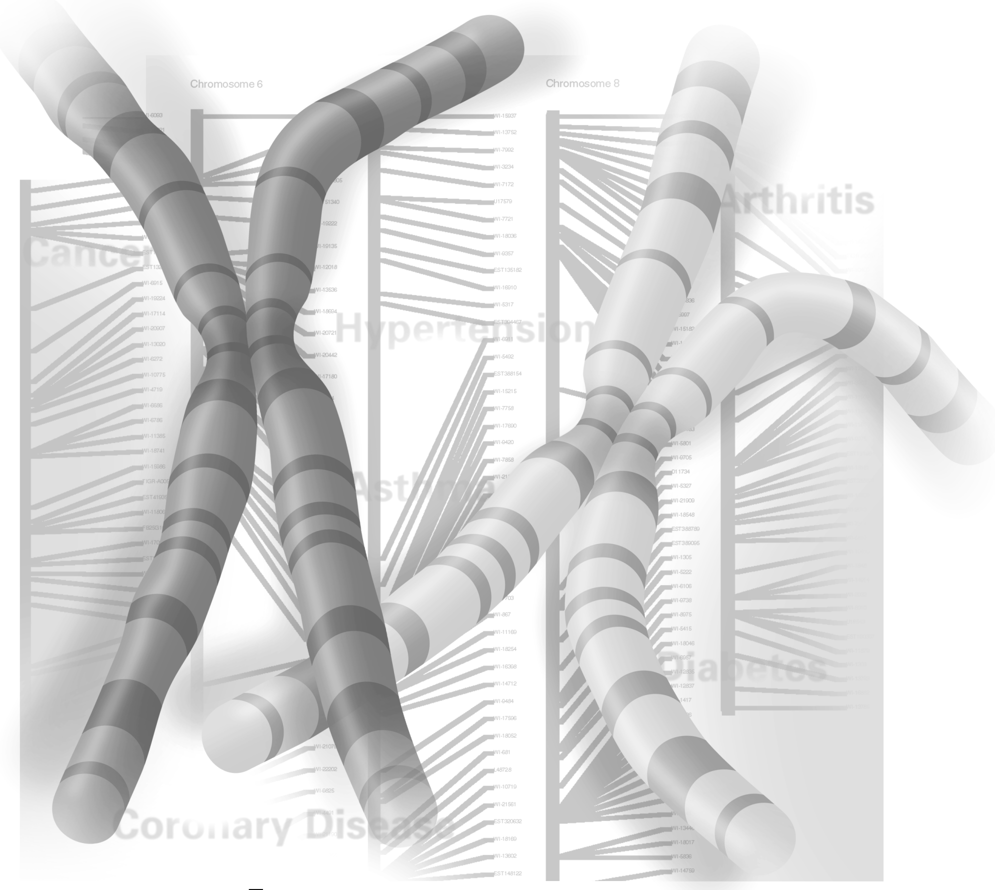
GeneChip®

HuSNP™ Mapping Assay



User's Manual

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GETTING STARTED

1

INTRODUCTION AND OBJECTIVES

Welcome to the *Affymetrix® GeneChip® HuSNP™ Mapping Assay User's Manual*. This manual is a technical guide for using GeneChip HuSNP probe arrays. All protocols included in this manual have been tested and used successfully by the scientists at Affymetrix.

As an Affymetrix® GeneChip Array User, we welcome your feedback. Please contact our technical support team with any input on how we can improve this resource.

This manual applies to the following products:

- GeneChip® HuSNP™ Probe Arrays (5 pack), P/N 900194
- GeneChip® HuSNP™ Reagent Kit (25 reactions), P/N 900193, which includes the following components:

  Multiplex Primer Pools 1-24

  Labeling Primer, Biotin-T3

  Labeling Primer, Biotin-T7

  HuSNP™ Reference DNA (4.0 ng/ µL)

  Control Oligo B1

EXPLANATION OF GENECHIP® PROBE ARRAYS

GeneChip® probe arrays are manufactured using technology that combines photolithographic methods and combinatorial chemistry. Tens to hundreds of thousands of different oligonucleotide probes are synthesized in a 0.81 cm x 0.81 cm area on each array. Each probe type is located in a specific area on the probe array called a probe cell. Each probe cell contains millions of copies of a given probe.

Probe arrays are manufactured in a series of cycles. A glass substrate is coated with linkers containing photolabile protecting groups. Then, a mask is applied that exposes selected portions of the probe array to ultraviolet light. Illumination removes the photolabile protecting groups enabling selective nucleoside phosphoramidite addition only at the previously exposed sites. Next, a different mask is applied and the cycle of illumination and chemical coupling is performed again. By repeating this cycle, a specific set of oligonucleotide probes is synthesized, with each probe type in a known location. The completed probe arrays are packaged into cartridges.

OVERVIEW OF GENECHIP® HUSNP™ MAPPING ASSAY

Step	**Process Duration**
Genomic DNA	
⇩ 120 ng of sample DNA	
Multiplex Amplification	2 hours
⇩ 1:1000 Dilution	
Labeling Amplification	3 hours
⇩ Pool, Concentrate	
Hybridization	overnight
⇩	
Wash and Stain	50 minutes
⇩	
Scanning	8 minutes per sample
⇩	
Data Analysis	1 minute per sample

Figure 1.1 GeneChip® HuSNP™ Mapping Assay

Starting with 120 ng of genomic DNA, a set of 24 simultaneously run multiplex PCRs will amplify the Human Single Nucleotide Polymorphisms (SNPs) represented in the GeneChip HuSNP Genetic Mapping Assay. The amplified SNPs are further amplified and concomitantly labeled using biotinylated primers in a second set of 24 simultaneously run labeling PCRs. The biotinylated PCR products are then pooled, concentrated, and prepared for hybridization.

The biotinylated amplification products, which reflect the bi-allelic genotype in the sample DNA, are hybridized to the GeneChip® HuSNP™ probe arrays during an overnight incubation at 44°C in the GeneChip Hybridization Oven. On the following day, the probe arrays are thoroughly washed and stained with a complex of streptavidin phycoerythrin (SAPE) and biotinylated anti-streptavidin IgG antibody. The automated wash and stain procedures are run on the GeneChip® Fluidics Station 400, under the control of Affymetrix® Microarray Suite software running on a PC-compatible workstation with a Windows NT™ operating system.

The stained probe arrays are then scanned in the Agilent GeneArray™ Scanner, also under the control of Microarray Suite. Each probe array will be scanned twice to capture the light emitted at wavelengths of 530 nm and 570 nm, generating two scan image files.

Affymetrix® Microarray Suite will process the two scan images to calculate all of the signal intensities on the probe array. Using the signal intensity data, statistical algorithms in Microarray Suite will generate SNP genotyping results in the Nucleotide Analysis Window, as well as tabular and graphical reports.

SCHEMATIC REPRESENTATION OF THE PCR AMPLIFICATIONS

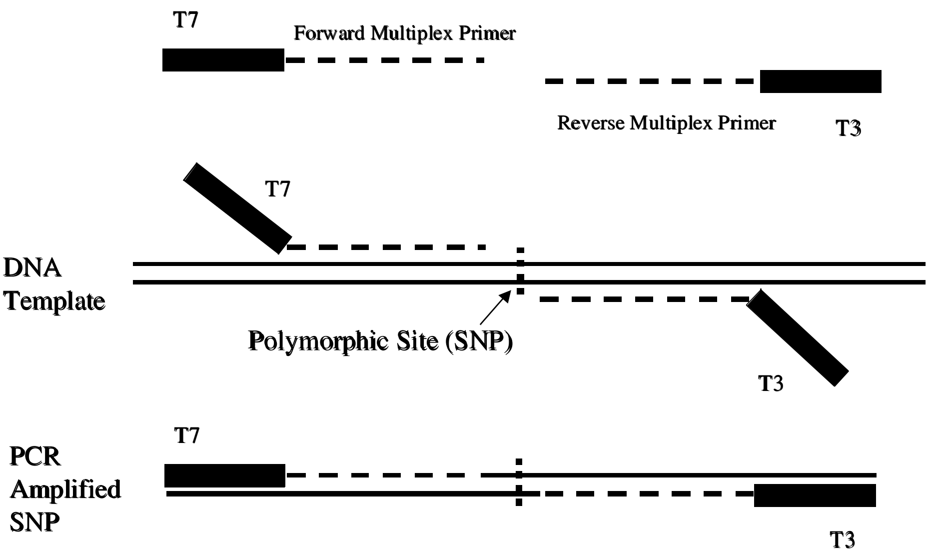

Figure 1.2 Multiplex PCR diagram

The SNP-specific primers also contain T7 and T3 sequences at their ends; consequently, all of the products from the multiplex PCR have the same T7 and T3 sequences in common.

