen and the **Select User** window open. (Figure 473)

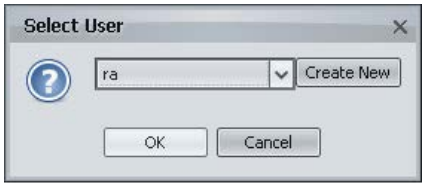


Figure 473 Select User window.

2. Click **Create New** in the Select User window.

   The **Create New User** window opens. (Figure 474)

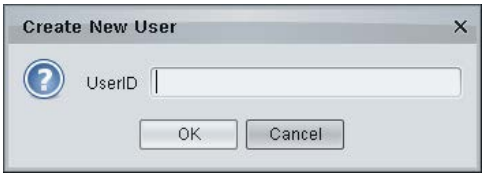


Figure 474 Create New User window.

3. Enter a name for the new profile in the User ID field.
4. Click **OK** in the Create New User window.

   The new user appears in the dropdown User list in the **Select User** window. (Figure 475)

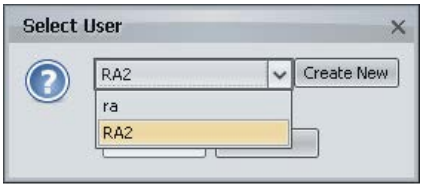


Figure 475 Select User window with new user profile.

5. Select the new user, then click **OK**.

   Any changes you make to the setup of the software that is listed in "User profiles" on page 408 are saved when you shut down the software and used the next time the software is opened with this user profile.

Delete a user profile

1. Go to the Windows™ folder where the user profiles are stored and delete the folder with the profile name you want to delete.

   You can see the location of the folder in the About window, as described in "Analysis file locations in Windows™ 11" on page 23.

Create and use named settings

You can save a snapshot of your favorite settings as a Named Setting. To apply a particular Named Setting to the active data (check marked in the Files List), make a selection from the Named Setting dropdown list. Some pre-configured Shared Named Settings may be available for use by all users. Only an administrator can add or remove Shared Named Settings, but any user can apply them to their data.

Save a named setting

1. Set the display data settings as desired.

   These can include:

   - Which graphs and segment types are turned on or off
   - Segment Filter Settings
   - Restricted mode on/off

2. From the Preferences menu, select **Save Named Setting**.

   The **Setting Name** window opens. (Figure 476)



Figure 476 Named Setting List.

3. Enter a name for the setting you want to create, then click **OK**.

   The setting is saved and appears in the Named Setting dropdown list. (Figure 477)

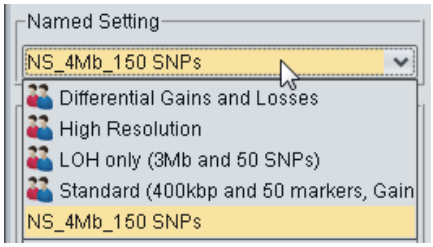


Figure 477 Named Setting dropdown list.

Note: The Named Setting saves the settings at the time it was created. Subsequent changes to the settings will not be saved in the Named Setting.

Select a named setting

1. From the Named Setting dropdown list, select the setting. (Figure 478)

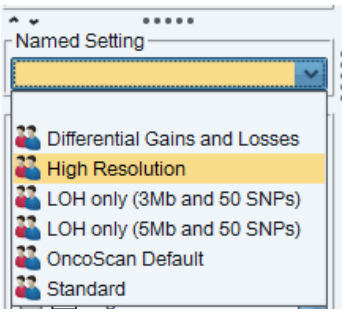


Figure 478 Named Setting dropdown list.

Alternatively, from the Preference menu, select **Apply Named Setting…**

The **Select Named Setting** window opens. (Figure 479)



Figure 479 Select Named Setting window.

2. Select the Named Setting from the dropdown list, then click **OK**.

   The selected setting is applied.

Note: A Named Setting is not modified by any changes that you make to the settings in ChAS. If you want to keep a copy of your new settings, you will need to save them as a new Named Setting.

Delete a named setting

1. From the Preferences menu, select **Delete Named Setting.**

   The **Delete Setting** window opens. (Figure 480).

Note: Shared Named Settings (the icon in the Named Setting list) do not appear in the Delete Setting dropdown list. Users cannot delete or modify a shared Named Setting.



Figure 480 Delete Setting window.

2. Select the setting you want to delete from the dropdown list, then click **OK**.

   The setting is deleted.

Export and import preferences

Preferences functions enable you to transfer most of the settings in a User Profile between one system and another.

Note: If you import "exported" preferences that reference a Shared Named Setting which no longer exists, such as a Shared Named Setting from ChAS 1.0.1 or ChAS 1.1, the profile is changed to point to the default Shared Name Setting.

Set directory for export preferences

1. From the Preferences menu, select **Export Preferences…**

   The **Select Directory to export preferences to** window opens. (Figure 481)

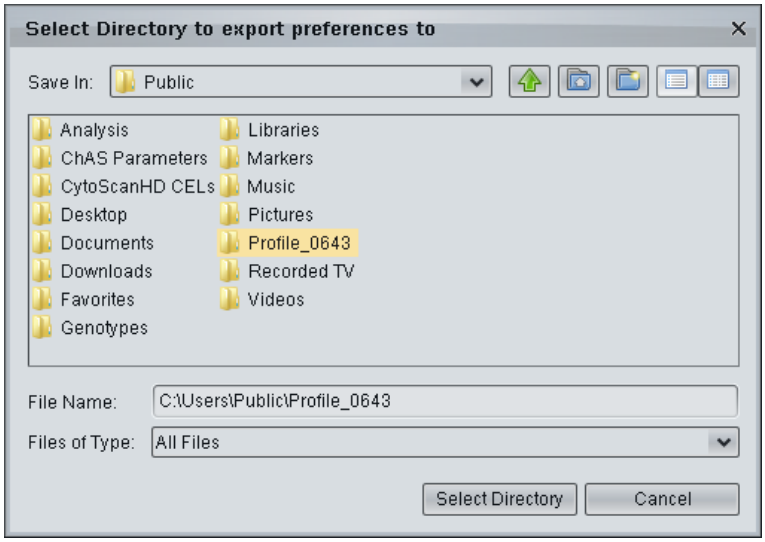


Figure 481 Select Directory to export preferences.

2. Use the navigation features of the window to select or create a directory for the preferences.

   Note: The software creates a folder named "preferences" in the directory you select or create. If you select a directory that already contains a "preferences" folder, it is overwritten. When you want to import the preferences, select the directory that contains the "preferences" folder that is indicated by the icon.

3. Click **Select Directory.**

   If the directory already contains a "preferences" folder, the Overwrite notice appears. (Figure 482)

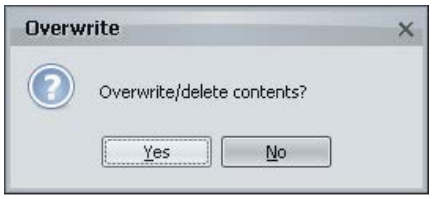


Figure 482 Overwrite notice.

4. Click **Yes** to export the preference files to the directory that you selected.

   You can then transfer the preferences to another user profile or system.

Set directory for import preferences

1. From the Preferences menu, select **Import Preferences…**

   The **Select Folder to import preferences from** window opens. (Figure 483)

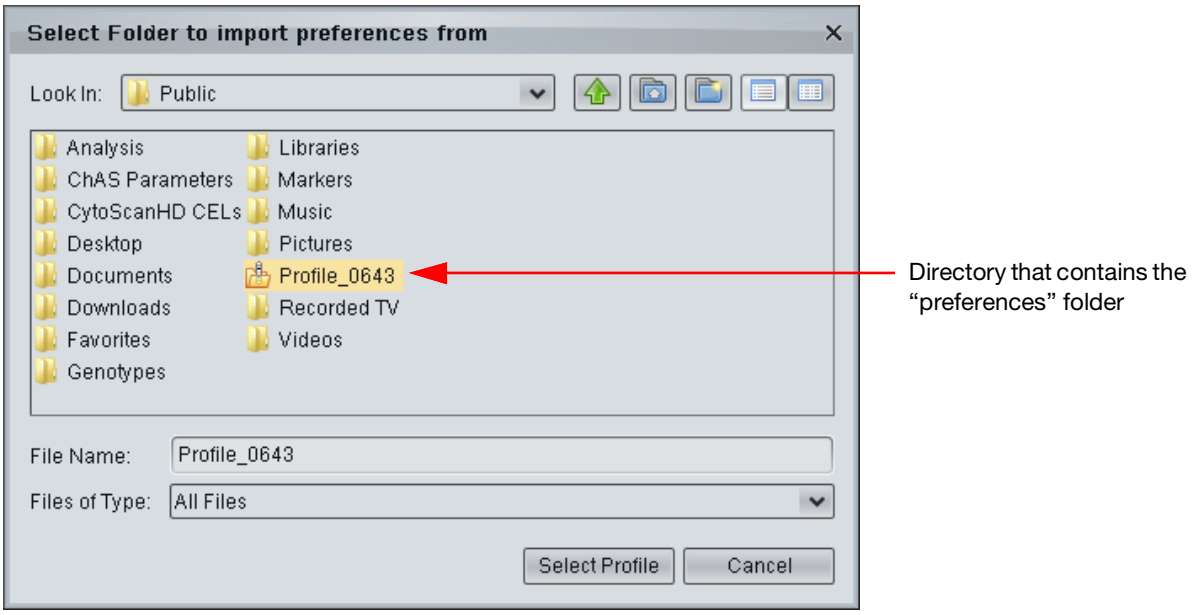


Figure 483 Select Folder to import preferences from.

2. Use the navigation features of the window to select the directory that the preferences were exported to (directories that contain a "preferences" folder are indicated by the icon.)
3. Click **Open to import the preference files.**

   If you selected a directory that doesn't contain the "preferences" folder, an error message appears. (Figure 484)

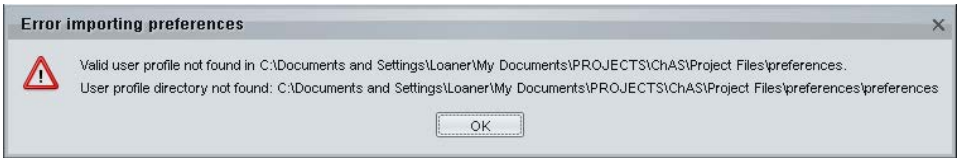


Figure 484 Error notice.

4. Click **OK**, then repeat steps 1–3, selecting the correct folder.

   The Restart notice appears. (Figure 485)



Figure 485 Restart notice.

Note: The imported preferences will not be applied until you restart ChAS.

Import hyperlinks

External websites may update their links from time to time. To remedy this, a feature as been added to update an outdated link(s) within ChAS.

1. Click **Preferences → Import Hyperlinks**.

   The Load Hyperlinks Configuration window opens.

2. Navigate to your updated hyperlinks (**.chaslink**) file, click to highlight it, then click **Select File**.
3. Restart the ChAS Browser to apply the link update(s).

Note: For **.chaslink** file help, contact Technical Support.

Configure the HTTPS service

The HTTPS service may enable external applications to interact with the ChAS Browser. By default, this service is off. Contact Technical Support before activating this feature.

1. Click **Preferences → Configure HTTPS Service**.

   The Configure HTTPS Service window opens.

2. Select the checkbox to enable the service, then enter the designated Port number.
3. Close the window.

   The service and Port are now activated.

21 Database tools

Connect to a remote ChAS DB server

Note: The ChAS v4.6 Browser cannot access previous ChAS Database versions.

You must upgrade any older version of the ChAS Database to ChAS v4.6 before the ChAS v4.6 browser can access it. To do this, see "Back up a database" on page 419 and "Restore a database" on page 419.

1. From the **Preferences** dropdown list, click **Edit Application Configuration…**

   The **Configuration** window appears.

2. Click the **Server** tab.

   The Server window/tab appears. (Figure 486)

3. Type in the name or IP address of the computer/server name that you would like to connect to in the Hostname or IP Address text field or contact your IT Department for help completing this form.

   Note: Up to 3 Hostnames/IP Addresses can be saved/stored.

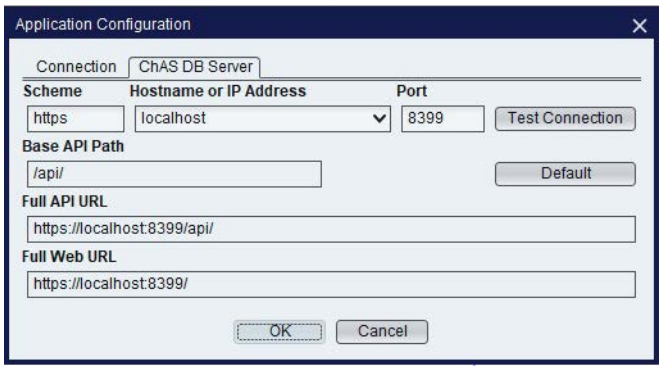


Figure 486 Server window/tab.

4. Click **OK**.

   If a connection to the ChAS DB cannot be established or the server/computer containing ChAS DB is not turned on, the following message appears (Figure 487). Check the name/IP address and ensure that the server/computer is turned on.



Figure 487 Error message.

Access the ChAS DB server tools

Note: The screen captures used in this chapter may vary depending on the browser you are using.

1. From the **Chas DB** dropdown list, click to select **Database Tools**. (Figure 489)

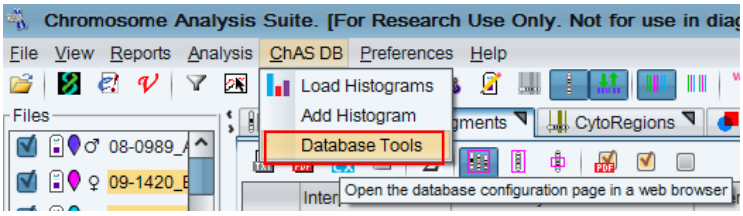


Figure 488 Select User window.

The following web page appears. (Figure 489)

IMPORTANT! The ChAS Server Home Page requires an active internet connection and browser (Google Chrome™ browser and Internet Explorer v11 are recommended). Also, if you are using the local ChAS DB, an active internet connection is not required.

After 30 minutes of inactivity, you will need to reenter your credentials to access the ChAS Database Tools page.

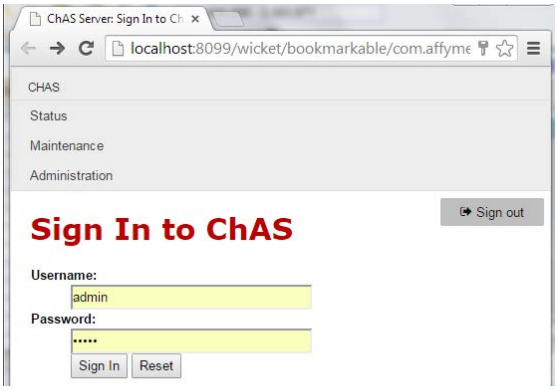


Figure 489 Sign in to ChAS window.

2. Log in using the installer's default Username: **admin** and Password: **admin**.
   After this initial log in, it is mandatory to change the admin password. Ensure that this password is stored securely as recovery of this admin account will require to reinstall the software. It is recommended to create New User(s) and assign User Roles. See "Administration" on page 423.

Note: Ensure that you look in the URL field to identify which ChAS Database the ChAS Database Tools is accessing.

The ChAS DB Home Page appears.

Status page

Use this page to view how many samples and segments are in the Database. (Figure 490)

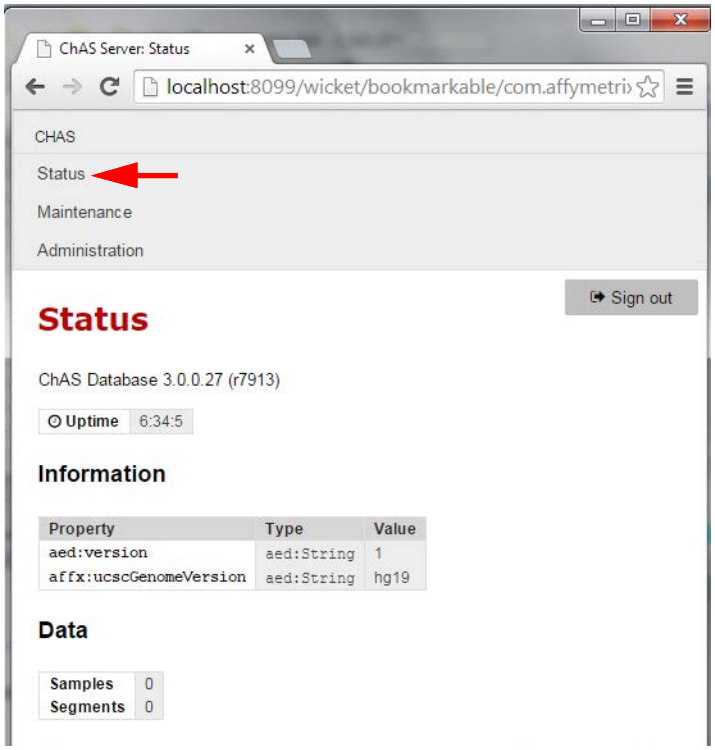


Figure 490 ChAS DB Status Page.

Maintenance

Use this page to perform a backup, restore, and database clean up. (Figure 491)

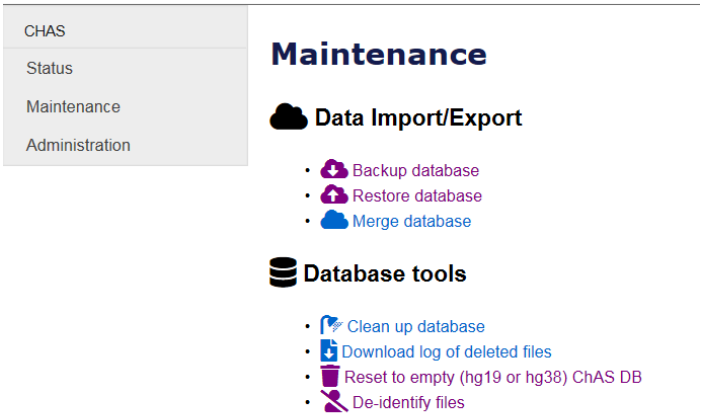


Figure 491 ChAS DB - Maintenance.

Back up a database

1. Click **Backup Database**, then click the **Backup** button.

   A backup is automatically generated and is stored in the Affymetrix directory shown in Figure 492. (\Affymetrix\ChAS\PostgreSQL\Backups\ChASDB_yyMMdd_HHmmss.backup)

Note: A backup is automatically done whenever a Restore operation is performed.

IMPORTANT! It is strongly recommended that you perform scheduled routine backups of the database.

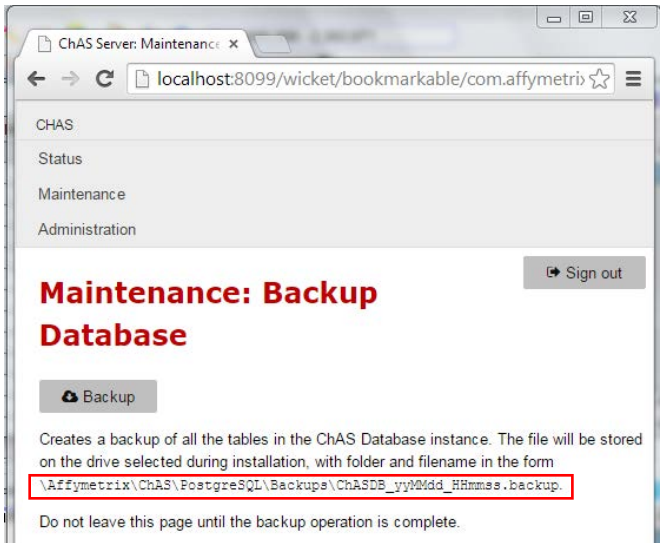


Figure 492 ChAS DB - Backup Database.

Note: The ChAS installer creates a disabled Windows Task that automatically backs up the ChASDB database on a weekly basis after it is enabled.

Restore a database

Note: Restoring a backup file created in ChAS 3.0/3.1/3.2/3.3/4.0 automatically upgrades it to ChAS 4.1.

1. Click **Restore Database**, then click the **Choose File** button. (Figure 493)

   A File Explorer window opens.

2. Navigate to the location where your ChAS DB was last backed up, then click **Open**.

   By default, a backup of your current database is stored/resides here:
   \\Affymetrix\ChAS\PostgreSQL\Backups

3. Click **Restore** to start the restore process.

IMPORTANT! Do not leave this page after choosing the Restore button until you see the message that the database has been successfully restored. Also, after the restore process has successfully completed, you must click **ChAS DB → Refresh ChAS DB Data** to view the data in the database using the ChAS Browser.