The 1:1000 dilution following the multiplex PCR effectively lowers the concentration of SNP-specific primers in the Labeling PCR and in the subsequent hybridization to the probe array.

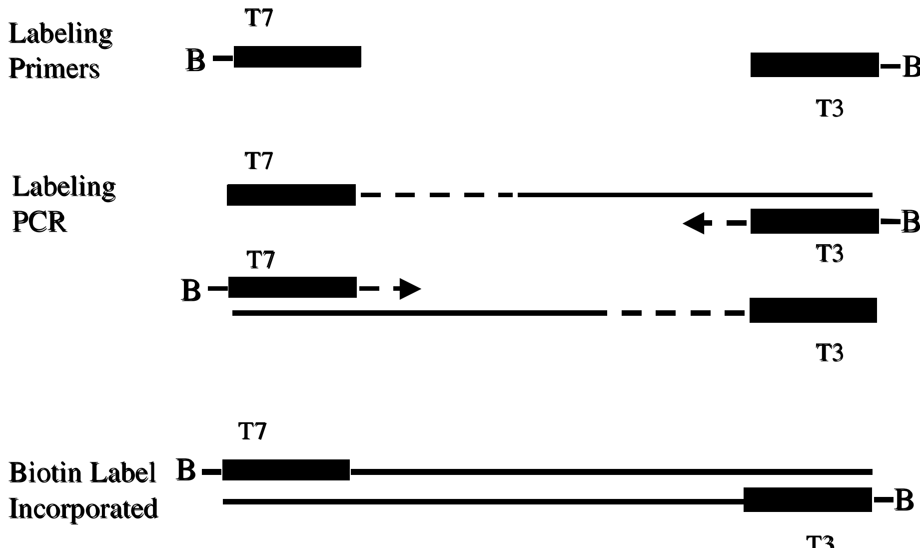

Figure 1.3 Labeling PCR diagram

The biotinylated T7 and T3 primers target the common T7 and T3 sequences at the ends of the multiplex PCR products; consequently, all of the products from the labeling PCR have a biotin on their 5´ ends.

PRECAUTIONS

1. FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES.

2. Avoid microbial contamination, which may cause erroneous results.

WARNING

All biological specimens and materials with which they come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. This includes adherence to the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) for blood-derived and other samples governed by this act. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.

3. **CAUTION:** Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic reagents.

4. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.

5. Use powder-free gloves whenever possible to minimize introduction of powder particles into sample or probe array cartridges.

TERMINOLOGY

1. **Probes:** The oligonucleotides synthesized on the surface of the probe arrays are called probes because they probe or interrogate the sample.

2. **Target:** The target is the labeled PCR product that is being interrogated. It is hybridized to the probes on the array.

3. **Probe Cell:** Specific areas on the probe array that contain multiple copies of a unique oligonucleotide.

INTERFERING CONDITIONS

Wear powder-free gloves throughout procedure. Take steps to minimize the introduction of exogenous nucleases. Water used in the protocols is molecular biology grade.

Proper storage and handling of reagents and samples is essential for robust performance.

All instruments used during this procedure should be calibrated and carefully maintained to ensure accuracy, as incorrect measurement of reagents may affect the outcome of the procedure.

INSTRUMENTS

The Affymetrix® GeneChip® HuSNP™ Mapping Assay is designed for use in a system consisting of a GeneChip Fluidics Station 400, a GeneChip Hybridization Oven, and an Agilent GeneArray™ Scanner.

REFERENCES

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2. Pease AC, Solas DM, Sullivan EJ, Cronin MT, Holmes CP, Fodor SPA. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc Natl Acad Sci*. 1994;91:5022-5026.
3. Cronin MT, Fucini RV, Kim SM, Masino Richard, Wespi RM, Miyada CG. Cystic fibrosis mutation detection by hybridization to light-generated DNA probe arrays. *Human Mutation.* 1996;7:244-255.
4. Wang DG, Fan J-B, et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science.* 1998;280:1077-1082.
5. Kwok SY, Higuchi RG. Avoiding false positives with PCR. *Nature*.1989;339:237-238.

WEB SITES

1. <<http://www.ncbi.nlm.nih.gov/SNP/ >>

   National Center for Biotechnology Information. Web site contains information on all SNPs used on the HuSNP™ array.

2. <<http://www.genome.wi.mit.edu>>

   Whitehead Institute for Biomedical Research/MIT Center for Genome Research web site contains information on map and sequence releases, software, and people at the Genome Center.

3. <<http://www.resgen.com>>

   Research Genetics, Inc. web site contains genome resources.

4. <<http://www.cidr.jhmi.edu/main.html>>

   Center for Inherited Disease Research web site provides genotyping and statistical genetics services for investigators seeking to identify genes that contribute to human disease.

5. <<http://www.rockefeller.edu>>

   Rockefeller University web site provides information on the latest scientific discoveries and other university information.

LIMITATIONS

- The results of the protocols are dependent upon the proper handling of nucleic acids and reagents.
- The results should be evaluated by a qualified individual.

PCR AMPLIFICATION OF DNA SAMPLES

2

REAGENTS AND MATERIALS REQUIRED

The following reagents and materials are recommendations only. For supplier phone numbers in the U.S., please refer to the Supplier and Reagent Reference List in Appendix A of this manual. Information and part numbers listed are based on U.S. catalog information. Affymetrix does not guarantee that licenses are not required to use these products. Additional reagents that are needed for the complete analysis are listed in the appropriate chapters. Appendix A contains a master list of all reagents used in this manual.

Do not store enzymes in a frost-free freezer.

Assay Components Required from GeneChip® HuSNP™ Reagent Kit

- Multiplex Primer Pools 1-24
- Labeling Primer, Biotin-T3
- Labeling Primer, Biotin-T7
- HuSNP™ Reference DNA (4.0 ng/ml)

Materials to Order

PCR Amplification

- 10X Buffer II*, Perkin Elmer, P/N N808-0245
- 25 mM $MgCl_2$*, Perkin Elmer, P/N N808-0245
- AmpliTaq® Gold*, Perkin Elmer, P/N N808-0245
- Stock dNTPs, Pharmacia Biotech, P/N 27-2035-01
- Water, Molecular Biology Grade, BioWhittaker, P/N 16-001Y

* supplied together as AmpliTaq® Gold, 10X Buffer II, and $MgCl_2$, Perkin Elmer

Equipment/Disposables Required

PCR Clean Room

- Thin wall tubes, 0.2 mL, (strip of 8), MJ Research, Inc., P/N TBS-0201
- Caps for 0.2 mL tubes (strip of 8), MJ Research, Inc., P/N TCS-0801
- 96-well polypropylene v-bottom microplates, MJ Research, Inc., MLP-9611
- Microseal 'A' film, MJ Research, Inc., MSA-5001
- Pipets, P-2, P-10, P-20, P-200, P-1000
- Vortex Mixer
- Microcentrifuge
- Microcentrifuge tubes, 1.5 mL
- Multichannel pipets, 1-10 µL
- Deep Well Titer plates, 1 mL, Beckman, P/N 267004
- Seal and Sample aluminum foil lids, Beckman, P/N 538619
- Tube rack
- Pipet tips - barrier

PCR Staging room

- Pipets –P-2, P-10, P-20, P-200, P-1000
- Tube rack
- Multichannel pipets, 5-50 µL
- Swing-bucket centrifuge
- Vortex mixer

Main Laboratory (Low, Medium, and High Copy Areas)

Low Copy Area

- Seal and Sample aluminum foil lids, Beckman, P/N 538619
- Multichannel pipets, 1-10 µL
- Pipet, P-10
- Vortex mixer

Medium Copy Area

- Multichannel pipets, 1-10 µL
- Pipet, P-2
- Vortex mixer

High Copy Area

- Thermal Cycler, MJ Research DNA Engine or Tetrad; or Perkin Elmer 2400, 9600, or 9700
- Microcon®-10 (microconcentrators), Millipore, P/N 42406
- Pipets, P-2, P-10, P-20, P-200, P-1000
- Microcentrifuge tubes, 1.5 mL, USA Scientific, P/N 1415-2600
- Microcentrifuge

- Vortex mixer
- Gel apparatus
- 4% NuSieve® 3:1 Plus Agarose gel (24 well format), FMC Bioproducts, P/N 54928
- 20X TBE Stock Solution, BioWhittaker, P/N 16-012Y
- Loading dye
- 100 bp DNA ladder, GIBCO BRL, P/N 15628

REAGENT PREPARATION

PCR dNTP Mix

Add 25 uL of each 100 mM dNTP stock solution to 900 uL of molecular biology grade water, for a final concentration of 2.5 mM each dNTP.

Store frozen at (minus) -20°C.

GENOMIC DNA EXTRACTION

Because of the highly complex composition of these reactions, successful PCR yields and consistent genotype assignment will benefit from using the highest quality DNA possible, well-purified from PCR inhibitors and other sources of interference. Genomic DNA can be extracted from samples such as blood, buccal swabs, etc., using standard laboratory techniques. Although the following commercial kits and extraction services have yielded generally good results in our work at Affymetrix, they have not been qualified extensively for compatibility with this assay. Be sure to read all the instructions that accompany any commercial kits.

Since the genomic DNA will subsequently be amplified by PCR, the extraction procedures should not be carried out in areas of the laboratory that are designated for the PCR procedures described below. Take steps to prevent the introduction of previously amplified PCR products. The Phone numbers for the following companies are listed in Appendix 1, List of Suppliers and Reagents.

DNA Extraction Kits

- PureGene®, Gentra Systems, Inc., P/N D-5500
- Krystal®, Cambridge Molecular Technologies, P/N 508333
- Wizard®, Promega, P/N A1120
- QIAmp®, QIAGEN, P/N 29304

Extraction Service

- Nucleic Acid Purifications (800) 711-2090

PREPARING WORKING CONCENTRATION OF GENOMIC DNA

1. Determine the concentration of DNA by absorbance at 260 nm using standard spectrophotometric methods.
2. Prepare Working Stocks of sample genomic DNA. Dilute at least 135 ng of the extracted DNA to a working concentration of 4.0 ng/µL in nuclease-free water (or other appropriate diluent such as 0.25x TE).
3. Store the Working Stocks of sample DNA, along with the HuSNP™ Reference DNA, at 4°C in the PCR Staging room (a designated room described in the section, *Running the Multiplex PCR Amplification*).

SETTING UP THE MULTIPLEX AND LABELING PCRs

Location: PCR Clean Room

IMPORTANT

Absolutely NO template DNA or PCR should be allowed into this designated room; we recommend gloves and gowns to minimize the risk of PCR carryover.

Setting Up the Multiplex PCR

Overview – For each genomic DNA sample, set up 24 amplification reaction tubes, each containing one of the 24 Multiplex Primer Pools. Next, prepare PCR Master Mix I, which contains Taq Polymerase, dNTPs and buffer. For each genomic DNA sample, aliquot enough of the mix to run 24+ reactions.

Optional – We recommend running an additional set of 24 reactions for the HuSNP™ Reference DNA (4.0 ng/ µL), as a control set for the PCR amplifications and subsequent hybridization to HuSNP probe arrays.

1. To efficiently process multiple samples with the minimum risk of liquid handling errors, we highly recommend the use of amplification reaction tubes in a 96-well plate or 8-tube strip format. For each genomic DNA sample, label or identify a set of 24 reaction tubes as #1 through #24, representing each Multiplex Primer Pool.

2. In a PCR clean room, dispense 3.0 µL of each Multiplex Primer Pool into the appropriately labeled amplification reaction tube.

3. Use the following table (Table 2-1) to calculate the necessary volumes for preparing PCR Master Mix I. The multiplex amplification reaction volume is 12.5 µL. For each genomic DNA sample, prepare enough mix for 28 reactions (enough for more than 24) to compensate for pipetting inaccuracies. When running multiple genomic DNA samples, prepare enough master mix for 28 times the number of samples.

Table 2.1 PCR Master Mix I (for one DNA sample)

Stock Solution	1 Reaction volume (µL)	28 Reaction volume (µL)	Final Concentration
10X Buffer II	1.25	35	1X
25 mM $MgCl_2$	2.5	70	5 mM
2.5 mM dNTPs	2.5	70	0.5 mM
5 U/µL AmpliTaq® Gold	0.25	7	1.25 U/12.5 µL reaction
Water	1.75	49	
Total	8.25	231	

4. In a PCR Clean room, mix together the components of the PCR Master Mix I in a microcentrifuge tube. Vortex thoroughly to mix.
5. If running multiple genomic DNA samples, aliquot 222.75 µL of the PCR Master Mix I (enough for 27 reactions) to microcentrifuge tubes for each DNA sample.

Setting Up the Labeling PCR (Part 1)

Overview – For each genomic DNA sample, setup a second set of 24 amplification reaction tubes and prepare PCR Master Mix II, which contains biotinylated T3 and T7 primers, dNTPs and buffer. Although the Taq Polymerase may be included in the Master Mix II, our recommendation is to add the enzyme just before actually running the Labeling PCR.

1. For each genomic DNA sample, label or identify a second set of 24 reaction tubes as #1 through #24. Clearly mark these tubes for labeling PCR to distinguish them from the first set of 24 tubes used for the multiplex PCR.

2. Use the following table (Table 2-2) to calculate the necessary volumes for preparing PCR Master Mix II for the labeling amplification. The labeling amplification reaction volume is 25.0 µL. For each genomic DNA sample, prepare enough mix for 28 reactions (enough for more than 24), to compensate for pipetting inaccuracies. When running multiple genomic DNA samples, prepare enough mix for 28 times the number of samples.

Table 2.2 PCR Master Mix II (for one DNA sample)

Stock Solution	1 Reaction volume (µL)	28 Reaction volume (µL)	Final Concentration
10x Buffer II	2.5	70	1X
25 mM $MgCl_2$	4	112	4 mM
2.5 mM dNTPs	4	112	0.4 mM
10 µM bio-T7 primer	2	56	
10 µM bio-T3 primer	2	56	0.8 µM
5 U/µL AmpliTaq® Gold	0.5	14	2.5 U/25 µL reaction
Water	7.5	210	
Total	22.5	630	

3. In a PCR Clean room, mix together the components of the PCR Master Mix II in a microcentrifuge tube. Vortex thoroughly to mix.

   As a precaution to maintain optimal enzymatic activity, we recommend leaving the Taq Polymerase out of the mix at this point and instead dispense the required volume of Taq Polymerase as a Working Stock in a separate tube.

4. Proceed to the PCR Staging room, bringing along the aliquots of PCR Master Mix I, PCR Master Mix II, Taq Polymerase Working Stock, and the two sets of amplification reaction tubes.

RUNNING THE MULTIPLEX PCR AMPLIFICATION

Location: PCR Staging room

IMPORTANT

PCR product should NEVER be allowed into this second designated room; we recommend gloves and gowns to minimize the risk of PCR carryover.

Overview – Add genomic DNA (enough for 24+ reactions) to an aliquot of the PCR Master Mix I and dispense into the set of 24 reaction tubes containing the Multiplex Primer Pools. At this point the reaction tubes will contain all of the components necessary for the SNP-specific multiplex amplification.

1. During the multiplex PCR, store the PCR Master Mix II at 4°C and the Working Stock of Taq Polymerase at -20°C in the PCR Staging room.

2. In the PCR Staging room, for each genomic DNA sample, add 33.75 µL of 4.0 ng/µL genomic DNA (enough for 27 reactions) to a 222.75 µL aliquot of PCR Master Mix I. Vortex thoroughly to mix.

3. Dispense 9.5 µL of the solution containing sample DNA and PCR Master Mix I to each of the 24 amplification reaction tubes containing the 3 µL of Multiplex Primer Pools.

   Each of the reaction tubes now contain 5 ng of genomic DNA, the SNP-specific primers at a concentration of 50 nM, and all the other components necessary for the multiplex PCRs in a final volume of 12.5 µL.