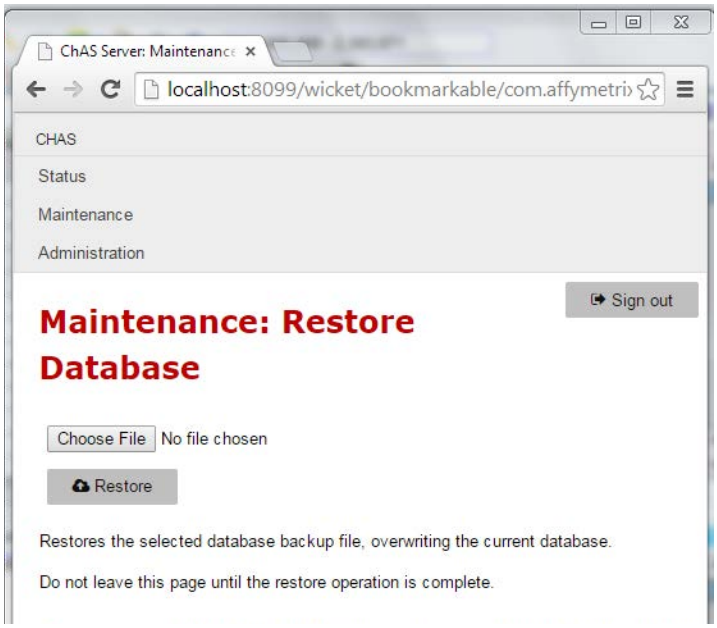


Figure 493 ChAS DB Home Page.

IMPORTANT! After restoring the database, you must click ChAS DB → Refresh ChAS DB data to view the newly restored data from the database.

Merge ChAS Databases

When merging the contents of 2 ChAS Databases into a single database, one database should be restored into ChAS and the other database must exist as a **backup.db** file.

When merging the segments from 2 databases, if a duplicate entry is found then the merge keeps the entry for the database currently active in ChAS. The duplicate from the **backup.db** is skipped.

Merge 2 ChAS Databases

IMPORTANT! The 2 ChAS Databases to be merged, must be from the same version of ChAS. Also, the library files for CytoScan HD and OncoScan CNV Plus must be present in your Library folder before merging the 2 databases.

Ensure that one of the ChAS Databases is restored into ChAS (for details on how to Restore a ChAS DB, see page "Restore a database" on page 419). Since any duplicate segments between the databases will keep the copy from the actively restored database, ensure that the database with the more complete content is the one that is actively restored in ChAS.

1. From the ChAS Browser, go to **ChAS DB → Database Tools**.

   If prompted, log into the ChAS Database as you normally would.
2. Click the **Maintenance** link.
3. Click the **Merge database** link.
4. Click the **Browse** button to navigate to the **backup.db** file you want to merge with the current database.
5. Click **Merge**.

Merge an older ChAS Database

If you want to merge a database (from an older version of ChAS) with a current ChAS 4.0 database, perform these steps:

1. Backup your current ChAS 4.0 database.
2. Restore the database from, for example, ChAS 3.1. See "Restore a database" on page 419. During the restore process, the older ChAS Database is automatically upgraded and is now compatible with ChAS 4.0.
3. Backup the 3.1 database you just restored.
4. Restore the ChAS 4.0 database you backed up in Step 1.

Note: If the databases to be merged contain duplicate entries, the copy that is in the currently restored database is kept. The entry from the backup.db that is being merged is skipped.

5. From the ChAS Browser, go to **ChAS DB → Database Tools**.

   If prompted, log into the ChAS Database as you normally would.
6. Click the **Maintenance** link.
7. Click the **Merge database** link.
8. Click the **Browse** button to navigate to the **backup.db** file you want to merge with the current database.
9. Click **Merge**.

Clean up a database

ChAS DB automatically runs re-indexing scripts to maintain optimal performance. You can also run these scripts manually if desired.

Note: You must have a **Manager** or an **Admin** role to clean up a database.

1. Click the **Clean up database** button (Figure 494) to run the Vacuum Analyze and Reindex Database optimization process.

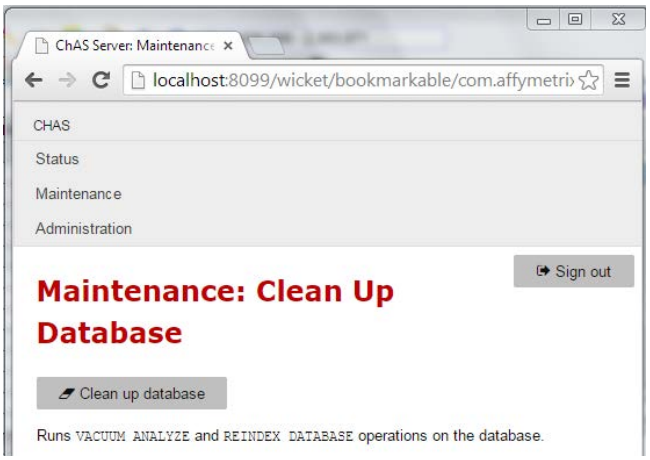


Figure 494 ChAS DB - Cleanup Database.

Download deletion logs

Use this feature to export a list of filenames that were deleted from ChAS DB.

1. Click the Download Log of Deleted Files
2. If asked, provide your ChAS DB Username and Password.
3. Save the genome-model log file.
4. Open with an Excel™ spreadsheet for easier viewing.

Create a blank ChAS DB

Use this feature to create a blank hg19 or hg38 ChAS DB. Ensure that you backup your previous database prior to creating an empty ChAS DB, as the database will be erased and recreated.

Note: xxCHP files are only compatible with a ChAS DB of the same genome version.

1. Back up the current ChAS DB.
2. Click the Reset to Empty (hg19 or hg38) ChAS DB
3. Use the dropdown list to select the genome version for your new database. (Figure 495)

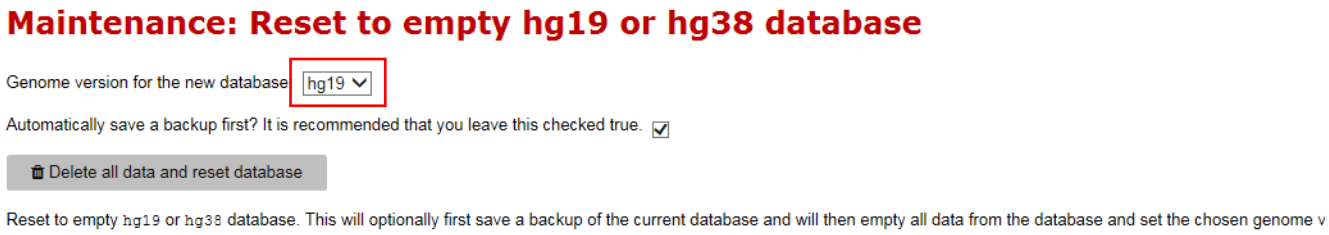


Figure 495 ChAS DB - Cleanup Database.

4. Check the box to have a backup of the current ChAS DB before the database is deleted and an empty DB is created.
5. Click **Delete all data and reset database**.

De-identify files

De-identifying files will remove potentially sensitive information from the ChAS DB. By running De-identification, the file names stored in the ChAS DB will be replaced by an alpha numeric ID.

Note: If you have included sensitive information in custom database fields, this process will not remove that information.

1. Click **De-identify Files**.
2. Optional: Create a backup of the database to preserve the original content, then click the **Start De-identification** button to replace the file names in your ChAS Database.

Administration

IMPORTANT! The default **admin** password assigned at installation must be changed before you can access the ChAS Database. This applies to new ChAS installations and restored ChAS Databases that still use the default **admin** password.

When logging into the ChAS DB for the first time, a message appears prompting you to change the default **admin**/**admin** password.

Change the default admin password

1. Click **ChAS DB → Database Tools**.

   A window appears.

2. Log in using the **admin**/**admin** for the username and password.

   The **Profile** option is enabled.

3. Click **Profile**.

   The Profile window appears

4. Go to the **Password** field and enter a secure password at least 8 characters in length, then enter it again in the **Password Confirmation** field.

5. Click **Accept**

Your new password is set and the ChAS Database is now accessible. **Note:** Ensure that you write down and store your new password in a safe place, as it cannot be reset.

Use the Administration feature

Note: You must have an Admin role to perform Administration functions. Log files for the ChAS Database can be found in: \ProgramData\Affymetrix\ChAS\Log

1. Click **Administration**.

   The following window appears: (Figure 496)

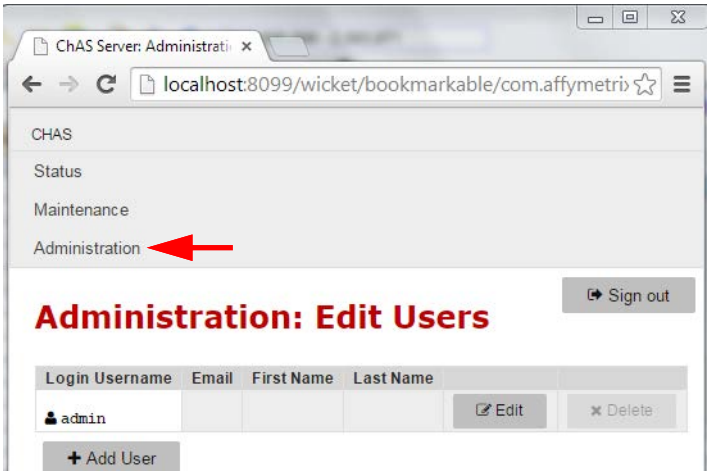


Figure 496 ChAS DB - Administration.

2. Click **Edit Users**.

   The following window appears: (Figure 497)

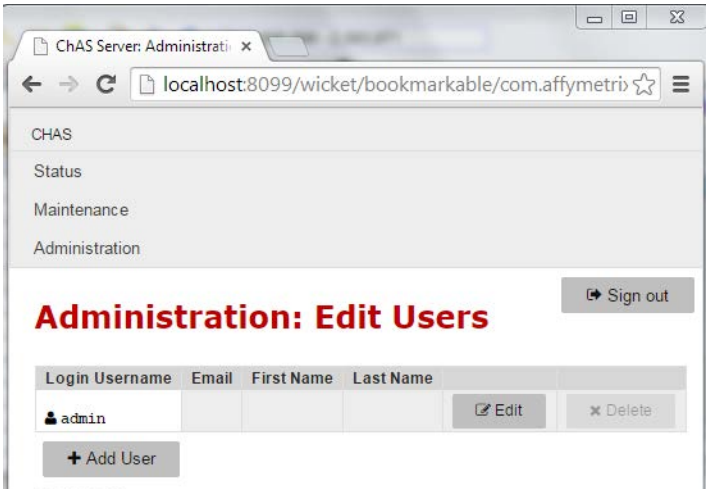


Figure 497 ChAS DB - Administration (Edit Users).

3. Do one of the following:

   - Click the **Edit** button to edit a current Username.

     Note: When changing the password for an existing user account, you must first enter your current password.

   - Click **Add User** to add (grant privileges to) a new ChAS DB user.

Permission Guidelines

- **User Role** has permission to query the database, edit segments, add segment and sample annotations, but does not have permission to upload data to the database, run backup/restore, maintenance or edit users.
- **Manager Role** has permission to query the database, edit segments, add segment and sample annotations, upload data to the database, run backup/restore, maintenance, but does not have permission to edit users.
- **Admin Role** has permissions to run all functions in ChAS Browser and ChAS DB.

Use a shared ChAS Database while off-line

IMPORTANT! If your Windows™ Firewall is enabled during the installation of ChAS and you want to Backup the ChAS Database and Restore it to your local ChAS DB, a message may appear indicating that you cannot connect to the shared folder. If this message appears, contact your IT department for help in allowing file sharing through the Windows™ Firewall.

Work off-line using a ChAS Database that resides on a shared server

1. Connect to the shared ChAS DB server click **Preferences → Edit Application Configuration**, **ChAS DB server** tab. For details on connecting to another ChAS DB, see "Connect to a remote ChAS DB server" on page 416.
2. Follow the procedure to perform a back up, locate the back up, then copy it to your local computer.
3. Return to **Preferences → Edit Application Configuration**, **ChAS DB server** tab, then click the default to redirect to your local ChAS DB server.

See "Restore a database" on page 419 for instructions on how to restore the back up database you just copied from the server.

IMPORTANT! You must have a Manager or Admin role and ensure that you log back into the local host before restoring your computer. While performing a restore from a backup of a shared server, the roles associated with the shared server are displayed, therefore any roles that were created on the local server are replaced by those used on the shared server (until local host ChAS DB is restored again).

Publish data you have analyzed in off-line mode to the shared ChAS DB server

1. Log back into the shared ChAS DB server. To do this. click **Preferences → Edit Application Configuration**, **ChAS DB server** tab, then enter the server name/IP address.
2. Click **OK**.

Note: After you are logged into the shared ChAS DB server, publish the samples to the database as you normally would. See "Publish data to the database" on page 377. If a xxCHP file has been previously Published to the database, you will receive a warning indicating this sample already exists in the database. You can choose to overwrite the existing information or cancel to keep the existing information.

Remap a hg19 ChAS DB to hg38

A ChAS DB populated with data from hg19 analyses can be remapped to hg38 coordinates using the ChAS DB Remapper feature.

The ChAS DB Remapper maps the segments from your hg19 ChAS DB to the hg38 genome. All segments in ChAS DB start and end with a marker currently mapped in hg19. The Remapper takes the probe locations for all markers in the segment from hg19 and remaps them to hg38. It locates the best representation of the remapped segment in hg38 by taking into account the number of additional markers in the remapped segment, as well as the number of markers removed from the original segment.

Remap confidence = (originalMarkerCount - addedCount - 2*removedCount)/originalMarkerCount

For remapped segment definitions in a ChAS DB, see the table on page 368.

Segments with a Remap confidence >0.75 are remapped to the hg38 ChAS DB. The Remapper makes a copy of the database prior to remapping. However, always make a backup or copy of the ChAS DB that you would like to remap for safe keeping.

1. Click **ChAS DB → ChAS DB Remapper**.
2. Click the **Browse** button (Figure 498) to select a ChAS db.backup to be remapped to hg38.

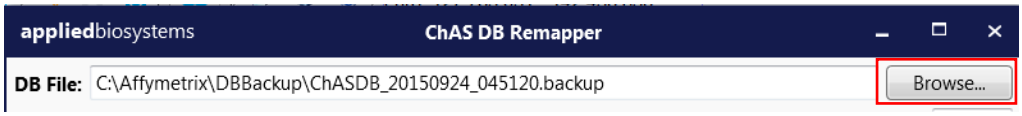


Figure 498 ChAS DB Remapper.

3. Click **Start**.

Note: Depending on the size of the ChAS DB.backup to be remapped, this process may take several minutes.

The ChAS Remapper window appears. (Figure 499)

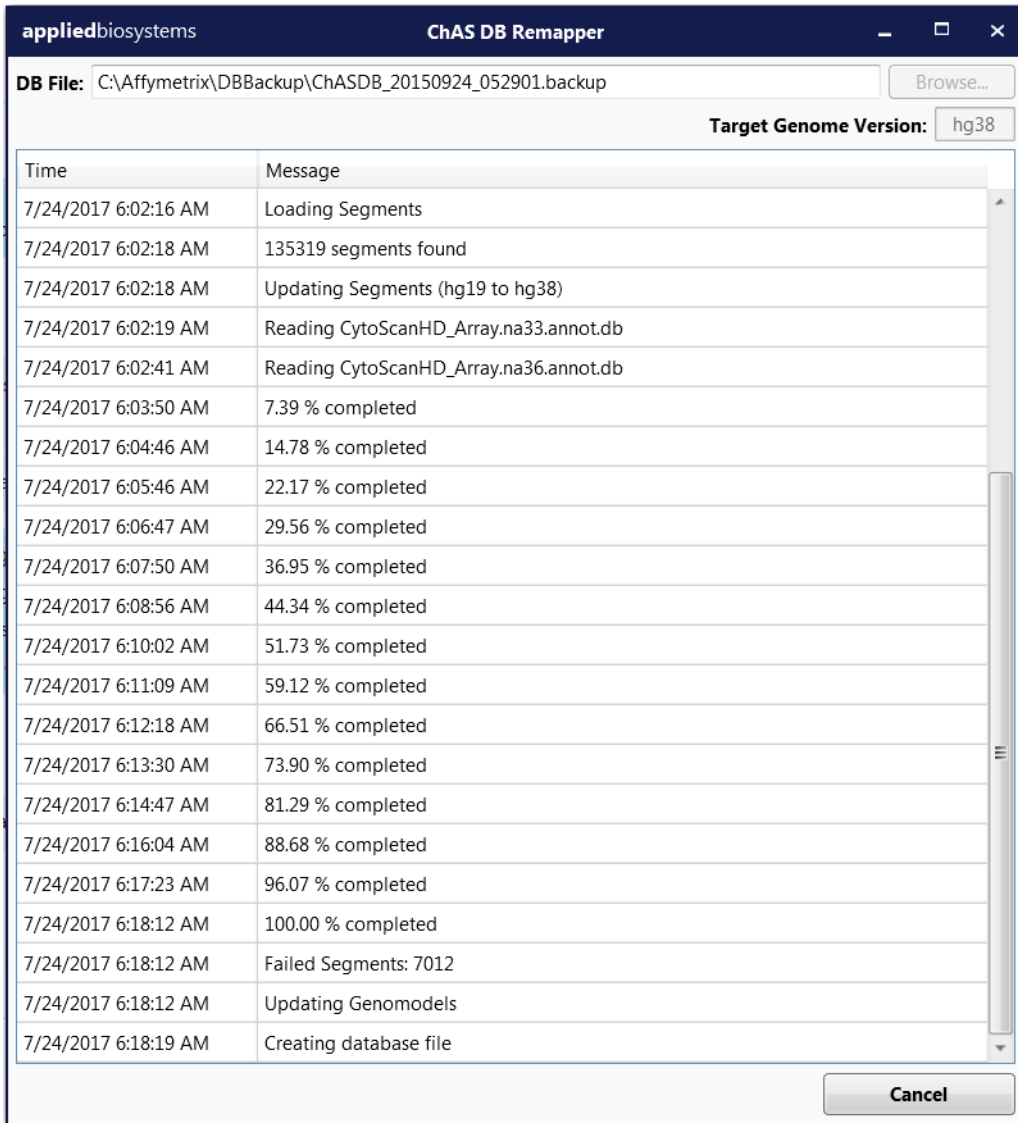


Figure 499 ChAS DB - Cleanup Database.

The following files are generated in the same folder as your original ChAS DB select to remap:

- ChAS DB.hg38.backup: This backup can be restored as the active ChAS DB for querying within the Browser.
- A TXT file listing all original segments: Provides a text file of the original segment information and the remapped segment information along with success or fail criteria.
- A TXT file listing the segments that failed to remap to hg38: Provides s text file of the original segments that did not remap to the new genome build.

For more information about viewing a remapped database, see "Additional segment intersection information" on page 370.

22 ChAS Database Loader (CDL)

The ChAS Database Loader (CDL) is now part of the ChAS Browser. CDL enables uploading of xxCHP files from any previous (including the current) version of ChAS. You may upload up to 500 xxCHP files at one time (as long as they are all analyzed from the same genome build).

CDL supports the following array types:

- Genomewide SNP 6
- CytoScan™ 750K
- CytoScan™ HD
- CytoScan™ HD Accel
- CytoScan™ Optima
- CytoScan™ XON
- CytoScan™ HTCMA
- OncoScan™ CNV Plus
- OncoScan™ CNV
- Cytogenetics 2.7M

Start CDL

1. Click **ChASDB → ChAS Database Loader**.

   The ChAS Database Loader window appears. (Figure 500)

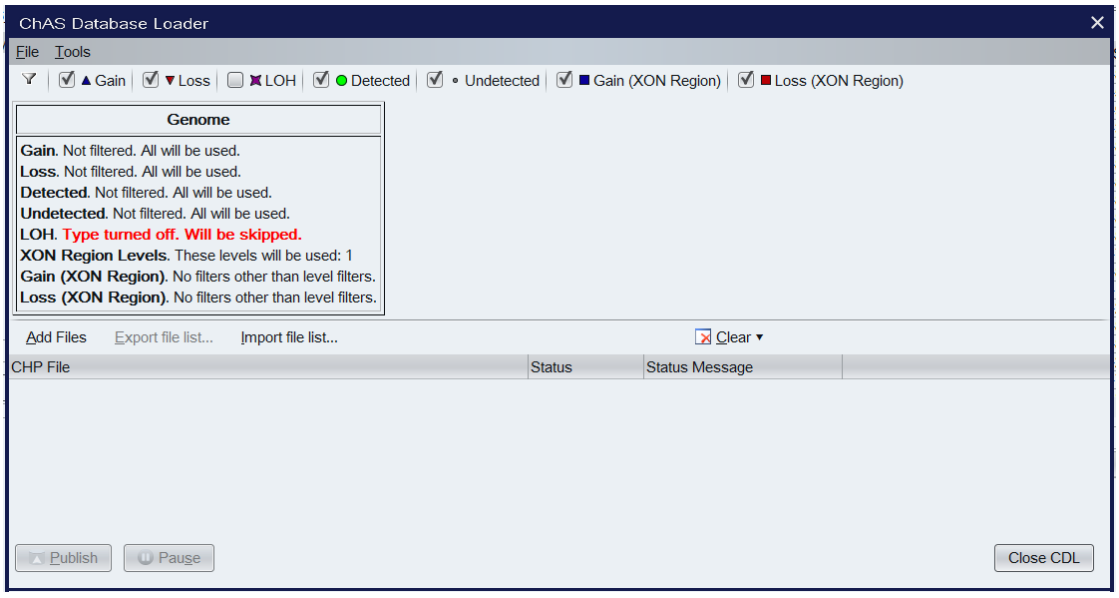


Figure 500 Main CDL window.