4. Seal tubes with caps.

5. Gently vortex the 96-well plate or 8-tube strips to thoroughly mix the reaction components.

6. Spin down at low speed (<2000 rpm) in a swinging-bucket centrifuge (use plate adapters if necessary).

7. Proceed to the main laboratory, bringing along the multiplex amplification reaction tubes.

Location: Main Laboratory

The PCR cyclers should be placed in the main laboratory.

1. Start the amplification reaction in the thermal cycler using the following protocol (Table 2.3).

Table 2.3 PCR Thermal Cycling Protocol for Multiplex Amplification (for MJ and Perkin Elmer Thermal Cyclers)

Step	Temperature	Time	Number of Cycles
Denaturing	95°C	5 minutes	1
Cycling with temperature ramp	95°C 52°C 72°C	30 seconds 55 seconds* (auto + 0.2°C/cycle) 30 seconds	30
Cycling	95°C 58°C 72°C	30 seconds 55 seconds* 30 seconds	5
Final Extension	72°C	7 minutes	1
Soak	4°C	Hold	

*50 seconds on Perkin Elmer Thermal Cycler

SETTING UP THE LABELING PCR (PART 2)

Location: PCR Staging room

IMPORTANT

PCR product should NEVER be allowed into this second designated room; we recommend gloves and gowns to minimize the risk of PCR carryover.

1. When the multiplex PCR is nearly finished, return to the PCR Staging room and add in the Taq Polymerase to complete the preparation of the PCR Master Mix II.
2. While still in the PCR Staging room, dispense 22.5 µL of Master Mix II to all of the amplification reaction tubes previously set up for the Labeling PCR in Part 1.
3. Proceed to the main laboratory, bringing along the amplification reaction tubes containing PCR Master Mix II.

DILUTING THE MULTIPLEX PCR AMPLIFICATION

Location: Main Laboratory

Designate three separate areas of the main laboratory as Low, Medium, and High Copy areas. The High Copy area should be near the PCR cyclers. Ideally, the Low and Medium Copy areas should be far away from the High Copy area and cyclers.

Overview– The multiplex amplification reactions will be diluted 1000-fold in nuclease-free water. The 1:1000 dilution effectively lowers the concentration of SNP-specific primers in the labeling PCR as well as in the subsequent hybridization to the probe array.

1. Place the amplification reaction tubes containing PCR Master Mix II in a designated Low Copy area of the main laboratory away from the PCR cyclers.
2. To simultaneously dilute the 24 multiplex amplification reactions, we recommend using 96 x 1 mL deep-well titer plates (available from Beckman). Fill 24 wells with 1.0 mL nuclease-free water.

   To minimize the risk of PCR carryover, our recommended practice is to fill deep-well titer plates with water in the PCR Clean Room beforehand, and continually maintain a stock of these plates out in the main laboratory. Once filled with water, the plates should be covered with aluminum foil lids before leaving the PCR Clean Room.
3. When the multiplex PCR is complete, spin down the multiplex amplification reactions at low speed (<2000 rpm) in a swinging-bucket centrifuge.
4. In a designated Medium Copy area of the main laboratory, carefully remove the caps from the tubes.
5. Using a 1-10 µL 8-channel pipette fitted with barrier plugged tips, transfer 1.0 µL of multiplex amplification reactions #1 to #8 to a row of 8 deep wells containing 1.0 mL water. Repeat for reactions #9 to #16, and reactions #17 to #24.
6. Seal the wells with an aluminum foil lid. Make sure that all of the wells are tightly sealed.
7. To thoroughly mix the PCRs in the water, gently vortex the plate.
8. Spin down the plates at low speed (<2000 rpm) in a swinging-bucket centrifuge fitted with plate adapters.

RUNNING THE LABELING PCR

Location: Main Laboratory

Overview – Since the SNP-specific primers in the multiplex primer pools also contain common T3 and T7 sequences, the biotinylated T3 and T7 primers in this second round of PCR will amplify and label all of the diluted multiplex PCR products from the first round of PCR.

1. In a designated Low Copy area of the main laboratory, carefully remove the foil from the wells. Alternatively, using an 8-channel pipette fitted with barrier plugged tips, firmly puncture the foil. This method will deform and damage the tips, so immediately discard them.

2. In the Low Copy area of the main laboratory, use a 1-10 µL 8-channel pipette fitted with barrier plugged tips to dispense 2.5 µL of the 1:1000 dilutions (from the section, *Diluting the Multiplex PCR Amplification*) of reactions #1 to #8 into the appropriate amplification reaction tube. Repeat for reactions #9 to #16, and reactions #17 to #24.

3. Seal tubes with caps.

4. Spin down at low speed (<2000 rpm) in a swinging bucket centrifuge (use plate adapters if necessary).

5. Start amplification reaction in the thermal cycler using the following protocol (Table 2.4).

Table 2.4 PCR Thermal Cycling Protocol for Labeling Amplification (for MJ or Perkin-Elmer Thermal Cyclers)

Step	Temperature	Time	Number of Cycles
Denaturing	95°C	8 minutes	1
Cycling	95°C 55°C 72°C	30 seconds 90 seconds 30 seconds	40
Final Extension	72°C	7 minutes	1
Soak	4°C	Hold	

POOLING AND CONCENTRATING THE LABELED DNA

Location: Main Laboratory

Overview – The 24 labeling reactions are pooled and concentrated (from approximately 600 µL down to 60 µL) for efficient hybridization to the probe arrays.

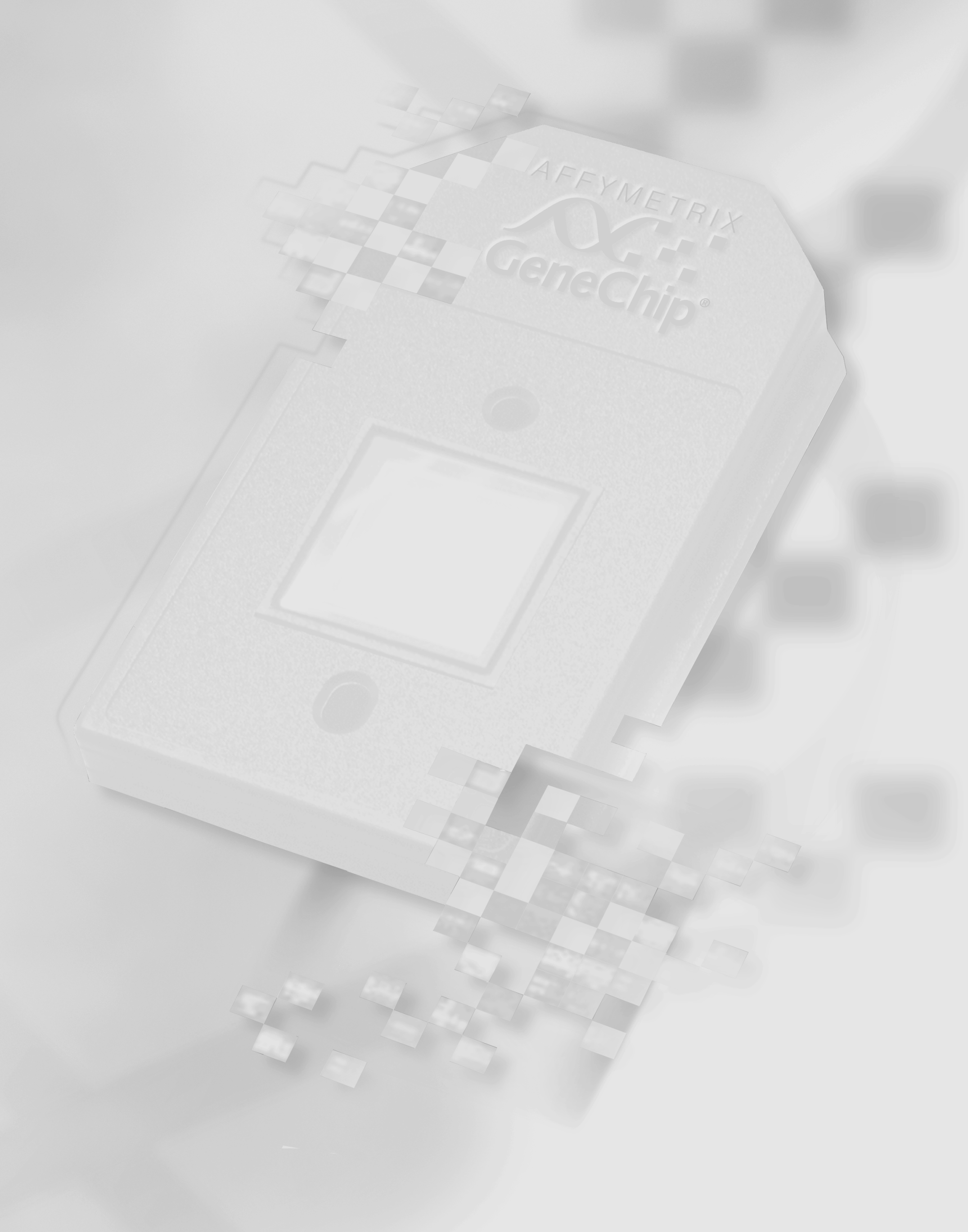
Optional – To confirm the PCR amplifications, in the High Copy area of the main laboratory, run 1.5 µL from each of the 24 labeling PCRs in a 4% NuSieve® agarose gel. A closely clustered family of bands running at approximately 100 bp should be visible in all 24 lanes of the gel(s). We recommend using the 100 bp Ladder available from GIBCO BRL for size markers.