Add files to CDL

1. From the main CDL window, click **Files → Add Files** (Figure 501)

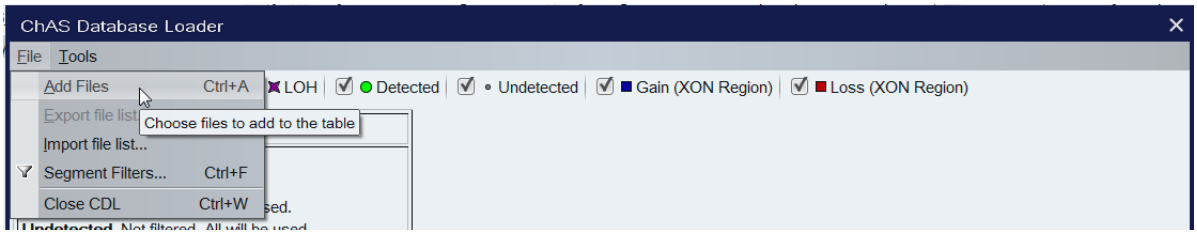


Figure 501 Add Files.

A File Explorer window appears. (Figure 502)

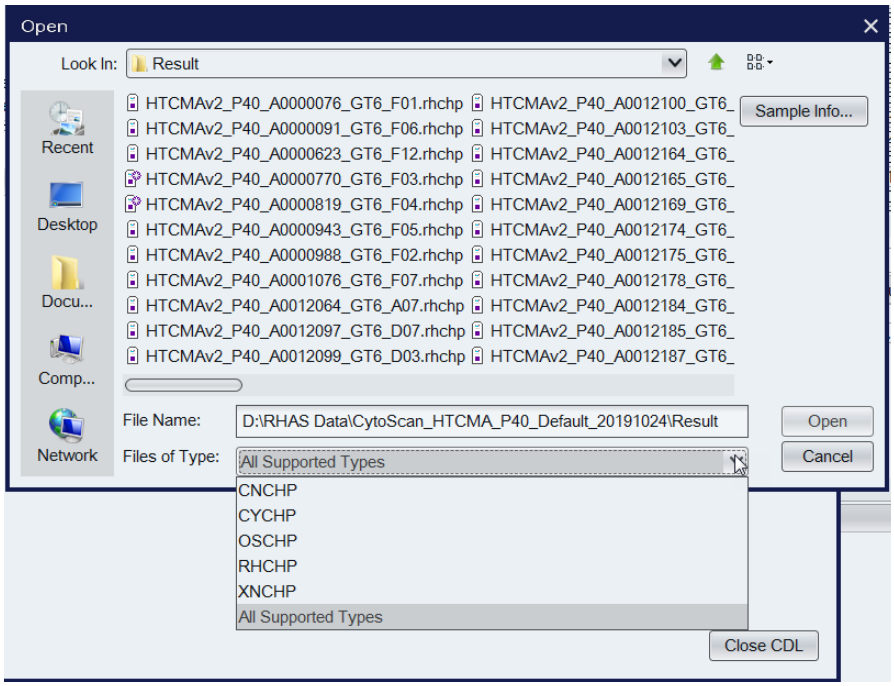


Figure 502 Add Files.

By default, the **Files of Type** is set to All Supported Types. (Figure 501) If you want to view a specific supported file type, click the dropdown, then select the file extension you want to display.

2. Click, Ctrl+click, Shift+click or Ctrl+a (to select multiple files).
3. Click **Open**.

   Your selected files now appear in CDL's main window. (Figure 504)

   Repeat steps 1–3 if you want to add files (up to 500) from different saved locations.

Sample info

1. If you want to view the properties of the files displayed in the File Explorer window, click Edit Configuration.

   The Sample Info window appears. (Figure 503)

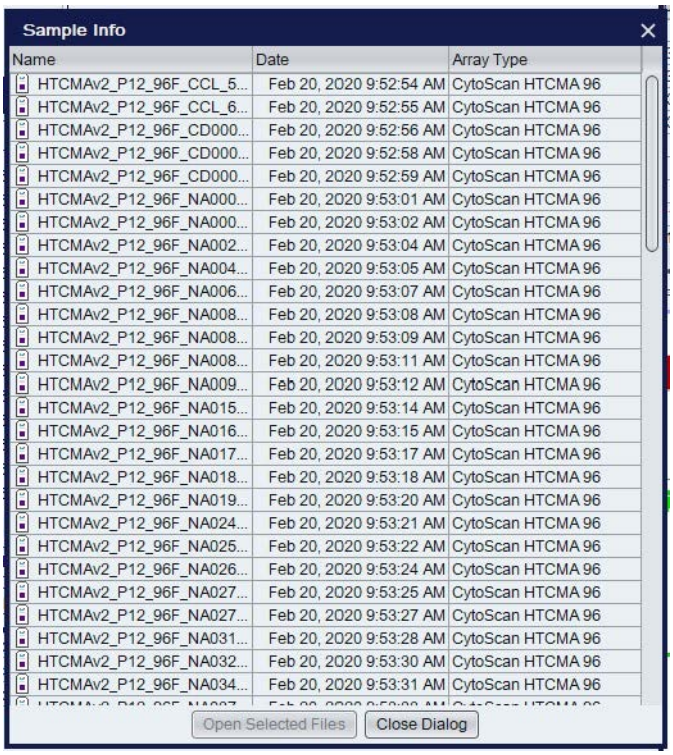


Figure 503 Sample Info window.

2. Click, Ctrl+click, Shift+click, or Ctrl+a (to select multiple files).
3. Click **Open Selected Files**.

   Your selected files now appear in CDL's main window.

Note: A special icon is used to indicate a CHPCAR or "sidecar" file, as shown in Figure 504. For more information on sidecar files, "Edit segment data overview" on page 211.

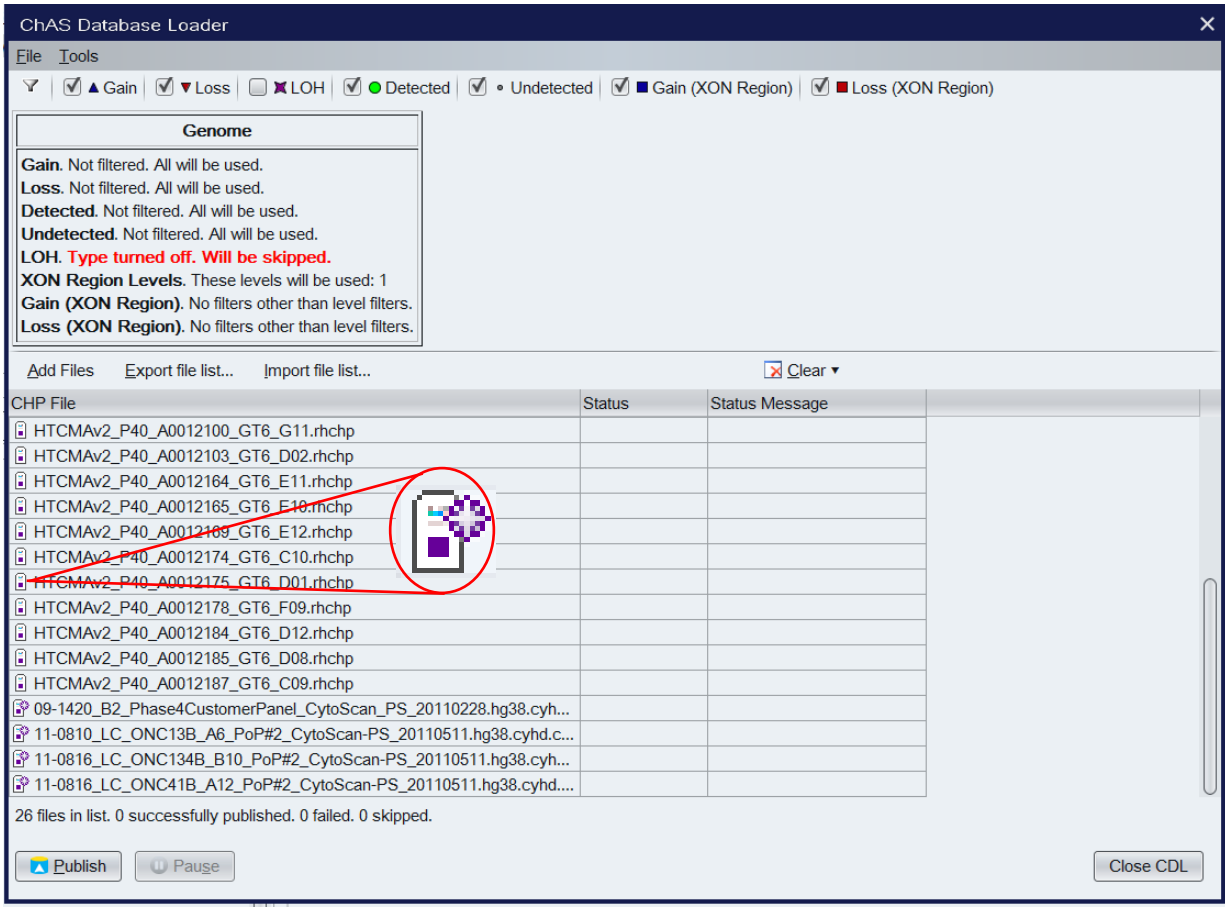
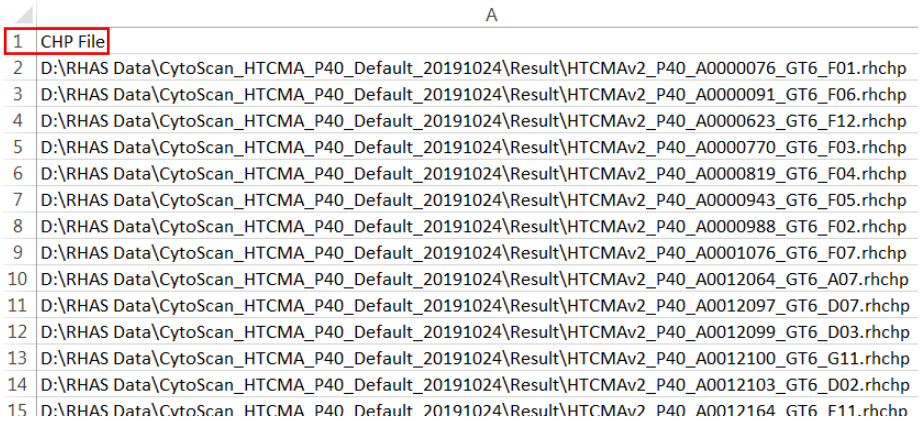


Figure 504 Main CDL window populated.

IMPORTANT! File level properties are optional fields that are available to CHP files analyzed in ChAS 3.0 or higher. Any file level properties entered are stored in the CHPCAR file, these properties will not populate in the CDL window. However, if those properties were entered for a CHP file and are contained in the CHPCAR file, they will be published to the database. Entries in a CHPCAR file supersede entries in the text file. File level properties for your xxCHP files can be added directly to the database after publishing has completed. For more details, see "Interact with the ChAS Database" on page 363.

Add files to be published using a text file

Files can be loaded into CDL using a tab-delimited text file. Place the file names, including their paths in the first column, then label the first column header **CHP File** as shown in Figure 505.



	A
1	CHP File
2	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000076_GT6_F01.rhchp
3	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000091_GT6_F06.rhchp
4	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000623_GT6_F12.rhchp
5	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000770_GT6_F03.rhchp
6	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000819_GT6_F04.rhchp
7	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000943_GT6_F05.rhchp
8	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000988_GT6_F02.rhchp
9	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0001076_GT6_F07.rhchp
10	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012064_GT6_A07.rhchp
11	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012097_GT6_D07.rhchp
12	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012099_GT6_D03.rhchp
13	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012100_GT6_G11.rhchp
14	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012103_GT6_D02.rhchp
15	D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012164_GT6_F11.rhchp

Figure 505 Tab-delimited text file Header 1 example.

1. Click **Import File List** or click **Files → Import file list**.

   A File Explorer window appears.

2. Navigate to, then select the tab-delimited text file containing path to the xxCHP files to be loaded into CDL.

Publish to the ChAS Database

IMPORTANT! Before you use CDL to publish your files, it is highly recommended you backup your ChAS Database first. For instructions on how to access and backup your database, see Chapter 21, "Database tools" on page 416. Also, xxCHP files can ONLY be published to a ChAS DB of the same genome version assignment.

Test your connection (optional)

Before publishing, you may want to test your ChAS Database connection. To do this:

1. Click **ChAS DB → ChAS Database Loader**
2. Click **Tools → Test Connection**

   A message appears if there is a successful connection to the ChAS Database.

Verify the ChAS Database

1. From the ChAS Browser, click the **Preferences** dropdown list, then select **Edit Application Configuration…**

   The **Configuration** window appears.

2. Click the **Server** tab.

   The Server window/tab appears. (Figure 506)

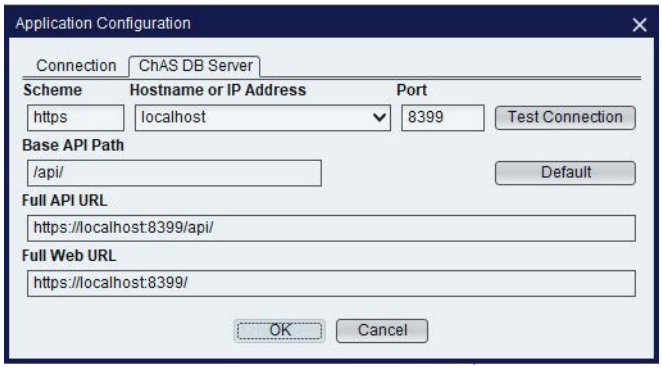


Figure 506 Server window/tab.

3. Verify the ChAS DB you are publishing to is correct, then click **OK**.

Before publishing files

Before publishing files, you must check the Genome dialog box (Figure 507) to ensure that your desired filter settings and data types are enabled.

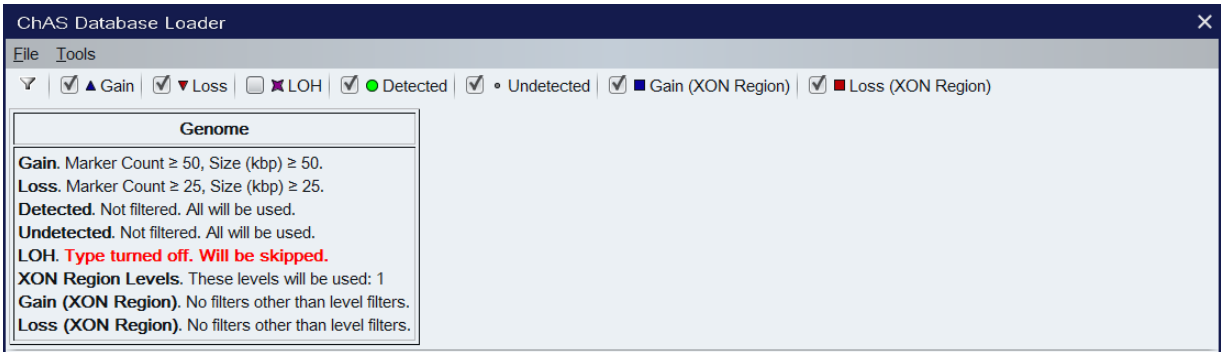


Figure 507 Genome dialog box.

Note: QC thresholds and Smooth/Joining settings in the ChAS Browser will be used when publishing xxCHP files using CDL. To use different QC thresholds and/or Smoothing and Joining settings, see "Set QC parameters in the ChAS Browser" on page 124.

Change segment filters (optional)

1. Click the **Filter** icon (or click **Files → Segments Filters**).

   The Segments Filters window opens. (Figure 508)

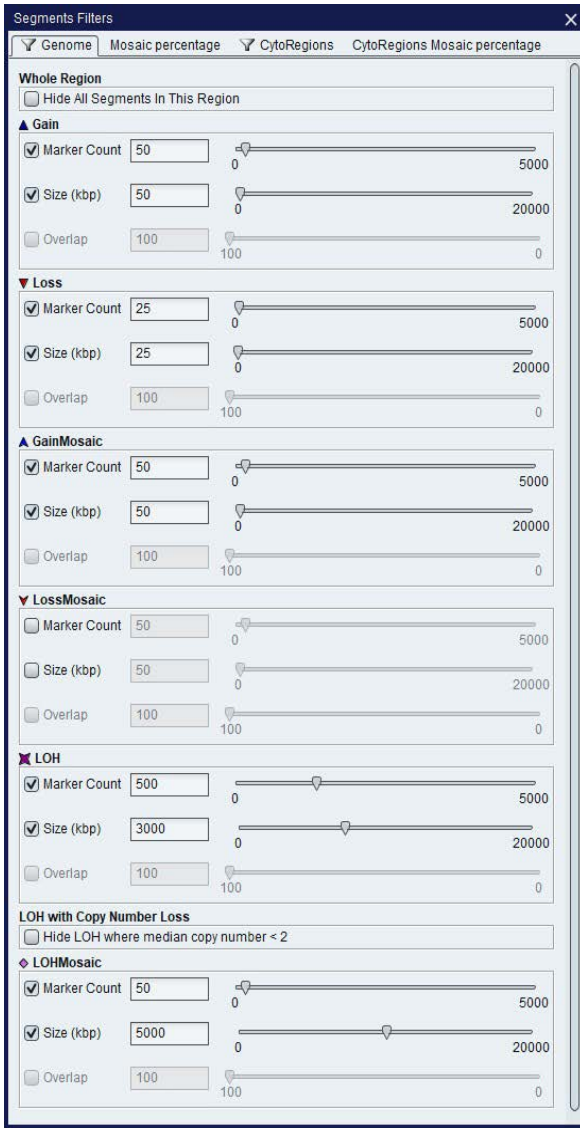


Figure 508 Segments Filters window.

2. Update the appropriate filters using the provided checkboxes, text boxes and sliders.
3. Click **X** to save your changes and close the window.

Manage data types (optional)

1. Use the Segments Filters window (Figure 508) to select the checkbox of the data type(s) you want to publish.
2. Click **X** to save your changes and close the window
3. Review the Genome dialog window (Figure 507) again to ensure that your data types to be published are displayed.

Publish your files

1. Check your table before publishing, as all displayed files are published.

Note: If there are specific files you do not want published, click, Ctrl+click, or Shift+click to highlight them, click the **Clear** dropdown list, then click **Clear Selected**. (Figure 509)

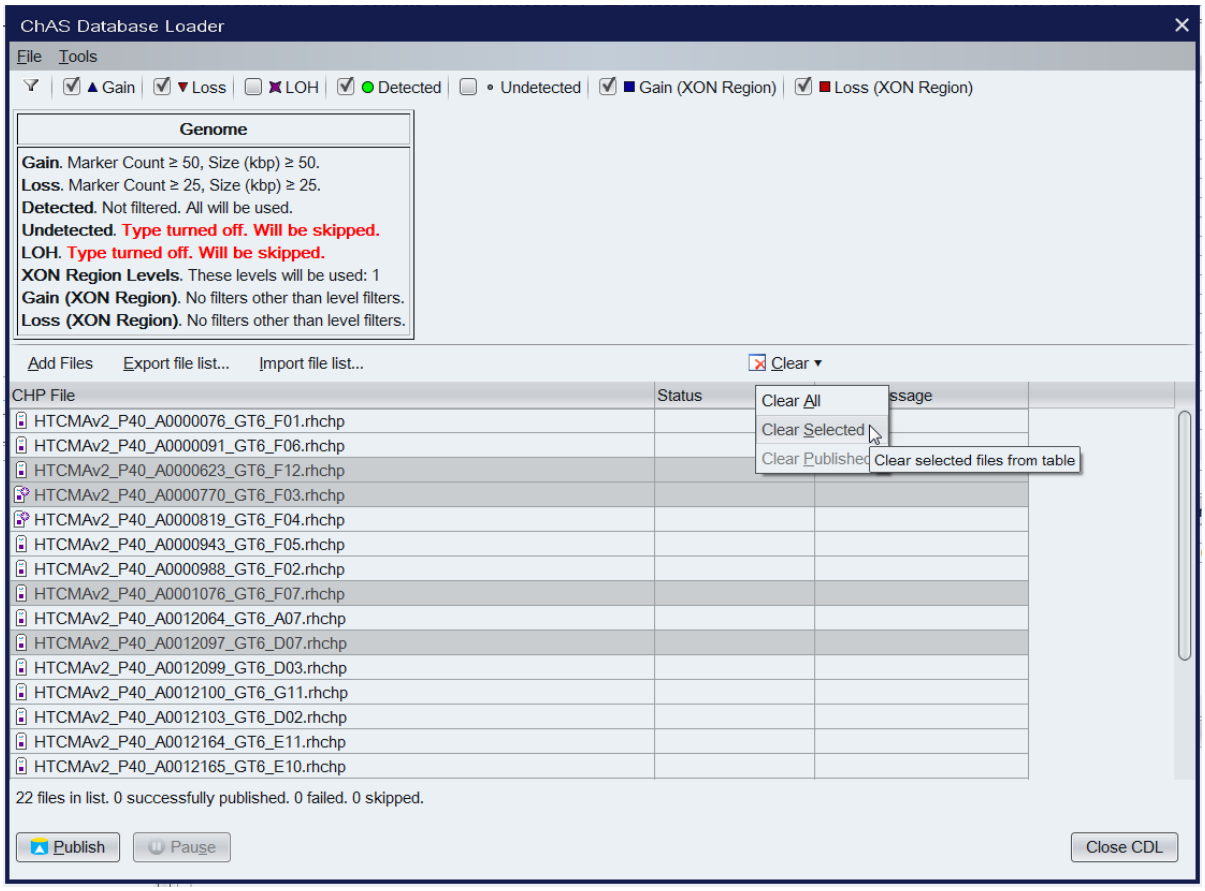


Figure 509 Selecting files not to publish.

IMPORTANT! You must have ChAS DB Manager or Admin privileges before you can publish. For information on setting up ChAS DB role assignments, see "Administration" on page 423.

2. Click Publish.

A **Publish?** window appears. (Figure 510)

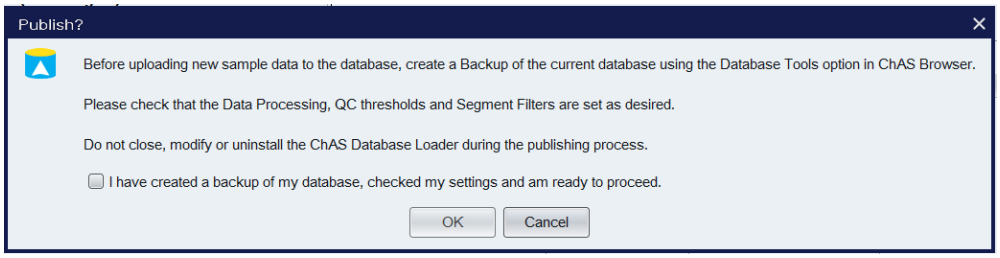


Figure 510 Publish? window.

3. Select the checkbox to acknowledge the message, then click **OK**.
4. An **Overwrite?** message may appear. (Figure 511) Click the appropriate button to continue.

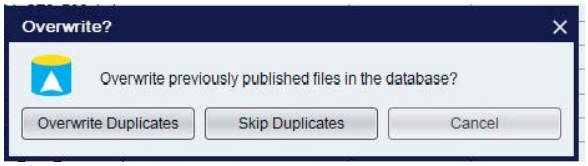


Figure 511 Overwrite? message.

The publishing process begins. (Figure 512)

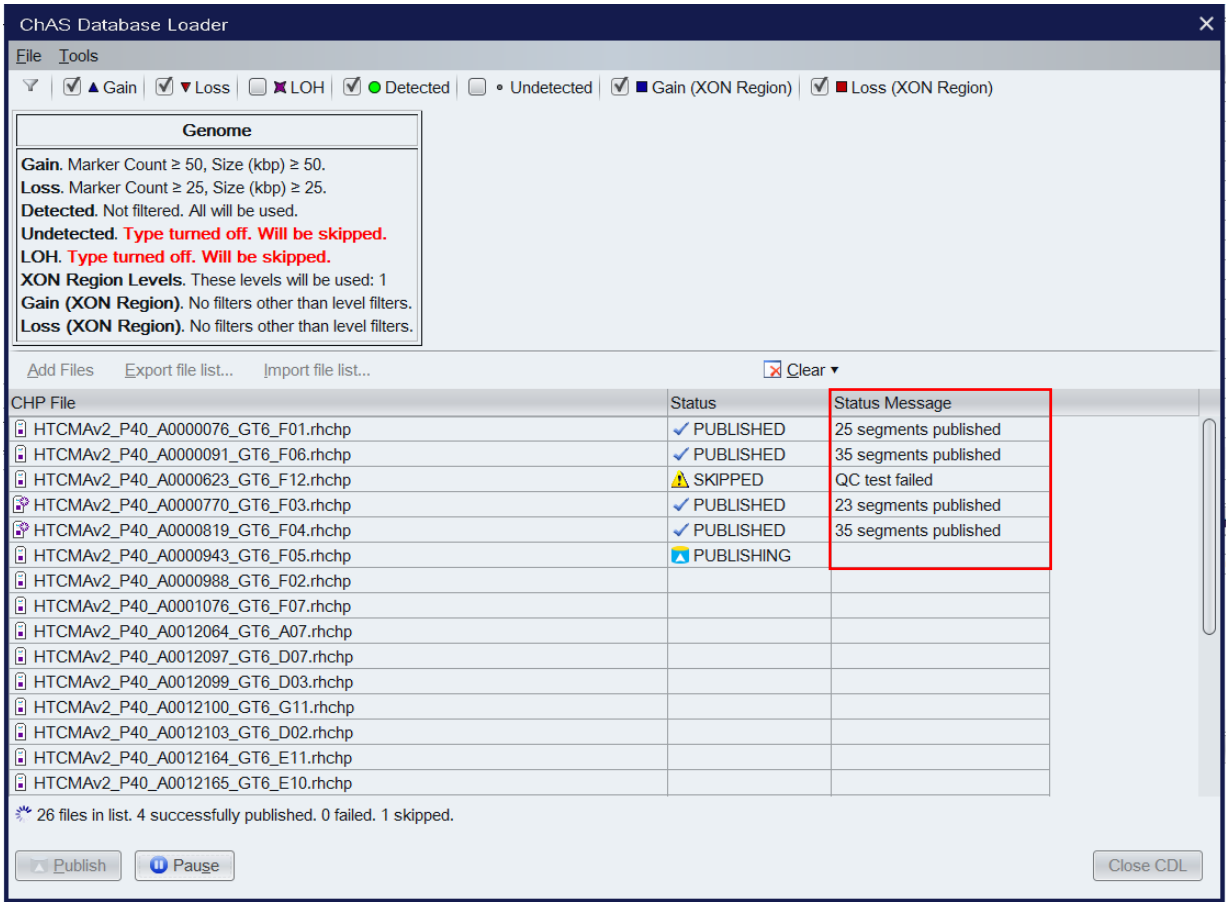


Figure 512 Publishing in progress example.

To pause the publishing process, click Pause. While in pause mode, you can add more files to the table, as described in "Add files to CDL" on page 429.

After the publishing process is complete, each **Status** column is marked with a result icon.

- = The file was successfully published to the ChAS Database.
- = The file was skipped over and not published, because it was already found in the database or it did not meet the assigned QC thresholds.
- = The file failed and was not published.

Note: See the table's Status Message column (Figure 512) for details regarding a skipped or failed file.

Clear Table Data

Clear Published files

After publishing, click **Clear → Clear Published** to remove all files that successfully published.

After clicking **Clear Published**, files with a Skipped or Failed status remain in the table. Click **Export properties...** to export these files for further investigation.

Clear All files

Click **Clear → Clear All** to remove all files from the table.

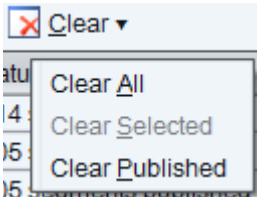


Figure 513 Clear table data dropdown list.

Close CDL

1. Click **Close CDL** or click **File → Close CDL.**

A Analysis parameters

Analysis parameters for single sample analysis

- For information on CytoScan algorithm parameters and their values, go to: https://media.affymetrix.com/support/developer/powertools/changelog/apt-copynumber-cyto.html

Reference model file creation

Reference Model File Creation is done with fixed parameters in the Reference Creation workflow.

It is essential that the input for Reference Model Creation include at least 44 total CEL files and at least 20 males and 20 females.

There are no user-adjustable parameters for the Reference Model File Creation

- For information on CytoScan algorithm parameters and their values, go to: https://media.affymetrix.com/support/developer/powertools/changelog/apt-copynumber-cyto.html

B AED file format

Affymetrix Extensible Data (AED) files contain data that annotate positions on a genome. AED allows custom, typed fields, can be edited in the ChAS Browser's AED Editor feature ("View and batch edit AED file contents" on page 286), and supports internationalization.

This appendix covers the formatting and use of Affymetrix Extensible Data (AED) files with ChAS.

- "AED file description"
- "Compatibility"
- "References"

AED files can be created by Chromosome Analysis Suite (ChAS) and loaded into ChAS as region information files to:

- Define CytoRegion and Overlap Map regions
- Record information of interest about features in the genome

AED files can be produced and edited in:

- ChAS (Recommended)
- Text-editing software (Not Recommended)
- Spreadsheet software such as an Excel™ spreadsheet (Not Recommended)

AED file description

An AED file contains a list of annotations, descriptions of features on a biological sequence such as a chromosome. This description is comprised of several properties—either properties defined by this specification, such as the annotation start and stop positions; or properties defined by users or third parties.

An AED file may also provide **metadata** which describe the particular group of annotations in the file as a whole, such as the author of the file or the genome assembly for which the annotations were produced.

Properties and metadata have certain types which define the semantics and constrain the range of values they may have. Properties should begin with a lowercase letter, while types should begin with an uppercase letter.

The AED file format uses a tab-delimited text format with the ***.aed** file extension. It uses Unicode character sets and has the following components: (Figure 514)

Header Row

Annotations

Regions_03_02.aed

	A	B	C	D	E
1	bio:sequence(aed:String)	bio:start(aed:Integer)	bio:end(aed:Integer)	aed:name(aed:String)	aed:value(aed:String)
2				aed:application(aed:String)	Chromosome Analysis Suite CytoB-N1.0.0.284 (r1764)
3		Metadata		aed:created(aed:DateTime)	2009-03-02T11:06:40.517-08:00
4				aed:modified(aed:DateTime)	2009-03-02T11:06:40.517-08:00
5	chr1	110034485	110041690	seg10	
6	chr1	112497197	112507652	seg12	
7	chr4	66574165	66587983	seg126	
8	chr4	69102606	69592855	seg130	
9	chr4	68970721	68970726	seg128	
10	chr4	70177951	70276607	seg132	
11	chr5	102163811	102287067	test_variation	
12	chr5	102622340	102642260	New_Gene	
13					
14					

Regions_03_02

Figure 514 AED file in an Excel™ spreadsheet with required header fields for properties and metadata.

IMPORTANT! AED supports only Unicode, which can be stored in one of various encodings (charsets such as UTF-8, UTF-16LE, and UTF-16BE). The AED file indicates the charset with an initial Byte Order mark (BOM). An AED file with no initial BOM is not recommended. An AED file that does not begin with a BOM will be interpreted as containing only the ASCII subset of Unicode, resulting in an error if any characters lie outside the range of ASCII. (With no indication of a charset, it is not possible to determine which non-ASCII characters were intended. File formats such as BED that make assumptions about non-ASCII characters have the potential to corrupt data when transported between systems.)

- "Header row": Names the properties that can be used in the annotations
- "Metadata records" on page 441 Optional: Provides information about the AED file itself and the group of annotations it contains.
- "Annotations Rows" on page 442: The annotation row displays values for the properties listed in the header rows (for each feature that is annotated).

Header row

The header row of an AED file is a tab-delimited list of the properties that can be used to describe a region of the genome.

Each AED file header represents a property. Normal records in the file represent annotations, and the record fields represent annotation properties. Special metadata records represent metadata properties for the file as a whole, rather than for a particular annotation.

A property name has the following format:

namespacePrefix:propertyIdentifier(namespacePrefix:TypeIdentifier)

- namespacePrefix
- A namespacePrefix is optional. It assigns the property or type to a vocabulary grouping called a namespace. The lack of a namespace prefix indicates that the property has been created by a user and is not part of the formal AED specification.

  The lack of a namespacePrefix indicates that the property is in the default/custom namespace; this namespace enables users to add properties to an annotation just by adding new columns, such as foo(aed:String) or bar(aed:Integer).
- propertyIdentifier

- The propertyIdentifier names the property that the values in the column are for.

  Each annotation can be assigned an unlimited number of properties. Each property has a certain meaning, and this meaning is usually defined by the documentation for the property namespace. The purpose of the AED file is to indicate values for certain properties for each annotation. For example, by use of the aed:name(aed:String) column, the AED file indicates a string value to be used as the name each annotation).
- TypeIdentifier
- The TypeIdentifier (always capitalized) specifies the data type of the value to be used for the property in the AED file. Examples include:
  - bio:sequence(aed:String)
  - aed:value(aed:String)
  - medianMarkerDistance(aed:Integer)
- Required Fields
- Fields may appear in any order, except that the following predefined fields must always appear in the following order at the beginning of the header:
  - bio:sequence(aed:String)
  - bio:start(aed:Integer)
  - bio:end(aed:Integer)
  - aed:name(aed:String)
  - aed:value(aed:String) **Optional:** You must use this property if you are including metadata information in the file.

IMPORTANT! ChAS verifies the property types when importing an AED file. If a file header specifies a known property, but includes an incorrect data type for the property, the file will not be loaded. For example, "fish:score" is a known property with "whole number" data type. An AED file header that specifies "fish:score(aed:String)" would be treated as an error.

Metadata records

Some records, instead of providing annotation about a location on a genome assembly, provide metadata information about the AED file itself (Figure 515). These metadata records are identified by the presence of an empty string in the **bio:sequence** field. The **bio:start** and **bio:end** fields must also be left blank for metadata records. If there are metadata records present, the **aed:value** field is required.

In a metadata record, the value in the **aed:name** field is interpreted as the name of the metadata property, with type identification rules identical to those of the header fields. The value in the **aed:value** field is interpreted as the value of the metadata property, and the characters that make up its string value must follow the lexical and semantic rules specified by the type indicated in the **aed:name** field.

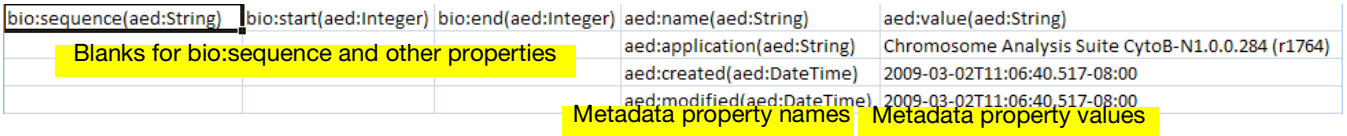


Figure 515 Metadata entries.

All other metadata record fields should be blank.

Annotations Rows

The rows below the Metadata properties are the annotations. Each row is a tab-delimited list of values. Each value must have the correct data type, as described in the property name for that value.

Compatibility

UCSC Browser Extensible Data (BED)

The BED file format, developed at UCSC, is widely used for transfer of simple region coordinates. However, the format has been interpreted and implemented in multiple ways by various software within and outside of UCSC. Some implementations require a TAB delimited format, others require a space-delimited format, and still others accept both. Characters outside of the ASCII character set are not well supported. We created the AED format with very strict and explicit definitions so as to avoid some of these compatibility issues.

Although the AED format is preferred, ChAS supports both the import and export of data in BED format. When exporting data in BED format, ChAS exports only the basic 4-column tab-delimited BED format containing the position and name of each item. If the names of any of your items contain spaces or non-ASCII characters, there is no guarantee that all programs will be able to interpret those names correctly.

When importing data in BED format, ChAS supports the reading of BED files with anywhere from 4 to 12 columns.

- The file must be TAB delimited
- Only ASCII characters should be used
- The values for thickStart and thickEnd will be ignored for display purposes
- The value for itemRgb will be honored for display purposes
- The values for blockCount, blockSizes and blockStarts can be used to import and display data with intron/exon structure, such as genes.
- Formatting rules in the BED header are ignored
- BED files containing multiple tracks are not supported; use a separate BED file for each track.

The UCSC Browser, as well as ChAS, uses the strict definition of BED where chromStart is not allowed to be greater than chromEnd. ChAS will accept import of BED files even if this convention is violated, but will auto-correct and export BED files properly with chromStart ≤ chromEnd.

AED has been structured to facilitate as much as possible migration of data rows to and from BED. Starting with existing AED and BED files, data records from AED may be transferred to BED by using:

- The “Export” function from inside ChAS (recommended)
- A text editor (not recommended) if the AED files are first prepared in the following manner:
- Remove all fields except for bio:sequence, bio:start, bio:end, and aed:name.
- Ensure that no non-ASCII characters are included. (The treatment of non-ASCII characters by a BED processor is undefined.)
- Ensure that no name contains whitespace characters.
- Data rows from the first 4 columns of a BED file can be transferred to AED with no constraints as long as the columns are delimited by TAB.

Microsoft Excel™ and other spreadsheet applications

Though not recommended, an AED file may be edited using most spreadsheet programs that support tab-separated value (TSV) files and that recognize a byte order mark (BOM). An AED file can be edited in an Excel™ spreadsheet, for example, using the following rules:

When loading an AED file into an Excel™ spreadsheet as a TSV file, ensure that the Unicode code page for the correct encoding is selected (preferred), or accept the default "Windows (ANSI)" code page (which should still recognize Unicode characters if the correct BOM is present in the file).

When saving an AED file from an Excel™ spreadsheet, ensure that the "Unicode Text" type is selected. This will result in a file encoded in UTF-16LE, which is still a valid AED file as it begins with the appropriate BOM.

Microsoft™ Notepad and other text editors

Though not recommended, an AED file may be edited by any text editor that supports Unicode and that uses a byte order mark (BOM) to indicate the charset. The version of Microsoft™ Notepad in Windows™ XP, for example, will both correctly read text files marked with a BOM and save text files using the appropriate BOM if the following rules are followed:

When saving an AED file from Microsoft™ Notepad, ensure that the encoding is set to "UTF-8" or "Unicode".

For other text editors, ensure that the correct preferences are set both to recognize and write BOMs for files.

Text editors

EmEditor <http://www.emeditor.com/> is a commercial text editor that has extremely good Unicode and BOM support, and is able to open up gigantic text files.

PSPad <http://www.pspad.com/> is a free text editor that has particularly extensive Unicode and BOM support and is available in many localizations.

UniPad <http://www.unipad.org/> is a shareware text editor that correctly handles Unicode and BOM, and provides a wide range of built-in glyphs for representing Unicode code points that cannot be viewed on most other text editors.

References

ISO 8601: ISO 8601:2004(E): *Data elements and interchange formats — Information interchange — Representation of dates and times.* International Organization for Standardization, 2004-12-01.

Microsoft Byte Order Mark: http://msdn.microsoft.com/en-us/library/ms776429(VS.85).aspx

RFC 3986: *RFC 3986: Uniform Resource Identifier (URI): Generic Syntax*. T. Berners-Lee, R. Fielding, and L. Masinter. Internet Engineering Task Force, 2005. http://tools.ietf.org/html/rfc3986

RFC 4122: *RFC 4122: A Universally Unique IDentifier (UUID) URN Namespace*. P. Leach, M. Mealling, and R. Salz. Internet Engineering Task Force, 2005. http://tools.ietf.org/html/rfc4122

RFC 4180: *RFC 4180: Common Format and MIME Type for Comma-Separated Values (CSV) Files*. Y. Shafranovich. Internet Engineering Task Force, 2005. http://tools.ietf.org/html/rfc4180

Unicode Byte Order Mark FAQ: http://unicode.org/faq/utf_bom.html

ChAS Browser NetAffx™ Genomic Annotations

Homo sapiens database files

NetAffx Genomic Annotation Files

Source of content

- NetAffx Genomic Annotation Files are used by the ChAS Browser to display recent snapshots of genomic annotations downloaded from public databases.
- The UCSC Genome Browser is the source of the data that populates the following Browser tracks: Genes, Ensembl, Segmental Duplications, sno/miRNA, and Cytobands. UCSC was also the source of OMIM™ data for ChAS Browser file versions NA31-NA33.1 and 32.1. NA33.2 and NA36 no longer contain annotation information for BACs and FISH Clones.
- The Database of Genomic Variants is the source of the data displayed in the DGV track.
- The ClinGen Resource is the source of the data displayed in the Triplosensitivity, Haploinsufficiency, and Recurrent/Curated Regions tracks.
- The OMIM™ database (with curation and processing assistance from UCSC and NCBI) is the source of the data displayed in the OMIM™ Genes, OMIM™ Phenotype Loci, and OMIM™ Region Phenotype Loci tracks.
- The NCBI ftp site (https://ftp.ncbi.nlm.nih.gov/refseq/MANE/) is the source of data displayed in MANE track.