1. In the High Copy area of the main laboratory, remove 23 µL of PCR product from each of the 24 PCR reactions and pool all the reactions together.

2. Concentrate samples in two Microcon®-10 microconcentrators by centrifuging at 13,000x g for 20 minutes at room temperature, or until the volume is reduced by at least ten-fold. Recover the sample by reversing the filters and

spinning at 3,000 xg for 3 minutes. Combine the sample and adjust the volume by adding water to 60 µL.

3. Aliquot 30 µL of the concentrated sample for the hybridization and store any remaining sample at (minus) -20°C.


AFFYMETRIX
GeneChip®

3

TARGET HYBRIDIZATION

REAGENTS AND MATERIALS REQUIRED

The following reagents and materials are recommendations only. For supplier phone numbers in the U.S., please refer to the Supplier and Reagent Reference List, Appendix A of this manual. Information and part numbers listed are based on U.S. catalog information. Affymetrix does not guarantee that licenses are not required to use these products. Additional reagents that are needed for the complete analysis are listed in the appropriate chapters. Appendix A contains a master list of all reagents used in this manual.

Required Assay Components

- Control Oligonucleotide B1 (from GeneChip® HuSNP™ Reagent Kit)
- GeneChip® HuSNP™ Probe Arrays

Required Reagents

- TMAC, Sigma, P/N T3411
- 1 M Tris-HCl, pH 7.8, Sigma, P/N T-2913
- Tween® 20, Pierce Chemical, P/N 28320
- 0.5 M EDTA, pH 8.0, Gibco BRL, P/N 15575-038
- 50X Denhardt's Solution, Sigma, P/N D-2532
- Herring Sperm DNA, Promega, P/N D1815
- Water, Molecular Biology Grade, BioWhittaker, P/N 16-001Y

Required Equipment

- GeneChip® Hybridization Oven

Miscellaneous Supplies

- Heat block
- Ice machine
- Microcentrifuge tubes (1.5 mL), VWR, P/N 2090122-641
- Micropipettors, (P-2, P-20, P-200, P-1000), Rainin Pipetman or equivalent
- Sterile-barrier pipet tips and non-barrier pipet tips (Tips must be pointed, not rounded, for efficient use with the probe arrays.)

REAGENT PREPARATION

Prepare the following stock solution.

1.0% Tween® 20

Prepare a 1% Tween® 20 solution in water. Mix thoroughly and filter through a 0.2 µM filter. Store at room temperature.

TARGET HYBRIDIZATION

1. Prepare the Hybridization Mix (Table 3.1). The volumes given are sufficient for one sample.

   For each genomic DNA sample, combine the following components in a microcentrifuge tube (Table 3.1).

   If running multiple samples, prepare enough Hybridization Mix for one additional sample to compensate for pipetting inaccuracies, and aliquot 105 µL of the mix into microcentrifuge tubes.

Table 3.1 Hybridization Mix

Stock Solution	Volume (µL)	Final Concentration
5M TMAC	81	3 M
Control Oligonucleotide B1	1.35	2 nM
1M Tris-HCl, pH 7.8	1.35	10 mM
1% Tween® 20	1.35	0.01%
0.5 M EDTA, pH 8.0	1.35	5 mM
10 mg/mL herring sperm DNA	1.35	100 µg/mL
50X Denhardt's solution	13.5	5X
Water	3.75	
Final volume	105	

2. Dispense 30 µL of the concentrated labeled DNA (From the section, Pooling and Concentrating the Labeled DNA on page 22) to the 105 µL aliquot of Hybridization Mix. The final volume of the hybridization sample is 135 µL.

3. Denature samples at 95°C for 5 to 10 minutes. Place tube immediately in ice/water mix. Incubate for 2 minutes on ice. Spin briefly in microcentrifuge.

4. Insert a P2/10 nonbarrier pipet tip into the upper septum on a HuSNP™ probe array. This is a vent (See Figure 3.1). Using a 250 µL nonbarrier pipet tip, draw up the hybridization sample. Mix sample up and down a few times in the sample tube to resuspend any material that dropped out of solution and inject the sample into the bottom septum of the probe array.

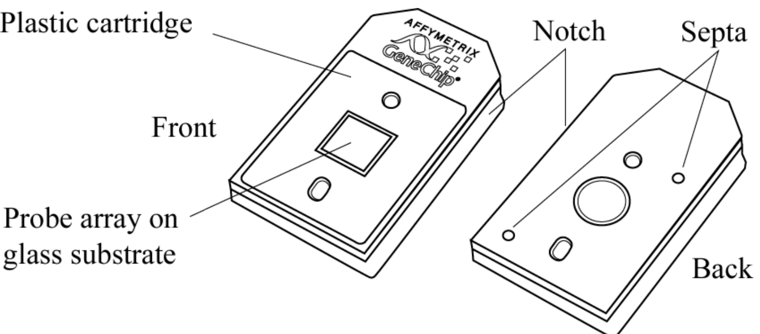


Figure 3.1 The GeneChip® probe array

5. Hybridize the probe array in a GeneChip® Hybridization Oven at 44°C overnight (about 16 hours), rotating at 40-50 rpm on the rotisserie.

WASHING, STAINING AND SCANNING

4

REAGENTS AND MATERIALS REQUIRED

The following reagents and materials are recommendations only. For supplier phone numbers in the U.S., please refer to the Supplier and Reagent Reference List, Appendix A of this manual. Information and part numbers listed are based on U.S. catalog information. Affymetrix does not guarantee that licenses are not required to use these products. Additional reagents that are needed for the complete analysis are listed in the appropriate chapters. Appendix A contains a master list of all reagents used in this manual.

Required Reagents

- Water, Molecular Biology Grade, BioWhittaker, P/N 16-001Y
- R-Phycoerythrin Streptavidin, Molecular Probes, P/N S-866
- 20X SSPE (3M NaCl, 0.2 M NaH_2PO_4, 0.02M EDTA), BioWhittaker, P/N 16-010Y
- 50X Denhardt's Solution, Sigma, P/N D-2532
- Anti-streptavidin antibody (goat), biotinylated, Vector Laboratories, P/N BA-0500
- Triton® X-100, Mallinkrodt, M282-01
- Tween® 20, Pierce Chemical, P/N 28320

Required Equipment

- GeneChip® Fluidics Station 400
- Agilent GeneArray™ Scanner

Miscellaneous Supplies

- Micropipettors, (P-2, P-20, P-200, P-1000), Rainin Pipetman or equivalent
- Sterile-barrier pipet tips and non-barrier pipet tips (Tips must be pointed, not rounded, for efficient use with the probe arrays)
- Microcentrifuge tubes, 1.5 mL
- 0.2 µm filters
- Tygon® Tubing, 0.04″ inner diameter, Cole-Palmer, P/N H-06418-04

Miscellaneous Reagent

- Bleach (5.25% Sodium Hypochlorite) VWR, P/N 21899-504

REAGENT PREPARATION

1. Wash Buffer A, 6X SSPE, 0.01% Triton® (1000 mL)

   Add 300 mL of 20X SSPE and 50 µL of 100% Triton® X-100 to 700 mL of water. Mix thoroughly and filter through a 0.2 µM filter. Store at room temperature.