IMPORTANT! Starting with ChAS v3.3, the naming convention of the NetAffx Genomic Annotation database file will change. This file will now include a date (as opposed to a specific NetAffx™ build number) and will be updated more regularly than its current schedule of being updated with each software release. The new naming convention is as follows:
NetAffxGenomicAnnotations.Homo_sapiens.hg38.naYYYYMMDD.db

For optimal use of the Segment Prioritization methods described in Chapter 17, "Prioritize segments" on page 347, you must download the NetAffx Genomic Annotation Files released with ChAS v4.6 or use more current ones when available.

IMPORTANT! It is VERY highly recommended to confirm findings obtained using the ChAS Browser's NetAffx Genomic Annotations File contents by linking out to external databases using the ChAS software coordinates for the most current annotation information. See "Link to external websites" on page 201.

D Genomic position coordinates

There are multiple conventions and file formats to describe locations in chromosomal DNA sequences. This appendix describes a few issues that relate to ChAS.

Genome assemblies

First, it is important to know which set of DNA sequences is being used as the reference. For the human genome, the reference assembly is available for download from public sources such as UCSC and Ensembl. Those 2 sites currently use identical genome assemblies, but refer to them by different names. UCSC uses names such as “hg18”, “hg19” and “hg38”. The identical genome assemblies are known as “NCBI36”, “GRCh37”and “GRCh37”at Ensembl. Assemblies at NCBI can have a decimal point as well, for example, “36.3” or “37.1”. For positions on the chromosomes 1–22, X and Y, there is no difference between assemblies “36.1”, “36.2” and “36.3” and we expect the same will be true for future “point” releases.

SNP and marker positions

When referring to individual positions on a chromosome, such as the positions of SNPs, it is sufficient to give a single coordinate. There are different conventions about whether to consider the first DNA base pair on the chromosome as position 0 or position 1.

For SNP marker positions, all of the following consistently use a 1-based index position coordinate: CYCHP files, CNCHP files, NetAffx™ detail pages for SNP markers, NCBI pages for SNP positions of dbSNP entries, and the Graphs Table in ChAS.

Consider the (randomly-chosen) SNP marker “S-3SRJC” from the CytoScan HD array. This marker is designed to correspond to the SNP with ID “rs4376202” in the dbSNP database. The NCBI website reports the position as chr4:1822637 on GRCh38. On the NetAffx™ website, the identical coordinate is also given for this SNP. The same coordinate value is given in CYCHP files and in the ChAS graphs table.
See http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=7641618 for this particular example.

For copy number markers which are not based on SNP positions, we continue to use a 1-based index position. For these markers, we continue to use a 1-based index position. Unlike the case for SNPs, there is no particular single base pair that the marker corresponds to. The convention in CYCHP files is to use the position of the first DNA pair corresponding to the position where the marker hybridizes with the DNA. When 2 or more markers have the same start position on a chromosome, the coordinate of one of them will be shifted by one or occasionally a few more bases such that each marker is reported at a unique position.

Segment positions

BED and AED file formats are used for storing and sharing region files between software. The BED format was created by UCSC for use with their genome browser, and is also used in other software. The AED format was created by Thermo Fisher Scientific for use with ChAS and possible future software, but used the BED format as a starting point.

The BED file format is explicitly defined to use a 0-based coordinate system where the second column (chromStart) in the file is the position of the first base-pair and the third column (chromEnd) is the position of the last base-pair plus one. Another way of saying this is that the start index is inclusive and the end index is exclusive. As an example, to refer to the first 100 based on the chromosome, you would use chromStart=0 and chromEnd=100. The length of any region is always given simply by chromEnd minus chromStart.

The UCSC browser strictly requires that chromStart not be larger than chromEnd. To support file outputs from non-conforming programs, ChAS will accept BED files where chromStart > chromEnd. It will simply switch those 2 coordinates and act as if the coordinates were given in the correct order.

Since a SNP has, by definition, a length of one base-pair, the proper way to represent a SNP position is with chromEnd = chromStart + 1. The UCSC browser does allow chromStart to be equal to chromEnd. But this is used for representing insertion points, and is not used to represent SNP positions. Because the AED format was intended to be compatible with BED format, we use the same coordinate system.

For example, suppose there are 3 markers with the following positions on a chromosome given in the CYCHP file: Marker A at 1000, Marker B at 2000, Marker C at 3000. Marker positions in the CYCHP file are 1-based index positions. To represent these in a BED file, we would need a file like this:

- Chr3 999 1000 markerA
- Chr3 1999 2000 markerB
- Chr3 2999 3000 markerC

If there were a segment starting at markerA and ending at markerC, we would need to represent it in a BED or AED file as:

- Chr3 999 3000 segment_1

Edit BED files

A BED file is essentially a tab-delimited text file, as shown in Figure 516.

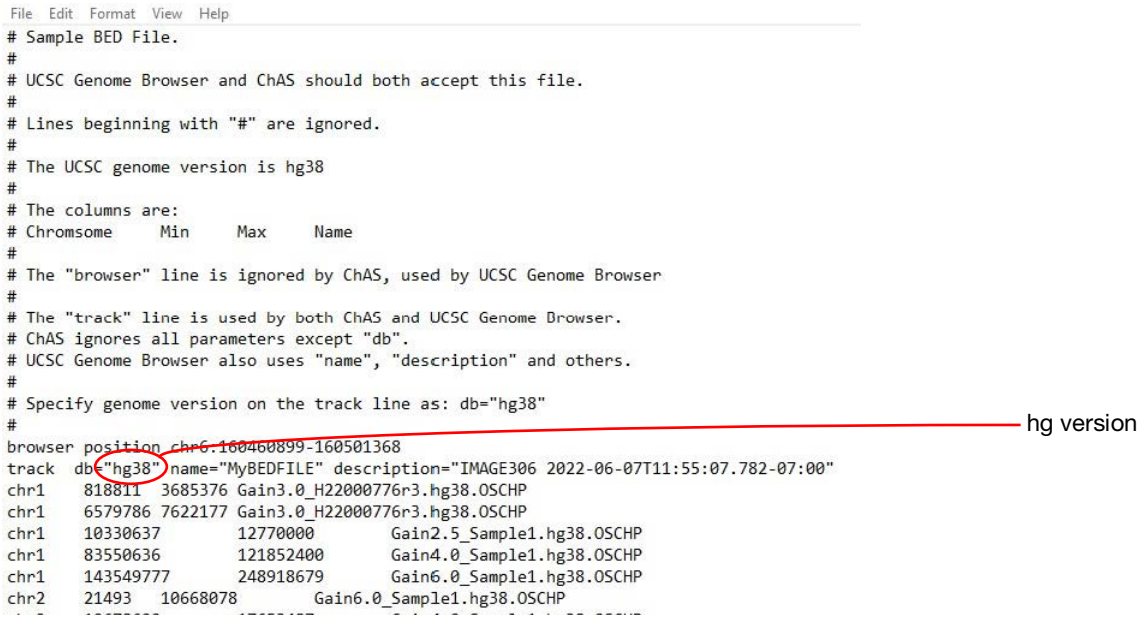


Figure 516 Example BED file.

Using a text editor such as Microsoft™ WordPad or NotePad (not a spreadsheet application like Microsoft Excel™ program) to edit BED files is recommended and work well.

Editing a BED file using a spreadsheet application such as Excel™ is not recommended because these programs may not preserve the correct BED file format. For example, when exporting data from an Excel™ spreadsheet into tab-delimited text, quotation marks may be added around some text, which would cause the file to be invalid and unusable with ChAS or other applications.

There is no easy way to prevent the Excel™ application from adding extra quotation marks which corrupt the output. Advanced Excel™ spreadsheet users can use macro programming to create special output formats. Other options include:

- Do not use an Excel™ spreadsheet to edit BED files. You may use a text editor, but be certain to separate the columns with TAB characters and do not use non-ASCII characters. The BED format was not designed with such characters in mind; therefore, problems may occur when you try to share these files with others. ChAS will reject BED files containing non-ASCII characters, and will never export non-ASCII characters into a BED file.
- After exporting a BED file from an Excel™ spreadsheet, edit the file in another application to remove extra quotation marks.
- Use AED format for the data, then use ChAS to export to BED format if needed.
- Be careful to create a BED file that does not cause the Excel™ spreadsheet to add quotation marks. Do not include the **itemRgb** column, quotation marks, or special characters in a track line. For example, the following is an acceptable track line: **Track db=hg19 name=My_Track description=This_is_my_data**

CytoScan™ algorithms and QC metrics

Algorithm overview

This section provides a high level overview of how copy number calls are generated within the software. The copy number workflow starts with the intensities on the array, include normalization and scaling, reference set ratios, Log2 transformation, CN state segmentation, and how CN segment calls are made.

Note: For CytoScan HTCMA algorithm and QC, see the *Reproductive Health Research Analysis Software User Guide* (Pub. No. MAN0027973).

Feature identification and signal extraction

GeneChip™ cartridge microarrays are scanned on the GeneChip™ Scanner and processed by the GCC scanner software package. GCC aligns a grid on the DAT file (the original scanned image) to identify each microarray feature and calculates the signal from each feature. This process uses the DAT file, containing the raw signal, and creates a CEL file, which contains a single signal intensity for each feature. The CEL file is used for all downstream analyses.

Single sample CytoScan™ workflow

Beginning with the raw signal data in the CEL file, the Single Sample CytoScan™ workflow implements a series of steps that perform probe set summarization, normalization, removal of variation caused by known properties and residual variation, and completing with calling genotypes, copy number segments and LOH segments.

A brief overview of each step performed by the CytoScan™ workflow is shown in Figure 518 on page 450. In addition, a rough sketch of Analysis Pipeline (for Single Sample Analysis) is demonstrated in Figure 518 on page 450.

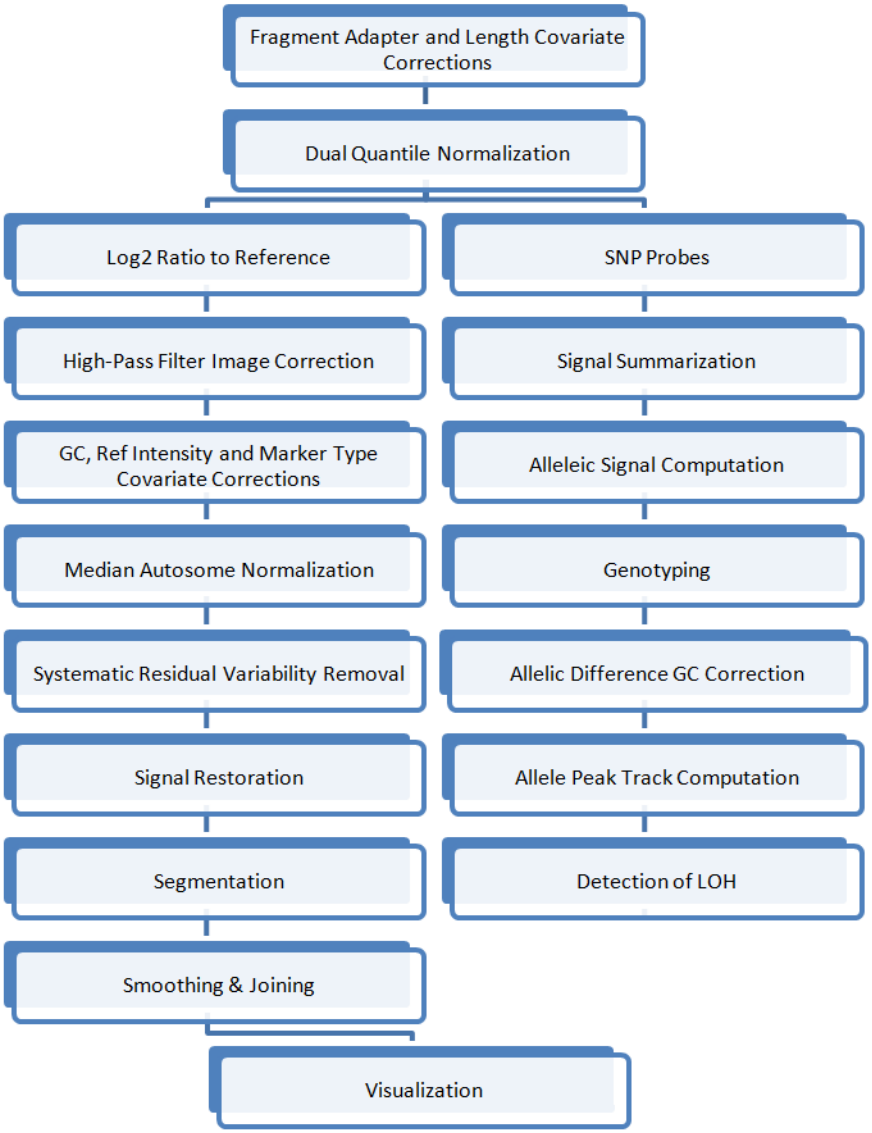


Figure 517 CytoScan Workflow Overview. Steps related to probes used for copy number determination run down the left and those steps used for SNP probes run down the right side of the diagram.

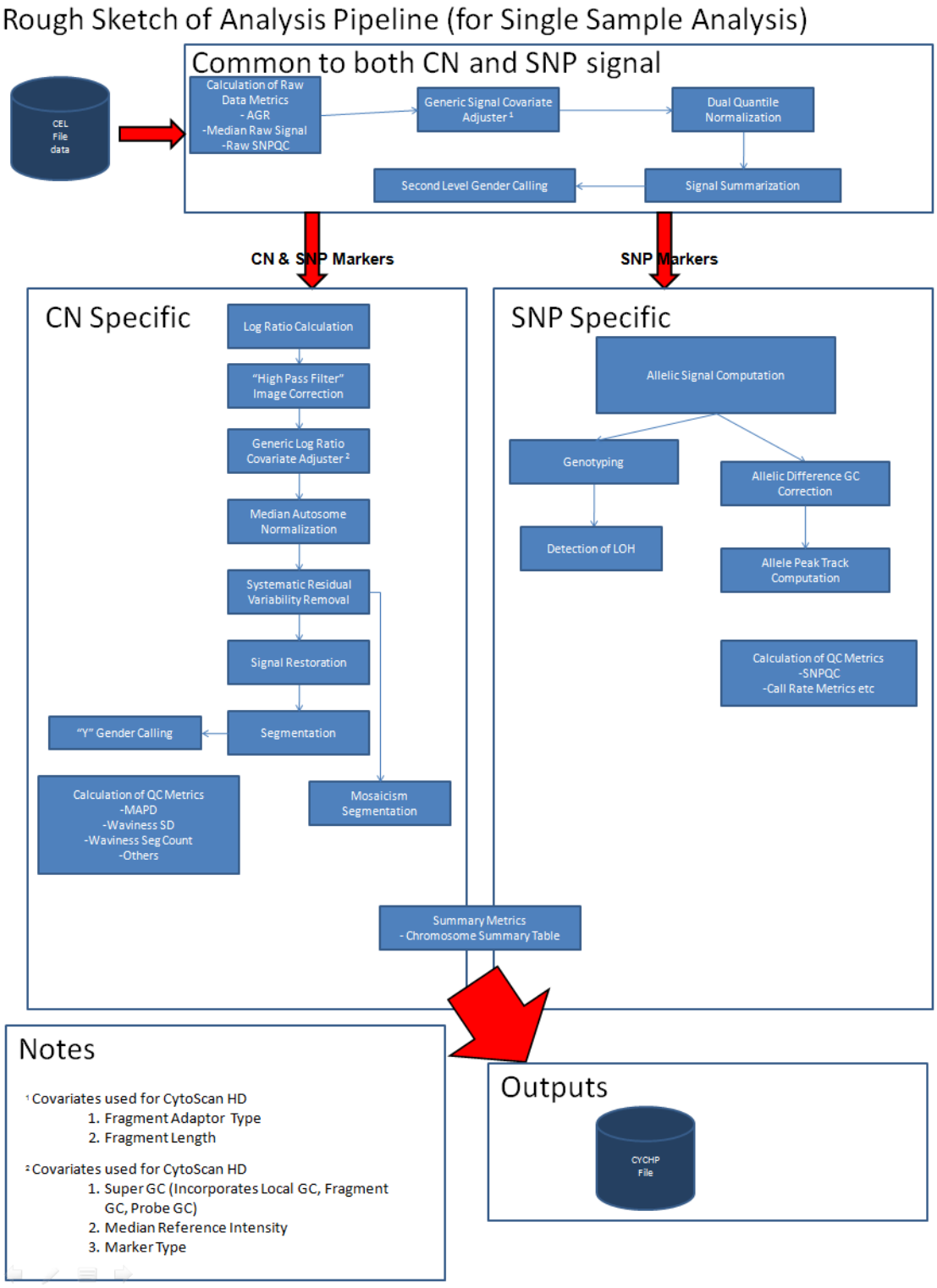


Figure 518 Rough sketch of Analysis Pipeline (for Single Sample Analysis).

Signal-level covariate adjustors

The first level of covariate adjustors operate on the raw signal.

Fragment adapter covariate adjustor

After the Nsp I restriction digest, an Nsp I-specific adaptor is ligated onto the cohesive end termini. Since Nsp I is a 6-nucleotide cutter with degenerate sites, meaning that they contain one or more base pairs that are not specifically defined, these ends are of various sequences and the ligated adaptors are a variety of sequences. The exact sequences of the cut site and ligation adaptor have an effect on the overall efficiency of ligation and subsequent PCR amplification. The Adaptor Covariate Adjustor corrects for these differences by normalizing the signals for each adaptor/cut site sequence class to an overall median.

Fragment length covariate adjustor

The length of each Nsp I fragment impacts the efficiency of PCR amplification and therefore the signal. Fragments of 300–500 bp are amplified with the highest efficiency and the degree of amplification tapers off as the fragments get longer. The Length Covariate Adjustor corrects for these differences by normalizing the signals for a series of fragment size bins to an overall median.

Dual quantile normalization

Dual quantile normalization is simply a 2-phase process where probes used for copy number detection and probes used for SNP genotype detection are normalized separately. In both cases, a normalization sketch is built using the autosomal probes in the reference set. The normalization sketch is the prototype distribution of probe intensities that defines what this distribution looks like for all arrays. The single sample autosomal probes are fit to the sketch and the X and Y probes are interpolated into the distribution.

Quantile normalization makes the assumption that the distribution of probes on the array is fairly consistent from array to array. Since the X-chromosome is one of the largest chromosomes (155Mbp, ~5% of the genome), differences between males and females would stretch this assumption. That is why the quantile normalization focuses on creating an autosomal sketch and normalizing the autosome to it. The X and Y chromosome probes are then handled in a special way. Each of them is matched to the closest pre-normalization signal value. Based on that match, their normalized signal should be close to the signal for the very same autosomal probe. So the normalized values for X and Y probes are simply "looked-up" in the pre-normalization autosomal sketch, and transformed to the post-normalization value.

Copy number workflow

Log2 ratio calculation

Log2 Ratios for each marker are calculated relative to the reference signal profile. The Log2 Ratio is simply $\text{Log2}(\text{sample}_m) - \text{Log2}(\text{reference}_m)$, for each marker, "m".

High pass filter image correction

Since most probes map to genomic markers associated with a normal copy number, most Log2 Ratios should be centered at a value of zero. Also, since markers from any genomic region are scattered across the surface of the microarray, regions of altered copy number will not appear as regional changes on the microarray image.

Some samples do reveal spatial trends away from zero that are gradual and this spatial bias when scattered back across the genome exhibits itself as added noise in the Log2 Ratios. The High Pass Filter Image Correction identifies these gradual spatial trends and adjusts Log2 Ratios to remove the spatial bias and lower the level of noise.Log2 Ratio-Level Covariate Adjustors

Log2 ratio-level covariate adjustors

Super GC covariate adjustor

The GC content of genomic DNA sequence impacts probe signal dose-response and therefore probe Log2 Ratios. The sequence GC content of the microarray probe impacts hybridization kinetics. In addition, the genomic GC content of the Nsp I fragment and the 500 kbp surrounding the probe (local GC) all impact the efficiency of target preparation in the genomic region of each probe. The super GC covariate adjustor combines the probe GC content, the fragment GC content and the local GC content into one covariate that corrects for Log2 Ratio differences based on the combination of GC contents associated with each probe.

Reference intensity covariate adjustor

Probes in different intensity categories have different dose responses in Log2 Ratio space. Using the Reference Set probes to define bins based on probe intensity, the single sample probes are binned and the median of the distribution of Log2 Ratios within each bin is adjusted to the median Log2 Ratio of the corresponding bin from the reference set.

Marker Type Covariate Adjustor

Polymorphic probes designed for SNP detection and non-polymorphic probes designed for copy number detection have different properties and different dose responses. The Marker Type Covariate Adjustor normalizes the median Log2 Ratios of SNP and CN markers to account for differences in Log2 Ratios between the 2 groups.

Median Autosome normalization

This final level of normalization simply shifts the median Log2 Ratio of the autosomes to a copy-number state equal to 2, that is, a Log2 Ratio of 0.

Systematic residual variability removal

Even after all of the Covariate Adjustors, there is some residual variation with unknown origins. During product development we have introduced variation into the protocol in an attempt to capture other forms of unanticipated variation. The Systematic Residual Variability Removal step matches sample variability to the residual variability of the reference set, and when matched, corrects the data to remove the residual.

Signal restoration

Signal restoration is an application of Bayes wavelet shrinkage to the Log2 Ratios associated with markers. The result of this transformation is an overall reduction in variation around the local mean of Log2 Ratios. In this context, “local” means a region consisting of a small set of markers upstream and downstream from a given marker. The resulting data can be viewed as the Weighted Log2 Ratio in ChAS and serves as the input to the segmentation algorithm. The wavelet shrinkage method is augmented by a reduction of influence of outliers when local means are computed.

Segmentation

Copy Number Calls for each Marker based on Log2 Ratios

For CytoScan™ arrays, markers are individually assigned a copy number call by a Hidden Markov model (HMM). The sample specific inputs to the HMM are the Weighted Log2 Ratios generated by the Signal Restoration module.

The weighted Log2 Ratios are centered on copy number (CN) = 2. In theory, when Log2 Ratio = 0 then CN = 2, when Log2 Ratio = –1 the CN = 1, etc. In truth, microarrays, or any hybridization-based technology, exhibit Log2 Ratio compression due to many factors, so the Log2 Ratios never exhibit the amplitude expected by the math. The following table shows theoretical and actual Log2 Ratios for different Copy Number States.

Table 57 Copy number states and Log2 Ratios.

Copy number truth	Theoretical Log2 ratio	Actual Log2 ratio
1	–1	–0.45
2	0	0
3	0.58	0.3

The actual Log2 Ratios observed are best derived from a very large data set with well-characterized copy number changes. To this end, we have analyzed over 1400 samples that have copy number changes across 75% of the genome and have established stable empirical values for these expected Log2 Ratios. These values, as well as the dispersion characteristics of the Log2 Ratio data, are used as inputs to the HMM along with the weighted Log2 Ratios of the sample data.

The HMM uses these inputs to convert observed Log2 Ratios into a CN state for each marker. It uses a table of transition probabilities that express the probability of changing from any CN state to another. As can be seen in the following example (Figure 519), there are many potential paths through the possible CN states of a set of markers.

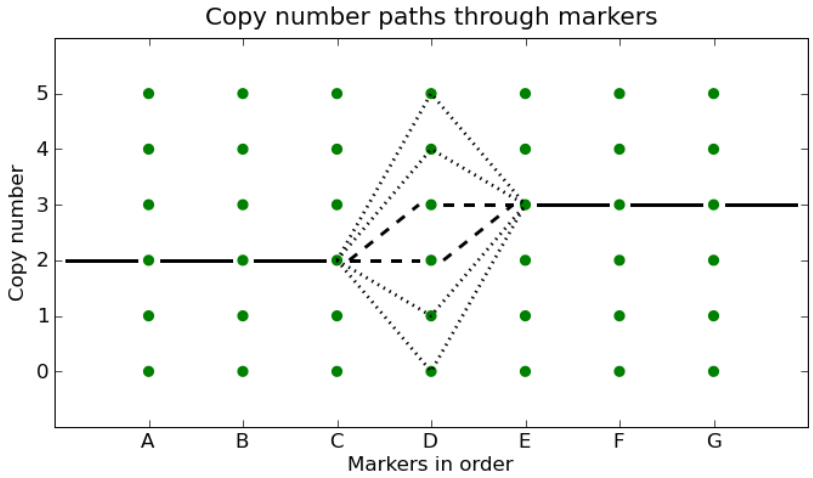


Figure 519 Potential CN paths through a set of markers.

The HMM uses the Viterbi algorithm to calculate the most probable path through the set of markers using the transition probabilities between each pair of CN states. Essentially, the graph of potential CN states is the "hidden" layer of the HMM, and the measure Log2 Ratios are the observed layer. The HMM algorithm finds the most probably CN states given the observed Log2 Ratios.

Segment Formation

After markers are assigned Copy Number States by the HMM, contiguous stretches of adjacent markers ordered by chromosome position having the same state are aggregated into segments. These segments are described in a segment table within the resulting CYCHP file that provides for each segment, the common Copy Number State, the number of markers in the segment, the genomic marker position that initiates the segment and the genomic marker position that terminates the segment.

Enforce Minimal Segment Length

Default parameters enforce a minimum segment length of 5 markers. This is a subjective choice of parameter that implicitly states that the user is not interested in segments with fewer markers than the minimum. The algorithm that enforces minimum segment size distributes markers from any segment with fewer than the minimum to its larger neighboring segments by changing the copy number call on the modified markers to conform with those of the neighbors.

Smoothing & Joining

To stabilize the calling of copy number gains or losses, the ChAS software implements a smoothing step. Smoothing will combine adjacent segments that are both gains, even if they are not the same Copy Number State. For example, smoothing will combine a set of adjacent segments of Copy Number State 3 and 4 into one segment and assign it the most prevalent Copy Number State of the markers in the original segments, (rounding up for gains and rounding done for losses in case of a tie). Smoothing will also combine Copy Number States 0 and 1. But smoothing will not combine gains with losses or either with normal segments.

Joining combines segments of gains or losses if they are separated by small spans of normal copy number segments. The default value defining "small spans" in ChAS is ≤50 markers and ≤200 kbp. Small segments of less than 50 markers/200kbp of normal copy number are removed, and the adjacent gain segments are joined. Likewise for flanking loss segments. This is a dynamic process in ChAS, in that smoothing and joining can be turned on and off, and parameters altered, resulting in modifications of the displayed segments, but not altering the underlying CNState graph.

Segment table output

The final result of the copy number pipeline is a table of segments identified in the sample. The table in the CYCHP file includes segments of normal and non-normal copy number. Segments called on the X- and Y-chromosomes are characterized as normal or non-normal using gender information and adjusting for the Pseudo Autosomal Regions (PAR) that are present on the X and Y. In ChAS, the segment table display only shows segments of non-normal copy number.

Copy number mosaicism algorithm

The algorithm for detection of copy number aberrations in the presence of mosaicism considers single copy deletions and gains. The algorithm is tuned to be most accurate when the normal/expected Copy Number State is 2. The algorithm targets detection of changes of approximately 3MB or more in size (for CytoScan™ HD). Copy number change events less than this size may be detected; however, sensitivity and specificity will be reduced.

Limitations in mosaicism segmentations

- The algorithm is designed to detect only mosaicism between approximately15–70% mosaicism for copy numbers between 1 and 3 for regions on the order of 3 MB in size or larger for autosomes and chrX .
- The algorithm is designed to detect only mosaicism between approximately 15–70% mosaicism for copy numbers between 0 and 2 for regions on the order of 3 MB in size or larger for chrY.
- The algorithm will not call events below 1 MB in size irrespective of number of markers.
- The algorithm will not call events below 10% mosaicism irrespective of genomic size or number of markers.
- The algorithm does not use allelic difference or BAF data for segmentation purposes. It is recommended that allelic difference and/or BAF data tracks be examined in conjunction with the reported mosaicism segmentation.

SNP marker workflow

Signal summarization

CytoScan arrays contain 6 probes for each SNP probe set, 3 targeting each allele. The first step of the SNP-specific workflow is to summarize the previously-normalized probe intensities for the A and B alleles, yielding allelic signal values.

Allelic signal computation

For each marker, the Allelic Difference is calculated as the difference between the summarized signal of the A allele minus B allele, standardized such that an A-allele genotype is scaled to a positive value, and the B allele is scaled to a negative value. The standardization is determined based on median values for this difference under different genotype configurations determined by the reference set. In this way a homozygous AA maps to approximately +1, and a homozygous BB allele maps to approximately –1, with the heterozygote mapping to approximately 0. Additionally, single A and B allele signals will map to 0.5 and –0.5, respectively. This scaling provides a useful way of discerning 2 copies of an A allele from a single copy, enabling detection of regions of copy-neutral LOH (for example, IBD) from hemizygous LOH.

Genotyping

Genotyping for CytoScan™ arrays is accomplished using the BRLMM-P algorithm described in the White Paper: BRLMM-P: A Genotype Calling Method for the SNP Array 5.0 (2007).

Allelic difference GC correction

Systematic changes in Allelic Differences can be related to differences in GC content. For instance, on a given sample Allelic Differences representing AA and BB genotype markers might get progressively closer or further from each other as the GC content changes. It is assumed that such changes represent unwanted variability. The Allelic Difference GC correction determines differences in the structure of the allelic differences associated with GC and then removes these differences. For CytoScan HD the super GC covariate is used. For CytoScan™ 750K the Local GC covariate is used.

Detection of LOH

The LOH algorithm frames the problem in terms of a statistical hypothesis test. Given a 1 MB sliding window containing *N* SNP markers with heterozygous and homozygous genotype calls, decide between the following 2 hypotheses:

Null Hypothesis: Region is LOH
Alternative Hypothesis: Region is non-LOH

To decide between the 2 hypotheses the number of heterozygous calls is compared with a critical value that is computed for each sample. When the number of heterozygous calls is above the critical value, then the alternative hypothesis is favored, that is, region is not LOH. If there are not a sufficient number of heterozygous calls then the decision is made in favor of LOH. The algorithm moves the region of *N* markers along the genome to determine LOH events. Due to the 1 MB sliding window, the algorithm will not generate LOH segments smaller than 1 MB. Further details are provided in the While Paper: The Loss of Heterozygosity (LOH) Algorithm in Genotyping Console 2.0.

Detection of mosaic LOH segments

The algorithm is designed to identify mosaic regions where the overall copy number remains at 2, but a subset of cells has lost one allele within a genomic region and becomes homozygous, while the remaining cells maintain normal heterozygosity. Because only a portion of cells is affected, this results in a region of mosaic loss of heterozygosity (LOH). This algorithm is available for use with CytoScan™ HD, CytoScan™ HD Accel, CytoScan™ 750K, and CytoScan™ 750K Accel arrays. It is optimized to detect mosaic LOH present in approximately 20% to 70% of cells for regions around 10 Mb in size

Array data QC metrics

This section provides a high level overview of the key QC metrics used with the CytoScan™ arrays.

Median of the Absolute values of all Pairwise Differences (MAPD)

MAPD is a global measure of the variation of all microarray probes across the genome. It represents the median of the distribution of changes in Log2 Ratio between adjacent probes. Since it measures differences between adjacent probes, it is a measure of short-range noise in the microarray data. Based on an empirical testing dataset, we have determined that array data with MAPD >0.25 (for CytoScan™ 750K and HD, MAPD >0.29 for CytoScan™ Optima) has too much noise to provide reliable copy number calls.

Waviness SD

Waviness-SD is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. Based on an empirical testing dataset, we have determined that array data with Waviness-SD >0.12 has either sample or processing batch effects that will reduce the quality of the copy number calls. Elevated Waviness-SD is not always an indication of too much noise. Elevated Waviness with good MAPD and SNPQC metrics can occur in samples with many copy number changes or very large regions of change. It is therefore advised to check the data when observing elevated Waviness with good MAPD and SNPQC.

SNPQC

SNPQC is a measure of how well genotype alleles are resolved in the microarray data. Based on an empirical testing dataset, we have determined that array data with SNPQC < 15 (for CytoScan™ 750K and HD, SNP QC < 8.5 for CytoScan™ Optima) is of poorer quality than is required to meet genotyping QC standards.

ndSNPQC (SNP Quality Control of Normal Diploid Markers)

The metric, SNPQC is a measure of how well genotype alleles are resolved in the microarray data. ndSNPQC is the same metric but only applied to normal diploid markers (that is those that have been determined to have Copy Number =2 in the sample). Larger ndSNPQC values are better.

ndWavinessSD (Normal Diploid Waviness Standard Deviation)

ndWavinessSD is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. ndWavinessSD is computed on normal diploid markers.

MAPD – Detailed Description

For quality assessment purposes, we define metrics that assess whether the microarray data is useful for copy number (CN) analysis. One of these metrics is Median of the Absolute values of all Pairwise Differences (MAPD).

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between Log2 Ratios for a given chip. Each pair is defined as adjacent in terms of genomic distance, with SNP markers and CN markers being treated equally. Hence, any 2 markers that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every marker belongs to 2 pairs (Figure 520).

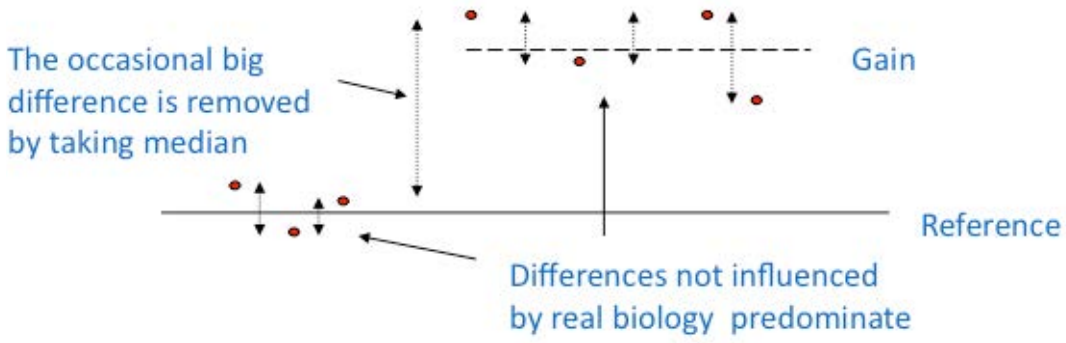


Figure 520

Formally, if xi: is the Log2 Ratio for marker i:

MAPD = median($|x_{i-1} - x_i|$, with i ordered by genomic position)

MAPD is a per-microarray estimate of variability, like standard deviation (SD) or interquartile range (IQR). If the Log2 Ratios are distributed normally with a constant SD, then MAPD/0.96 is equal to SD and MAPD*1.41 is equal to IQR. However, unlike SD or IQR, using MAPD is robust against high biological variability in Log2 Ratios induced by conditions such as cancer.

Variability in Log2 Ratios in a microarray arises from 2 distinct sources:

- Intrinsic variability in the starting material, hybridization cocktail preparation, microarray or scanner
- Apparent variability induced by the fact that the reference may have systematic differences from this microarray

Regardless of the source of the variability, increased variability decreases the quality of CN calls.

Effect of MAPD on functional performance

As a measure of performance, we measured copy number gain and loss using samples with large chromosome aberrations that spanned approximately 70% of the genome. With this dataset of nearly 1,500 microarrays we measured the sensitivity for detecting regions of copy number change across all of these regions. The sensitivity of detecting an aberration on each array was binned into groups of varying sensitivities, and plotted versus MAPD for each array in the following graph. (Figure 521)

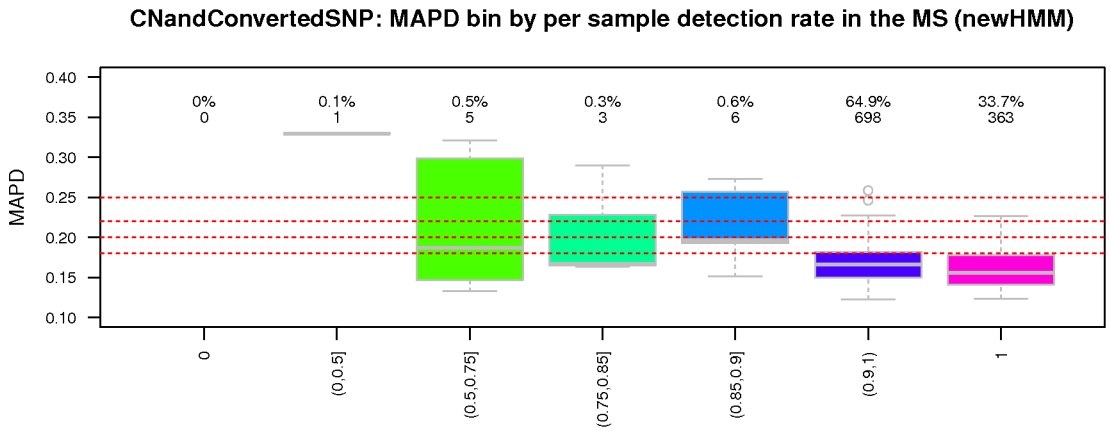


Figure 521

The bins of detection sensitivity are displayed as coordinates along the x-axis, with 0% detection at the left and 100% at the right. The number of arrays is listed above each box plot. The majority of the arrays had sensitivities above 90%. Based on this analysis, we established a QC cutoff for MAPD of 0.25. Arrays with MAPD above 0.25 cannot be reliably used to determine copy number.

Waviness-SD – Detailed Description

For quality assessment purposes, we define metrics that assess whether the microarray data is useful for copy number (CN) analysis. In addition to MAPD (above) we define an alternate form of measurement of variance in the array data that is called Waviness-SD, where SD stands for Standard Deviation.

Waviness refers to an effect seen in all genomic microarrays (see Maroni et al. (2007) Genome Biology **8**:R228) where long-range variation is observed, often associated with regional genomic differences like local GC-content changes.

Waviness-SD is a QC metric that focuses on measuring these long-range effects. As described separately, MAPD is a metric that measures short-range variation, the variation of adjacent probes. The long-range variation measurement is accomplished by calculating the variation in Log2 Ratios across the whole genome and subtracting out the short-range variation, specifically, for autosomal probes:

Define:

X_i as the Log2 Ratios of autosomal probes

And Z_i as the variance between adjacent probes:

$Z_i = X_{2i+1} - X_{2i}$

Waviness-SD is the total variance (X_i) minus the local variance (Z_i):

Waviness-SD = sqrt(Var(X_i)-Var(Z_i)/2)

While this metric is useful in most cases, it does make the assumption that most of the genome is of normal copy number. This assumption may not be reasonable for some types of cancer samples with large amounts

of genomic copy number variations, or for multiple-chromosome constitutive trisomies, where a considerable fraction of the genome is duplicated.

For most samples, a Waviness-SD value below 0.12 for CytoScan arrays indicates that the long-range variation is within levels that can be accommodated by the CytoScan algorithms. But a high Waviness-SD measure on a sample with good MAPD and SNPQC metric values should be checked for the presence of large regions of copy number change to assess whether it is a sample effect or a QC failure.

Waviness-SD can be a good indicator of process drift since it measures long-range variation relative to the CytoScan HD or CytoScan 750K reference profile. A general rise of Waviness-SD for all samples coming from your laboratory may be an indication of a change of protocol, technique or reagents.

SNPQC – Detailed Description

SNPQC is a metric that estimates the distributions of homozygous AA, heterozygous AB and homozygous BB alleles and calculates the distance between them. The better the separation of these distributions, the better the ability to identify a genotype based on its cluster position (Figure 522).

Propose we go towards a “single chip FLD” like metric

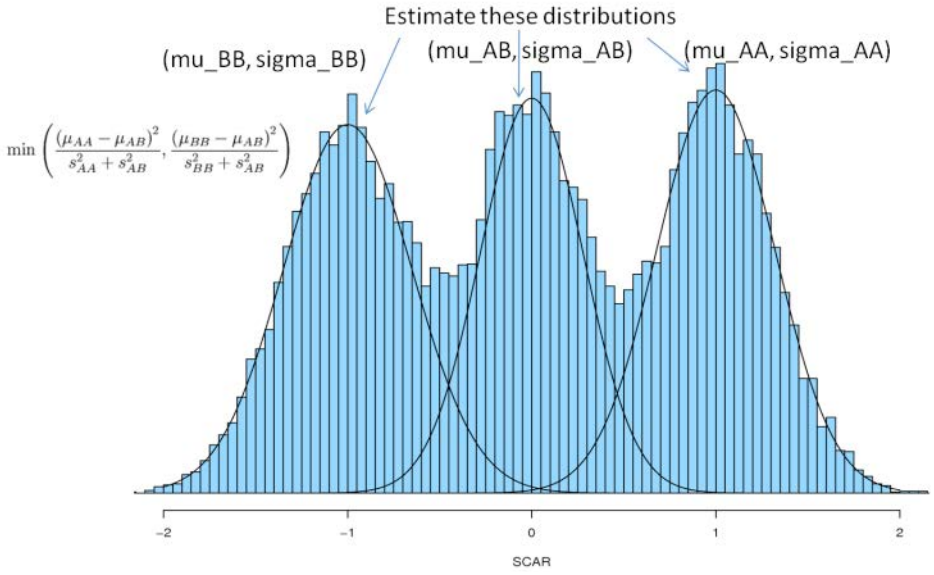


Figure 522 .

SNPQC correlates well with genotype performance, as measured by Call Rate and Concordance to published HapMap genotypes. To establish this relationship, we scored 380 microarrays from the Reference Set by calculating SNPQC, Call Rate and Concordance. The following graphs show the relationships between SNPQC and the other 2 metrics (Figure 523).