2. Wash Buffer B, 4X SSPE, 0.01% Triton® (1000 mL)

   Add 200 mL of 20X SSPE and 50 µL of Triton® 100% X-100 to 800 mL of water. Mix thoroughly and filter through a 0.2 µM filter. Store at room temperature.

EXPERIMENT AND FLUIDICS STATION SETUP

File Locations Settings

There are three file locations when Microarray Suite is run in disk files mode and experiment data are stored on disk files. There are four file locations when Microarray Suite is run in LIMS mode and experiment data are stored on a network drive.

The file location paths determine where Microarray Suite looks for the files needed to run a fluidics protocol or analyze data from a particular probe array type, and specifies where to save experiment data. Some of the default file locations may be changed and any modifications made by one user (identified by the logon name) do not affect the file locations of other users.

Note

The procedures outlined in this manual are for running Microarray Suite in Disk File mode only. For LIMS mode, please refer to Affymetrix® Microarray Suite User's Guide.

1. Select **Tools** → **Defaults** from the main menu.

   ⇒ The Defaults dialog box opens.

2. Click the **File Locations** tab (Figure 4.1).

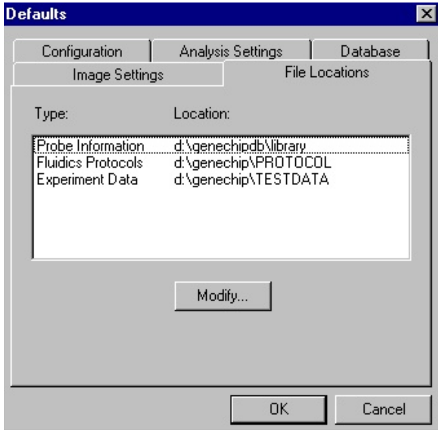


Figure 4.1 Defaults dialog box, File Locations tab.

3. To modify a path, highlight the file type of interest and then click **Modify** (or double-click the file type).
   ⇒ The Modify Location dialog box opens (Figure 4.2).

Note

The paths on the network server for Probe Information and Experiment Data cannot be modified.

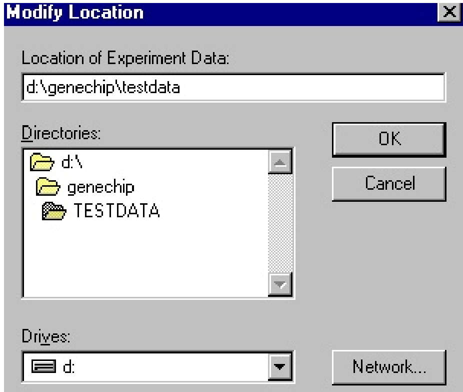


Figure 4.2 Modify Location dialog box

4. Select a drive from the **Drives** drop-down list. Then select the desired directory path in the **Directories** field.

Note

Microarray Suite can detect a network drive and writes a local temporary image data file (*.dat). This file is deleted *after* the scan image has been transferred to the network drive.

5. Click **Network** to map a network drive that is not already mapped. Network drives may also be mapped using Windows Explorer.

6. Click **OK** to close the Modify Location dialog box. Then click **OK** to close the Defaults dialog box.

Microarray Suite Files

There are three types of Microarray Suite files (see Appendix D for more information about the file types):

- Probe Information or *library* files are unique for each probe array type and contain information about the probe array design characteristics, scanning parameters, and default analysis parameters.
- Fluidics Protocol files define the hybridization, wash, or stain protocols run by the GeneChip®Fluidics Station 400.
- Experiment Data files include the *.exp created by the user when an experiment is defined and other file types Microarray Suite generates during an analysis (for example, *.dat, *.cel, *.chp).

SETTING UP AN EXPERIMENT

After the target is prepared, an experiment must be defined in Microarray Suite before the probe array can be processed in the fluidics station or scanned.

To set up an experiment, the user specifies a name, the probe array type, and other relevant information that defines an experiment. Microarray Suite relies on this experiment information to direct the fluidics station and scanner, and apply the correct cell and data analysis algorithms.

Setting Up an Experiment in Disk Files Mode

1. Click the **Experiment Info** button in the shortcut bar or main toolbar. Alternatively, select **File** → **New Experiment (Ctrl+N)** from the main menu.
   ⇒ The Experiment Information dialog box opens (Figure 4.3).
2. In the **Experiment Info** tab, enter a unique **Experiment** (file) **Name** for the probe array (up to 230 alphanumeric characters) and make a selection from the **Probe Array Type** drop-down list. This information is required by Microarray Suite.

The experiment name also serves as the file name for subsequent data files generated during the analysis. (See Appendix D for a description of Microarray Suite file types).

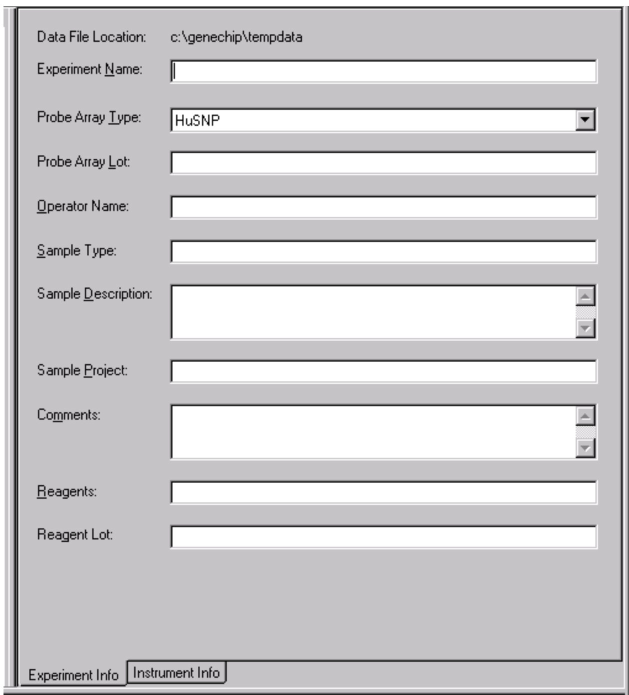


Figure 4.3 Experiment Information (disk files mode)

3. Enter the remaining information as desired to help track pertinent information about the experiment.

4. When finished entering the experiment information, click the **Save** button in the main toolbar or select **File** → **Save** from the main menu.

   ⇒ This creates an experiment information file (*.exp) in the current experiment data directory and displays the *.exp name in the data file tree.

Microarray Suite uses the information in the *.exp file to identify the probe array type and establish the file name under which to record hybridization and scanning parameters.

The Data File Location in the Experiment Information dialog box (for example, c:\genechip\tempdata in Figure 4.3) displays the directory location for the *.exp file and other types of experiment data files generated during the analysis.

5. Click the **Instrument Info** tab to view information about the fluidics protocol and scanning parameters captured by Microarray Suite after the probe array is processed in the fluidics station and scanned (Figure 4.4).
   - This page is blank until the probe array has been processed in the fluidics station.