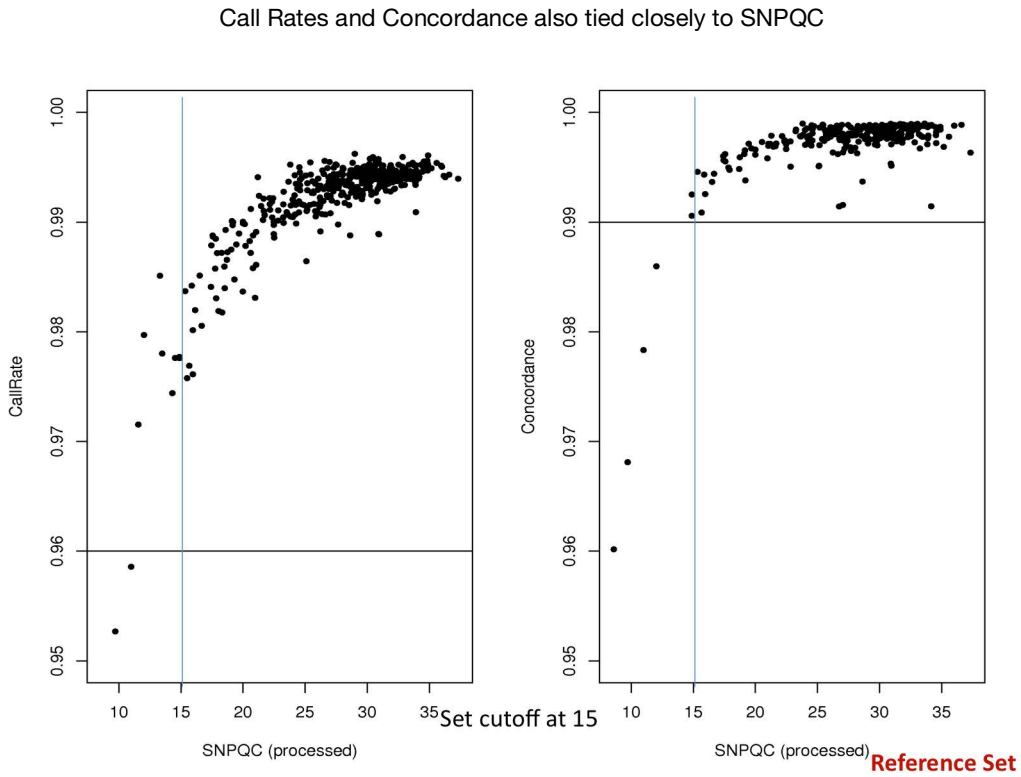


Figure 523 .

The left panel shows that when SNPQC >15, Call Rate is above 98%. The right panel shows that when SNPQC >15, Concordance is above 99%. This functional mapping of SNPQC has allowed us to set a functional threshold for this QC metric at 15. Microarrays with SNPQC >15 are considered of high quality and interpretation of the data is possible.

Effect of SNPQC on functional performance

SNPQC provides insight into the overall level of data quality from a SNP perspective. The key consideration when evaluating the SNPQC value is to ensure the threshold is exceeded. The quality of the SNP allele data is compromised, and is noisier and more difficult to interpret when the SNPQC values are below the recommended acceptance threshold as illustrated in the following figure. When the SNPQC value is below 15, the noise within the array is higher than normal which compromises the overall data quality and clarity of results. However, when the SNPQC value is above 15, the data is of excellent quality and can be relied upon as robust with regard to performance (Figure 524).

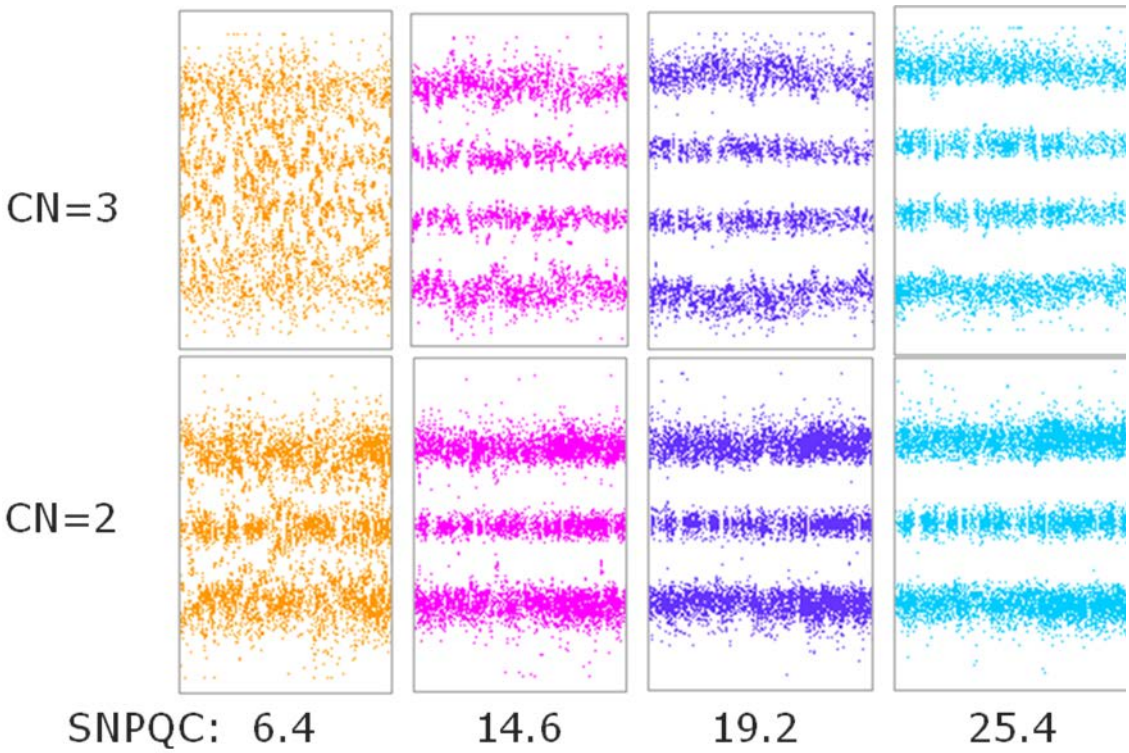


Figure 524 Exampl.s of Allele Track data quality at various levels of SNPQC. The lower row of figures show data for a CN=2 and the upper for CN=3 regions. The panels from left-to-right represent increasing SNPQC quality. The functional threshold for SNPQC is 15, so all values above 15 show excellent data quality.

The key consideration is whether the SNPQC value is above or below the threshold value and not the absolute magnitude. As long as the SNPQC value exceeds the threshold there is a retention in the data quality as illustrated by the graphs to the right which demonstrate clear allelic data across a broad range of SNPQC values which exceed the recommended threshold. SNPQC is one of the metrics used to assess array quality and should be helpful towards determining which experimental data sets are of satisfactory quality to continue with subsequent interpretation.

Note: For detailed information on algorithms and QC metrics for the OncoScan™ array, see the *OncoScan™ Console User Manual* (Pub. No. 703195).

TuScan algorithm

The TuScan algorithm uses B-allele frequencies (BAFs) and log2 ratios to estimate the ploidy and percentage of aberrant cells in the sample (%AC) which in turn are used to calculate copy number calls (CN). The BAFs and log2 ratios contribute equally to CN determination. TuScan first uses the BAFs and log2 ratio data to identify segments of equal CN. Next TuScan uses the BAFs, log2ratios and segment data to find the combination of %AC and ploidy that best fits the data. When TuScan can successfully determine %AC, the algorithm assigns each aberrant segment an integer copy number representing the copy number in the tumor portion of the sample. This is possible because CN is well approximated by an integer when the tumor is nearly homogeneous. If the tumor is highly heterogeneous (that is, lacks a dominant clone), or contains a large amount of "normal" cells %AC cannot be determined. In other words, if the percentage of aberrant cells contributing to the various aberrations in the sample varies across all aberrations, %AC and ploidy cannot be determined. When %AC cannot be determined, the segmentation algorithm will still identify segments of equal CN, but the CN in just the aberrant cells cannot be determined. In this case, TuScan bins the copy numbers and returns fractional CN values in 1/3 increments (for example, 2, 2.33, 2.66, 3 etc.). This fractional copy number is derived from the normal contamination as well as the heterogeneous population of tumor cells; therefore, the fractional CN calls represent the average CN observed for that segment. Users should look at the value of %AC to determine whether the CN value represents the CN in the tumor (%AC= number) or the average CN in the sample (%AC=NA). Tumor heterogeneity also affects the interpretation of the CN number calls when %AC cannot be determined. For example, a TuScan call of 2.33 can result from 40% of the aberrant cells having 3 copies, 10% of aberrant cells having 5 copies, or a more complex heterogeneous mixture of copy numbers. Since nearly every tumor sample will have some amount of normal contamination combined with tumor heterogeneity it is not possible to predict how often TuScan will be able to determine the %AC, it will vary depending on the sample.

Manual re-centering algorithm (OncoScan™ data)

TuScan identifies normal diploid markers in a sample of interest, determines the copy number for these markers (2, 4 or 6) and ensures that markers with CN=2 have a log ratio of 0. This is referred to as "centering" the sample.

When no or an insufficient number of normal diploid markers are found, the automatic recentering does not occur. In addition, occasionally the automatic recentering misses the true CN =2 markers and does not correctly center the sample. In these cases, it is advised to center the sample manually to get correct CN calls. Manual recentering is now available through the CHAS software and the recentered sample is re-run through TuScan (described above) to provide integer copy number.

The new RC.OSCHP files can be viewed in ChAS or the BioDiscovery software, Nexus.

To manually recenter samples, an offset (median log2 ratio) is provided that tells the algorithm how much a sample should be pushed up (positive value) or pushed down (negative value) so that this region resides at the log 2 ratio = 0, indicative of normal diploid.

In the following example (Figure 525), the sample should be centered at chromosome 4q. The median log ratio on 4q is –0.17, therefore the manual recentering adjustment would be given this offset value, resulting in an increment adjustment of 0.17 for all log ratios.

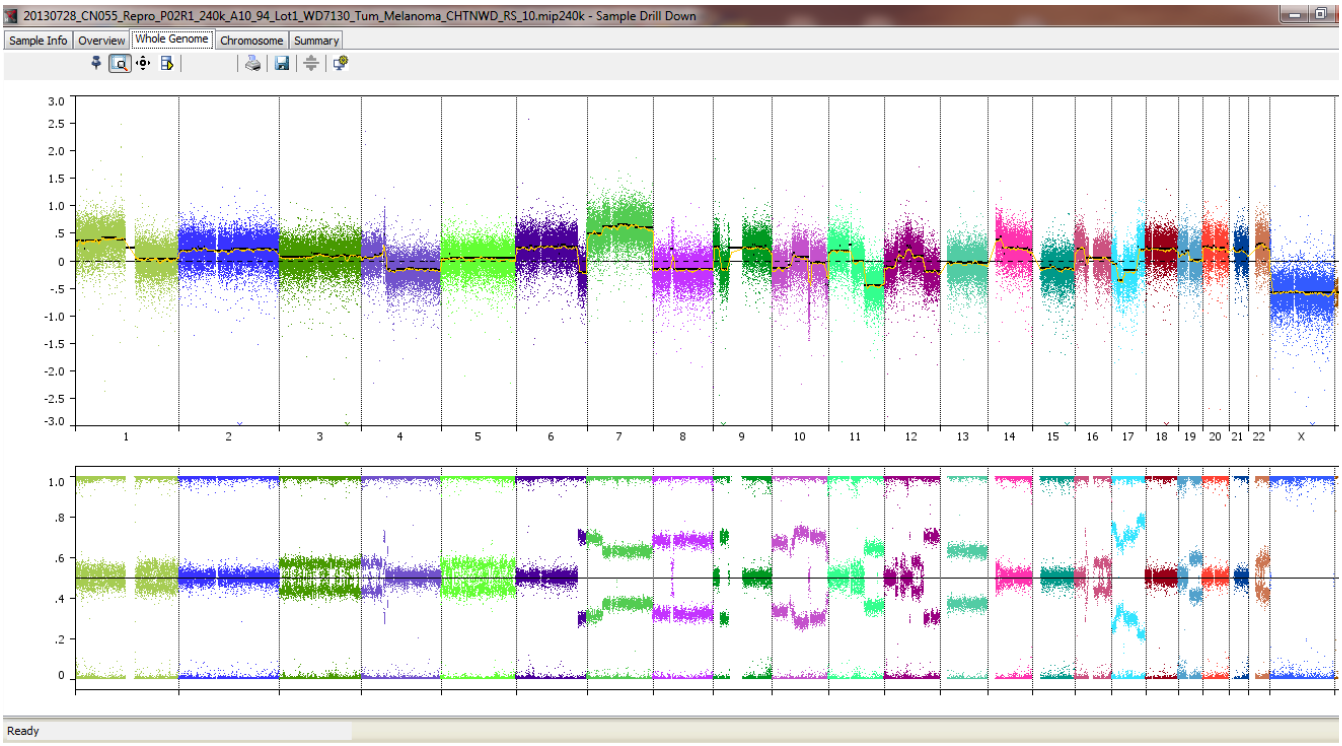


Figure 525

Copy number effect on somatic mutations

Somatic mutation probesets in the OncoScan™ FFPE Assay are designed to selectively respond to the presence of mutation sequences. However, large copy number amplifications spanning the somatic mutation targets can sometimes lead to falsely reporting the presence of mutations in amplified regions.

If the copy number state is greater than ~15, you may observe false positive somatic mutation calls. The only region for which we have observed this problem is the EGFR gene, which is prone to very high copy number in certain cancer types.

In the following example (Figure 526), the predicted copy number state for the EGFR gene is greater than 30, which affects the somatic mutation score. Another side effect shown in the example below is that 3 mutations are called in the high Copy Number region, a contradictory event for at least 2 of these mutations.

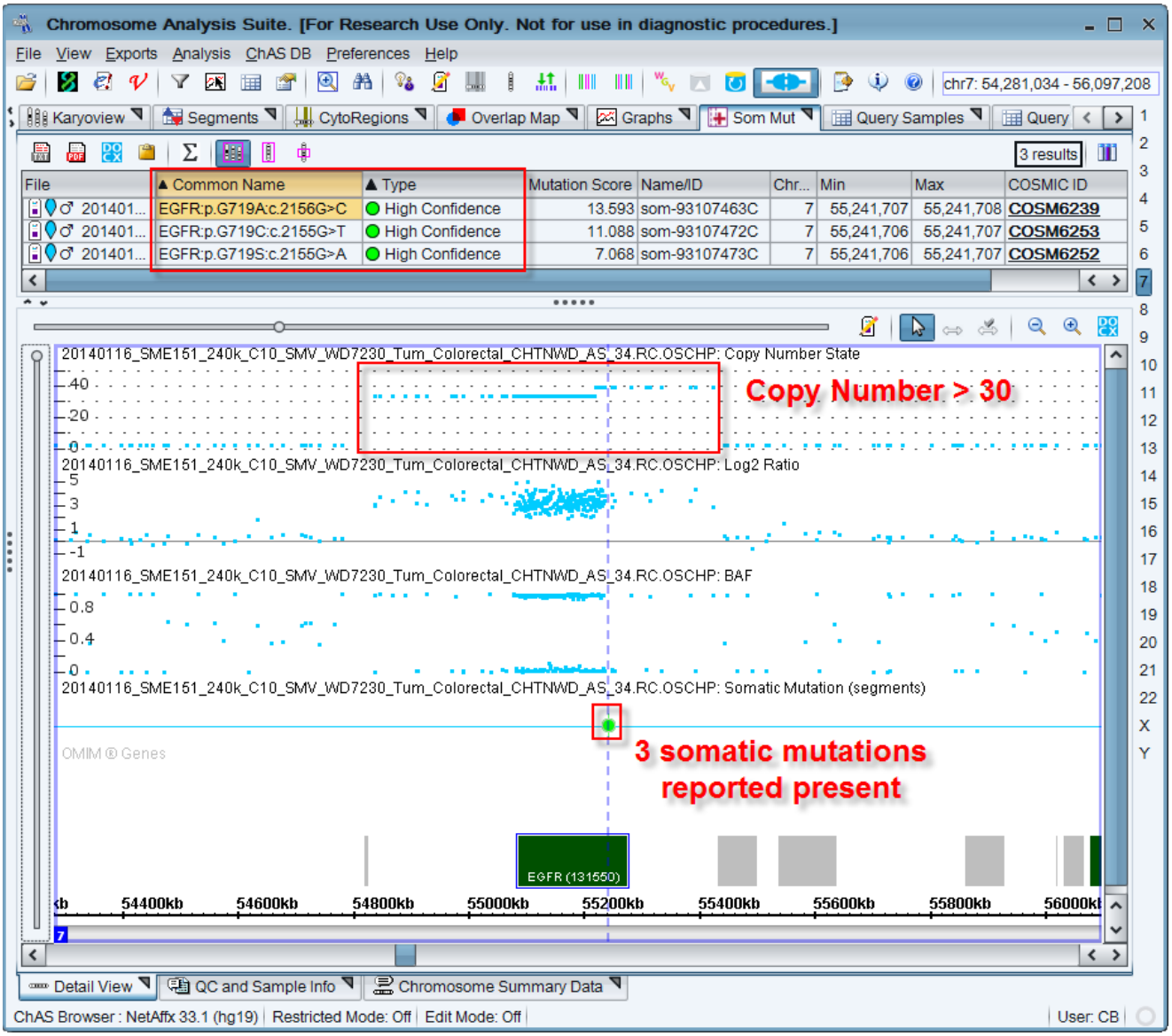

Chromosome Analysis Suite. [For Research Use Only. Not for use in diagnostic procedures.]
File View Exports Analysis ChAS DB Preferences Help
chr7: 54,281,034 - 56,097,208
Karyoview Segments CytoRegions Overlap Map Graphs Som Mut Query Samples Query
3 results
File | Common Name | Type | Mutation Score | Name/ID | Chr... | Min | Max | COSMIC ID
201401... | EGFR:p.G719A:c.2156G>C | High Confidence | 13.593 | som-93107463C | 7 | 55,241,707 | 55,241,708 | COSM6239
201401... | EGFR:p.G719C:c.2155G>T | High Confidence | 11.088 | som-93107472C | 7 | 55,241,706 | 55,241,707 | COSM6253
201401... | EGFR:p.G719S:c.2155G>A | High Confidence | 7.068 | som-93107473C | 7 | 55,241,706 | 55,241,707 | COSM6252
20140116_SME151_240k_C10_SMV_WD7230_Tum_Colorectal_CHTNWD_AS_34.RC.OSCHP: Copy Number State
Copy Number > 30
20140116_SME151_240k_C10_SMV_WD7230_Tum_Colorectal_CHTNWD_AS_34.RC.OSCHP: Log2 Ratio
20140116_SME151_240k_C10_SMV_WD7230_Tum_Colorectal_CHTNWD_AS_34.RC.OSCHP: BAF
20140116_SME151_240k_C10_SMV_WD7230_Tum_Colorectal_CHTNWD_AS_34.RC.OSCHP: Somatic Mutation (segments)
OMIM ® Genes
3 somatic mutations reported present
EGFR (131550)
54400kb 54600kb 54800kb 55000kb 55200kb 55400kb 55600kb 55800kb 56000kb
Detail View
QC and Sample Info
Chromosome Summary Data
ChAS Browser : NetAffx 33.1 (hg19) | Restricted Mode: Off | Edit Mode: Off
User: CB

Figure 526

CytoScan™ XON region calling algorithm

For CytoScan™ XON Arrays a region focused calling algorithm was developed to distinguish between neutral, gain and loss copy number. Regions are defined by genomically contiguous stretches of closely spaced markers. Each region is assessed independently.

A 3 state conditional random field (CRF) model is used to segment each region. The CRF model uses the log2 ratio measurements corresponding to that marker and 4 markers on either side, plus the state of the previous marker, to assess whether a given marker is in one of the 3 states. Before using the CRF model the log2 ratio is independently adjusted for every marker to account for expected differences in responsiveness to copy number.

For resistance to outliers, the t-distribution is used for computation of emission probabilities. The mean parameter for the emission distributions are fixed for each state across all markers, but the standard deviation parameter includes a component that differs for each marker, based on expected marker variability. Adjacent regions with the same copy number determination are not joined into larger segments so large copy number aberration events may be represented by multiple region based calls.

Recommended CytoScan™ XON Array workflows

IMPORTANT! The Segment Prioritization feature outlined in Chapter 17, "Prioritize segments" on page 347 can be used in conjunction with the recommended steps in this appendix to quickly prioritize XON regions.

Whole exome analysis

The markers on the CytoScan™ XON Array have been categorized into these 4 levels (based on the annotation in the region of the genome).

- **Level 1:** Includes genes with the highest level of evidence: developmental delay, epilepsy, ASD, XLID, Metabolic disorders, hereditary cancer OMIM™ Morbid genes.
- **Level 2:** ClinVar genes not covered in Level 1.
- **Level 3:** Other OMIM™ genes not identified as Level 1.
- **Level 4:** Other Ref Seq, UCSC, Ensembl genes, LOVD.

Recommended workflow for analyzing the whole exome

Load XNCHP file(s)

1. Click **File → Open**.
2. Select the XON-Level 1 Named Setting.

   The XON Region Segment Calls in genomic regions assigned as Level 1 are shown.
3. Turn off any filters that are based on markers or size.