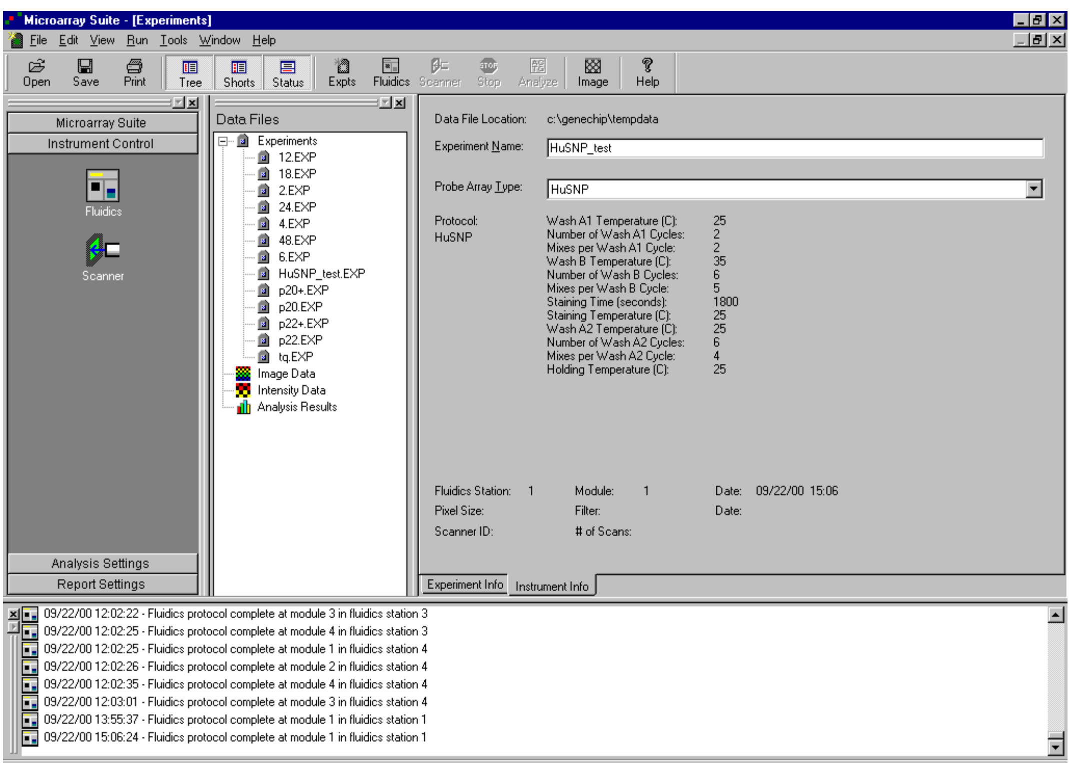


Figure 4.4 Hybridization and scan information

6. To close the Experiment Information dialog box, click the **Close** button or select **File** → **Close** from the main menu.

Entering Experiment Information

To wash, stain, and scan a probe array, an experiment first must be defined in Affymetrix® Microarray Suite.

1. Select **Experiment Info** from the **Run** menu. Alternatively, click the New Experiment icon on the tool bar.

   The Experiment Information dialog box will appear allowing the experiment name to be defined along with several other parameters such as probe array type, sample description, and comments.

2. Type in the **Experiment Name**. Click the down arrow and select the **Probe Array Type** from the drop-down list.

   Experiment Name and **Probe Array Type** are required. Complete as much of the other information as desired. The protocol information at the bottom of the dialog box will be imported to the experiment information dialog box after the hybridization and scan have been completed.

3. Save the experiment by choosing **Save**.

   The name of the experiment will be used by Microarray Suite to access the probe array type and data for the sample while it is being processed. Data files generated for the sample will be automatically labeled to correspond to the experiment name. Affymetrix® Microarray Suite software automatically fills in the **Protocol** section of this dialog box with information on array processing from the fluidics station.

4. Close the Experiment Information dialog box.

Configuring the Fluidics Station

Before running a fluidics protocol, check to make sure the fluidics station(s) is properly configured.

1. Select **Tools** → **Defaults** from the main menu.
   ⇒ The Defaults dialog box opens (Figure 4.5).
2. Click the **Configuration** tab.
3. Confirm that the number of **Fluidics Stations Installed** is correct or enter a new value.

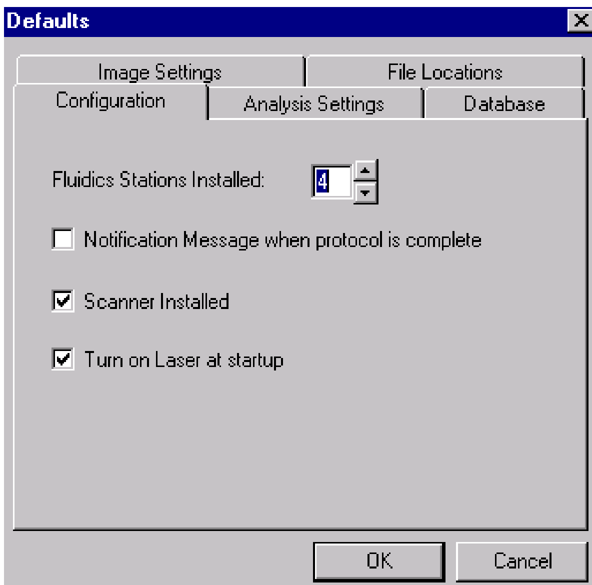


Figure 4.5 Defaults dialog box, Configuration tab

4. If desired, checkmark **Notification Message when protocol is complete** to display a notification message when a fluidics protocol is completed.
5. Click **OK** to close the Defaults dialog box.

Preparing the GeneChip® Fluidics Station 400

Before running fluidics protocols, the GeneChip® Fluidics Station 400 must be primed. This fills the fluidics station lines with appropriate buffer. Priming is performed when the fluidics station is first turned on or when the wash solution is changed, before washing after a shutdown has been performed, or when a module LCD window instructs the user to prime.

1. Click the **Fluidics** button in the Instrument Control section of the shortcut bar or in the main toolbar. Alternatively, select **Run** → **Fluidics** from the main menu.

   ⇒ The Station Selection dialog box opens if more than one fluidics station is installed on the workstation (Figure 4.6).

   ⇒ The Fluidics Station dialog box opens if only one fluidics station is installed on the workstation (Figure 4.7).

2. If more than one fluidics station is installed on the workstation, select the number designation of the current fluidics station from the **Station Number** drop-down list.

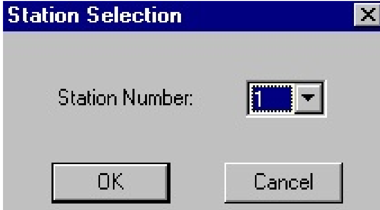


Figure 4.6 Station Selection dialog box

3. Set the fluidics station address designation using the switch block on the back panel of the fluidics station (refer to the *GeneChip® Fluidics Station 400 User's Guide*).

4. Click **OK** to close the Station Selection dialog box.

   ⇒ The Fluidics Station dialog box for the currently selected station automatically opens (Figure 4.7).

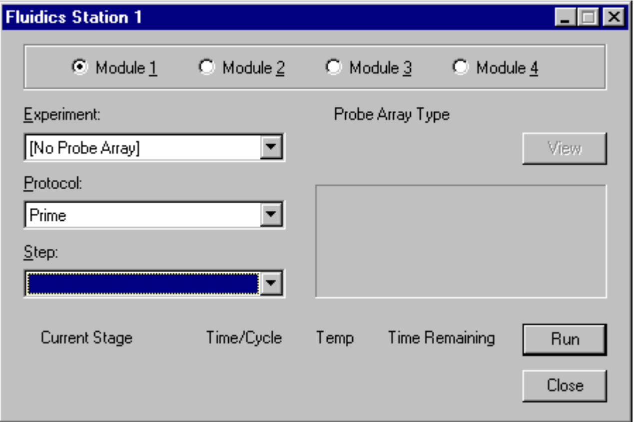


Figure 4.7 Fluidics Station dialog box, Prime protocol selected, disk files mode.

5. Select *Prime* from the **Protocol** drop-down list and *No Probe Array* from the **Experiment** drop-down list for each module to be used.

6. Fill the intake buffer reservoirs A and B with the appropriate priming buffer.

7. Empty the waste bottle and fill the water reservoir with Molecular Biology Grade water.

8. Load an empty, standard 1.5 mL microcentrifuge tube in the sample holder of each module to be primed.

9. Click **Run** for each module to be primed and follow the prompts in the fluidics station dialog box (also shown in the module LCD window).

   The status of the procedure is displayed in the Fluidics Station dialog box and the module LCD window. The fluidics station is ready to use when priming is completed and *Module primed, Ready* appears in the module LCD window.

PROBE ARRAY WASH AND STAIN

1. In the Fluidics Station dialog box (Figure 4.8), select the current fluidics station **Module**.
2. Choose an **Experiment** and the HuSNP™ **Protocol** from the drop-down lists.
3. Click **View** to display information about the selected experiment.
4. Click the **Start Protocol** button to begin the protocol on the selected module.
5. Repeat as necessary for other modules in the fluidics station(s).