   The XON gain/loss segments that are contained in/or overlap with Level 1 regions will be exposed on the XON Region Segment Track. The data in the Level 1 regions (Log2 Ratio, Weighted Log2 Ratio, Smooth Signal, Allele Difference, B-allele Frequency) will show in color (based on the sample color assignments in the User Configuration). The data in the remaining Level 2–4 regions will remain gray and no XON segments will be revealed.

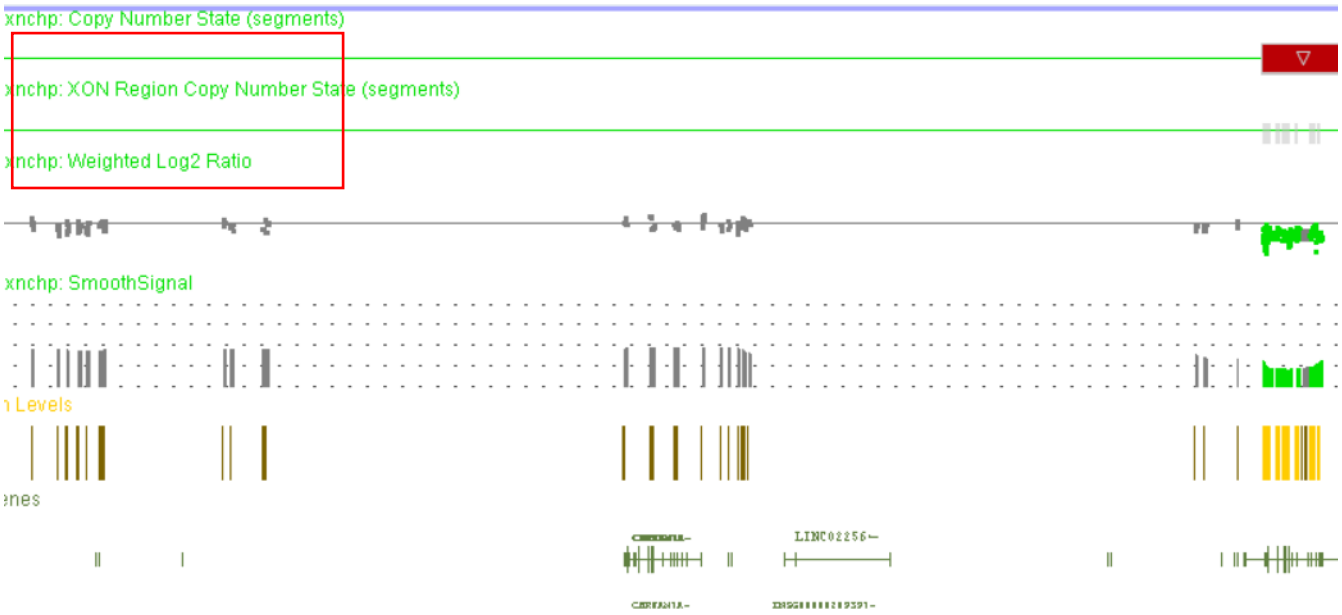


Figure 527 Example: Level 1 check only.

4. Add the XON Region Level and Summarized Log 2 Ratio columns to the segment table, then use the Segments Table to review the XON Region segment calls.
5. Optional: Annotate the XON Region segments as you normally would using the Call and Interpretation columns, as described in "Add annotations at the sample (xxCHP) file level" on page 236.
6. Optional: Exon Region segment calls from other Levels can be displayed by selecting the checkbox(es) in the Filters Tab.

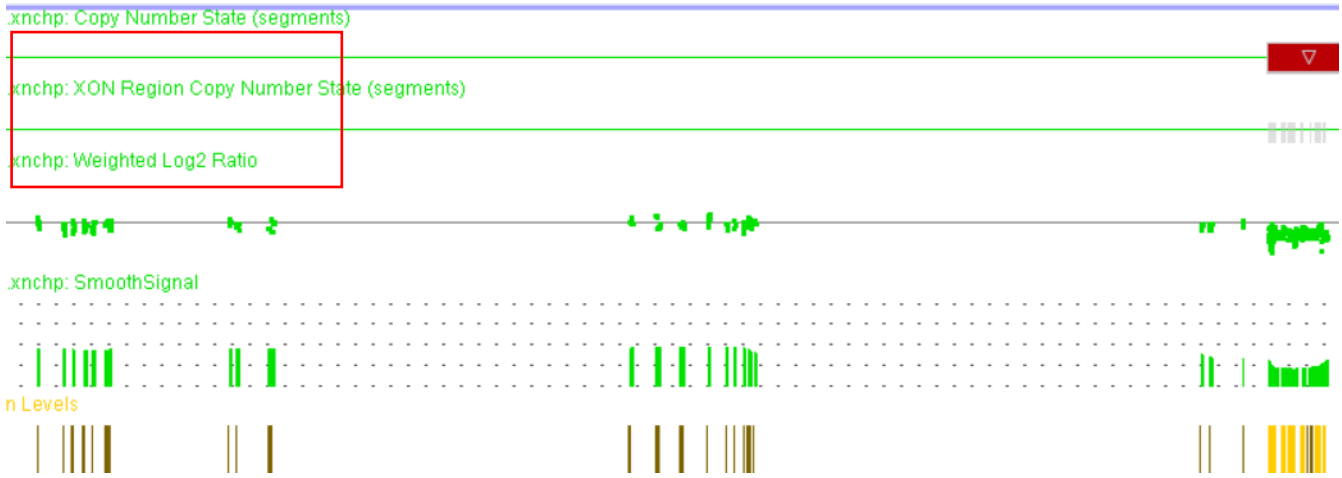


Figure 528 Example: Level 1 and 2 checked.

7. Optional: Publish the XNCHP file to the ChAS DB, as described in "Publish data to the database" on page 377.

Run a targeted analysis

When you have a specific set of genes that you want to search for XON Region segment calls, a targeted analysis can be performed using CytoRegions for Targeted XON analysis. Details on this feature can be found in "Assign a CytoRegion for targeted XON analysis" on page 261.

1. Create an AED file from your list of genes. See "Create an AED File from a gene list" on page 261. If you already have an AED/BED file containing your genes of interest, go to step 2.
2. Click **File → Open** to load the AED/BED file.
3. Locate the file, then click **Open**.
4. Right-click the loaded file, then select S**et File as CytoRegion for Targeted XON Analysis**.

   This assigns the file as a CytoRegions File, as well as automatically sets the appropriate filters for Targeted XON analysis. The filters hide all segments in the Genome region and colors all the data gray and turns on all levels in CytoRegions to expose any XON Region segment call and colors the data the assigned sample color.

   Note: Only XON Regions calls within the CytoRegions are displayed in the views and tables.

5. Optional: Annotate the XON segments as you normally would, as described in "Add annotations at the sample (xxCHP) file level" on page 236.
6. Optional: Publish the XNCHP file to the ChAS DB, as described in "Publish data to the database" on page 377.

Automatic CEL file analysis

ChASCelUploader is installed on the workstation of the GeneChip™ 3000 Scanner (GCS3000). This application monitors a user specified folder for CEL files and then copies the CEL files to a user specified network share drive for analysis by the **Automatic CEL File Analysis Tool**. This tool is located in the ChAS Analysis Workflow module. It converts CEL files to CHP files and includes an option for automatically upload the CHP files to your QIAGEN Franklin account using ChAS AIR tokens.

ChASCelUploader

ChASCelUploader monitors a user specified folder for CEL files generated by the GCS3000 Scanner, then automatically uploads them to a user specified network drive.

Install and configure the uploader

1. Locate the **ChASCelUploader_Setup.exe** file in the CHAS ZIP file, then double-click to open. Follow the on-screen prompts to install it.
2. After the installation is successful, navigate to: **C:\ProgramData\ChASCelUploader**
3. Locate the **ChASCelUploader.config** file, then open it in a text editor such as Microsoft™ Notepad. (Figure 529)

*ChASCelUploader.config - Notepad

File Edit Format View Help

```
<?xml version="1.0"?>
<UploaderConfig DataDir="C:\Command_Console\Data\Default"
                DestDir="Q:\ChAS\User\Director1\Autoupoader"
                MaxRetryCount="10"
                PoolingIntervalSec="180"
                TimeSecBeforeExpired="604800"
                TimeSecBeforeReadingCELFile="360"
                TimeSecBeforeRetrying="1800"
                TimeSecBeforeRetryingMultiplier="1.5">
  <ArrayTypes>
    <ArrayType>CytoScanHD_Array</ArrayType>
    <ArrayType>CytoScan750K_Array</ArrayType>
    <ArrayType>CytoScan_XON_Array</ArrayType>
    <ArrayType>CytoScanOptima_Array</ArrayType>
    <ArrayType>OncoScan_CNV</ArrayType>
    <ArrayType>OncoScan</ArrayType>
    <ArrayType>CytoScanHD_Accel</ArrayType>
  </ArrayTypes>
</UploaderConfig>
```

Figure 529 ChASCelUploader.config file.

4. At the **DestDir** line, change the drive letter to the network drive letter and folder (directory) you want your data automatically uploaded to.

   Note: By default the ChASCelUploader monitors the GCC default directory for CEL files. You can change this default by updating the DataDir path to a different folder on the GCC Workstation. If you are using GCDC workstation, update the DataDir path to: **D:\Command_Console\Data\Default**

5. Save and close the Notepad window.
6. To start the tool, click the **ChASCelUploader** Desktop Shortcut to start it or click the Windows™ Start button and navigate to **Thermo Fisher Scientific → ChASCelUploader**.

   The ChASCelUploader icon  appears in your system tray to show it is running. To stop it, right-click the icon and select **Exit**.

   Note: After the Uploader starts, only newly added CEL files are uploaded. Existing CEL files are not.

Automatic CEL file analysis tool

The **Automatic CEL File Analysis** tool in ChAS converts CEL files into ChAS Browser compatible CHP files automatically.

Convert CEL files to CHP files

How it works

The Automatic CEL File Analysis tool continually scans up to 5 designated input folders for new CEL files to analyze. After a CEL file is detected, it is analyzed resulting in a CHP file (which is auto-saved to a designated output folder). After a CEL file is processed, the ARR and CEL files are moved to the designated Archive folder.

Supported array types

- CytoScan™ HD (Single Sample Analysis or Normal Diploid Analysis only)
- CytoScan™ 750K (Single Sample Analysis or Normal Diploid Analysis only)
- CytoScan™ HD Accel (Single Sample Analysis only)
- CytoScan™ 750K Accel Array (Single Sample Analysis only)
- CytoScan™ XON (Single Sample Analysis only)
- CytoScan Optima (Single Sample Analysis only)
- OncoScan™ CNV (FFPE Analysis only)
- OncoScan™ CNV Plus (FFPE Analysis only)

Note: To properly match up CEL files when running OncoScan™ CNV or OncoScan™ CNV Plus Arrays, the CEL files from the "AT" and "GC" channels must have the exact same root name and include the array type. Each CEL file must indicate which channel they are using a concluding A or C. For example, the following CEL files will be paired properly for OncoScan™ FFPE analysis:

- OncoScan_Sample1_(OncoScanCNV_Array)A.cel
- OncoScan_Sample1_(OncoScanCNV_Array)C.cel

The following CEL files will NOT be paired properly for OncoScan™ FFPE analysis because the root names do not match:

- OncoScan_Sample1_(OncoScanCNV_Array)A.cel
- OncoScan_Sample2_(OncoScanCNV_Array)C.cel

The following CEL files will NOT be paired properly for OncoScan™ FFPE analysis because the array names are not included:

- OncoScan_Sample1_A.cel
- OncoScan_Sample1_C.cel

Launch the tool

1. Click the **Utility Actions** button (top right of the Analysis Workflow window)
2. Click **Automatic CEL File Analysis**.

   The window opens. (Figure 530)

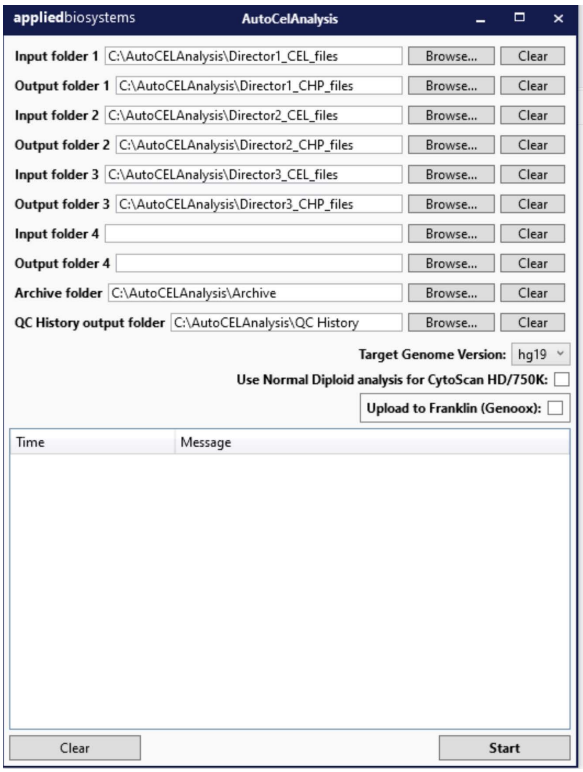


Figure 530 AutoCelAnalysis window.

Set up the tool

IMPORTANT! Input, Output, and Archive folder names and their assigned paths should not contain spaces. If a space is needed, use the underscore symbol [_].

Any non-ARR and non-CEL files detected in your assigned input folder will remain in this folder. Any detected ARR and CEL files will be processed and moved into your assigned Archive folder.

Assign input folder(s)

The Input folder is where your CEL files reside.

1. Click the **Input folder 1** the ••• button.

   A File Explorer window appears.

2. Navigate to your CEL file folder.
3. Click the folder to highlight it, then click **Select Folder**.

   Your CEL file folder path is displayed.

4. Optional: After the first Input folder is assigned, an option to designate a second Input folder appears (Input folder 2). Repeat steps above to assign a second Input folder. Up to 5 Input folders can be assigned.

Note: Ensure that you assign the Input folder using the folder path defined in **DestDir** (Figure 529) when you want to upload the data from the GCS3000 to Franklin. Just one input folder can be assigned for this work upload process.

Assign output folder(s)

After the tool produces a CHP file, it is placed in an Output folder.

1. Click the **Output folder 1** the ••• button.

   A File Explorer window appears.

2. Navigate to an existing folder where you want your newly converted CHP files saved or click **New Folder** to create a new Output folder.
3. Click **Select Folder**.

   Your Output folder 1 path is displayed.

4. Optional: After the first Output folder is assigned, an option to designate a second Output folder appears (Input folder 2). Repeat steps above to assign a second Output folder. Up to 5 Output folders can be assigned.

Assign an archive folder

Copies of the processed CEL files are placed in the Archive folder.

1. Click the **Archive folder** the ••• button.

   A File Explorer window appears.

2. Navigate to the folder where you want the CEL files saved or click **New Folder** to create a new Archive folder.
3. Click **Select Folder**.

   Your Archive folder path is displayed.

Assign a QC History file folder

1. Click the **QC History folder** button.

   A File Explorer window appears.

2. Navigate to the folder where you want the CEL files saved or click **New Folder** to create a new QC History folder.

3. Click **Select Folder**.

   Your QC History folder path is displayed.

Select a target genome version

1. By default, **hg19** is selected. If needed, click the **Target Genome Version** dropdown to select **hg38**. (Figure 531)

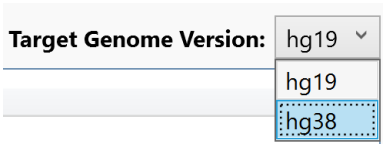
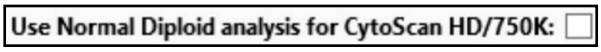


Figure 531 Version dropdown list.

Normal diploid analysis checkbox



1. Select the checkbox to use the Normal Diploid algorithm. Leave unselected to run the default single sample analysis.

Upload to Gennox Franklin

ChAS can upload segment data automatically to Franklin after AutoCelAnalysis.

1. Select the **Upload to Franklin (Gennox)** checkbox. (Figure 532)
2. Enter your Franklin credentials, email and password, then choose your ChAS profile in the expanded window to automatically upload your segment data to Franklin.



Figure 532 Franklin credentials.

Note: All Segment Filter settings, Smoothing and Joining rules in the selected ChAS profile will be applied to the segment data that is uploaded to Franklin. Data that has failed QC will not be uploaded to Franklin.

Run the tool

1. Click Start.

   The CEL file analysis begins and the **Time** and **Message** pane populates with information, as shown in Figure 533.

   Click **Cancel** to stop an analysis in progress. **Note:** The time for CEL file analysis to complete should be comparable to analysis times when manually setting up CEL files in the Analysis Workflow.

Note:

- During the analysis process, the tool detects the array type, then auto-generates an appropriately named array staging folder. As each CEL file analysis completes, the tool moves the newly created CHP file from its staging folder to the output folder you assigned earlier.
- If the Automatic CEL File Analysis stops for any reason, such as losing connection to the input folder or is inadvertently canceled, a log file of processed CEL files can be found in the designated Archive folder.

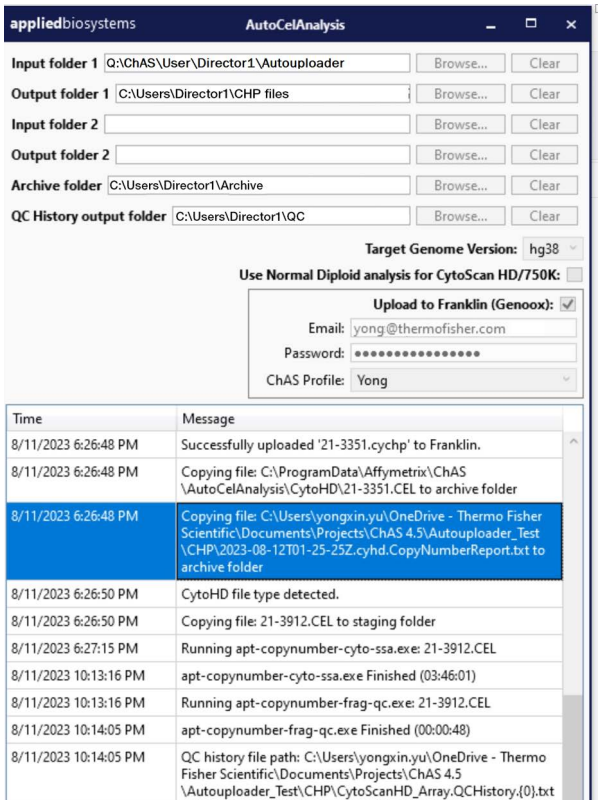


Figure 533 AutoCelAnalysis window - Populated.

Open the newly generated CHP file(s)

1. From the ChAS Browser window, click **File → Open**.

   A File Explorer window appears.

2. Navigate to your assigned Automatic CEL File Analysis output folder(s). See "Assign output folder(s)" on page 472.

3. Click or Ctrl+click to highlight the CHP files you want to open in the ChAS Browser, then click **Open**.

   The file(s) appear in the ChAS Browser.

View cases in QIAGEN Franklin

If using the automatic upload to Franklin features:

1. Go to https://franklin.genoox.com/clinical-db/home and sign in.
2. Click the **My Case** tab to view your case(s).
   The All Cases window appears. (Figure 534)

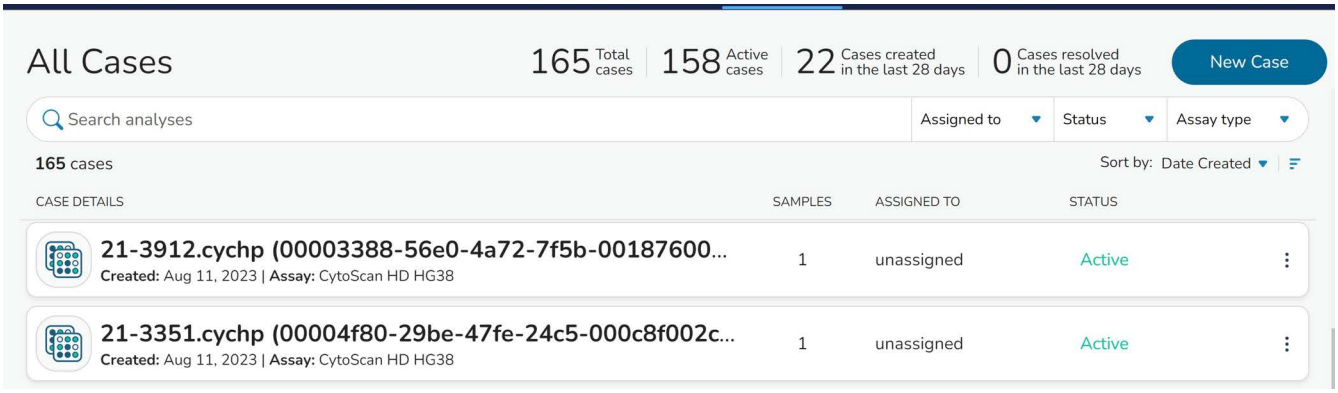


Figure 534 QIAGEN Franklin - All Cases window example.