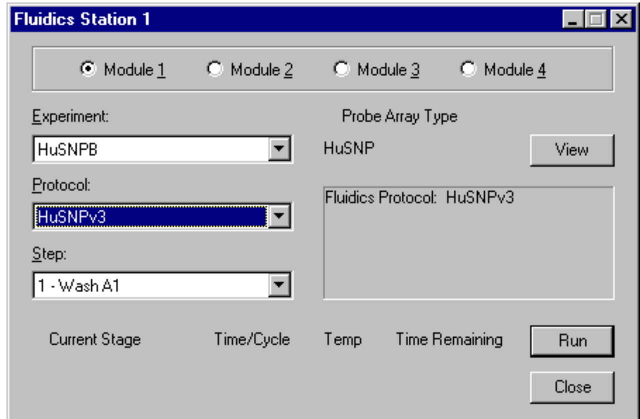


Figure 4.8 Fluidics Station dialog box, disk files mode.

The HuSNP™ protocol includes two post hybridization washes, staining, and a post-stain wash. The first wash consists of 2 cycles of 2 mixes per cycle with Wash Buffer A (6X SSPE) at 25°C. The next wash consists of 6 cycles of 5 mixes per cycle with Wash Buffer B (4X SSPE) at 35°C. The probe array is stained for 30 minutes at 25°C. The final wash consists of 6 cycles of 4 mixes

per cycle with Wash Buffer A (6X SSPE) at 25°C. The holding temperature is 25°C.

Note

The Fluidics Protocol dialog box is accessible from the **Tools** Menu, under **Edit Protocol**.

---

6. Remove any microcentrifuge tube remaining in the sample holder of the fluidics station module(s) being used.

7. Place a fresh microcentrifuge tube into the sample holder. The hybridization sample will be recovered into this tube, so label the tube accordingly.

8. Choose **Run** in the Fluidics Station dialog box to begin the washing and staining. Follow the instructions on the LCD window on the fluidics station.

   If you are unfamiliar with inserting and removing probe arrays from the fluidics station modules, please refer to the *GeneChip® Fluidics Station 400 User's Manual*.

9. Insert the appropriate probe array into the designated module of the fluidics station while the probe array lever is in the Eject position. When finished, make sure that the probe array lever is returned to the Engage position.

   Sensors in the fluidics station detect when the probe array and sample vial holder have been loaded. The process will proceed automatically from this point. The Fluidics Station dialog box and each module LCD window display the status of the procedure.

10. During the washing (approximately 15 minutes), prepare the following Staining Buffer Mix and Working Stock Solution of antibody. Volumes given are sufficient for one probe array.

Staining Buffer Mix

Streptavidin Phycoerythrin (SAPE) should be stored in the dark at 4°C, either foil wrapped or kept in an amber tube. Remove SAPE from refrigerator and tap the tube to mix well before preparing the Staining Buffer Mix. Do not freeze concentrated SAPE or diluted SAPE stain solution. Always prepare the mix immediately before use.

For each probe array to be stained, combine the following components in a microcentrifuge tube (Table 4.1).

Table 4.1 Staining Buffer Mix

Stock Solution	Volume (µL)	Final Concentration
20X SSPE	150	6X
50X Denhardt's solution	10	1X
1% Tween® 20	5	0.01%
1 mg/mL SAPE	25	50 µg/mL
Water	305	
Final volume	495	

If running multiple samples, prepare enough Staining Buffer Mix for one additional sample to compensate for pipetting inaccuracies, and aliquot 495 µL of the mix into microcentrifuge tubes.

Keep the aliquots of Staining Buffer Mix in the dark and on ice (or at 4°C) until needed.

Working Stock of Antibody

For each probe array to be stained, aliquot 5 µL of 0.5 mg/mL biotinylated anti-streptavidin as a Working Stock of antibody. If running multiple samples, aliquot enough antibody for one additional sample to compensate for pipetting inaccuracies. Keep the Working Stock of antibody on ice (or at 4°C) until needed.

1. When the wash is complete, the LCD window will display **REMOVE VIAL**. Remove the microcentrifuge tube containing the hybridization sample from the sample holder.

2. The LCD window will display **LOAD VIAL OF STAIN.**

3. For each probe array that has completed the wash step, dispense 5 µL of the Working Stock of Biotinylated Anti-Streptavidin to a 495 µL aliquot of Staining Buffer Mix. Vortex thoroughly.

4. Place the microcentrifuge tube containing the Staining Buffer Mix with Antibody into the sample holder, making sure that the metal sampling needle is in the tube with its tip near the bottom.

   The Fluidics Station dialog box and the LCD window will display the status of the staining and subsequent washing as they progress.

5. When the stain and post-stain wash are complete, the LCD window will display **EJECT CARTRIDGE**. Remove the microcentrifuge tube containing stain and replace with an empty microcentrifuge tube.

6. Remove the probe arrays from the fluidics station modules by first moving the probe array holder lever to the Eject position. When finished, make sure that the probe array holder is returned to the Engage position.

7. Check the probe array window for large bubbles or air pockets. If bubbles are present, proceed to the inset, *If Bubbles are Present,* on page 48.

   If the probe array has no large bubbles, it is ready to scan on the Agilent GeneArray™ Scanner. Proceed to the section, *Scanning a GeneChip® Probe Array,* on page 49. If there are no more samples to process, shut down the fluidics station following the procedure, *Fluidics Station Procedures,* on page 44.

   Scan the probe arrays immediately, or keep them at 4°C and in the dark until ready for scanning.

FLUIDICS STATION PROCEDURES

Resuming a Fluidics Protocol

Affymetrix® Microarray Suite tracks the progress of a fluidics protocol during a run. If the protocol stops before completion, the protocol may be resumed beginning at the point where it was interrupted.

> **Note** The resume feature is only available for fluidics protocols that display multiple steps in the Step drop-down list of the Fluidics Station dialog box.

1. When ready to resume the protocol, choose the protocol from the **Protocol** drop-down list in the Fluidics Station dialog box.
2. Click **Run** to continue the protocol from the point where it was interrupted.

Bypassing Steps in a Fluidics Protocol

Some multi-step fluidics protocols can be started at any step, so that part of a protocol may be bypassed.

1. Click the **Fluidics** button in the Instrument Control shortcut bar or the main toolbar. Alternatively, select **Run** → **Fluidics Station** from the main menu.
   ⇒ The Fluidics Station dialog box opens (Figure 4.9).

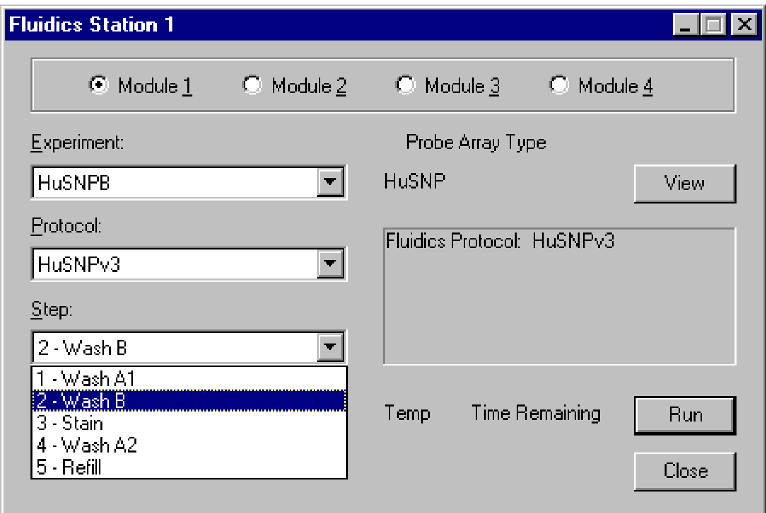


Figure 4.9 Fluidics Station dialog box, bypassing protocol step 1. Disk files mode.

2. Select the desired **Module**, **Experiment**, and **Protocol** from the drop-down lists (Figure 4.9).

Note

The bypass function is only available for fluidics protocols that display multiple steps in the Step drop-down list of the Fluidics Station dialog box.

3. Select the desired beginning **Step** from the **Step** drop-down list.

4. Click **Run** to start the fluidics protocol at the selected step.

Editing a Fluidics Protocol

Some hybridization and wash (Hybwash) protocols may be edited.

Note A Hybwash protocol must be modified before it is run. Protocol changes made during a run do not affect the run in progress.

1. Select **Tools** → **Edit Protocol** from the main menu.

   ⇒ The Fluidics Protocol dialog box opens (Figure 4.10).

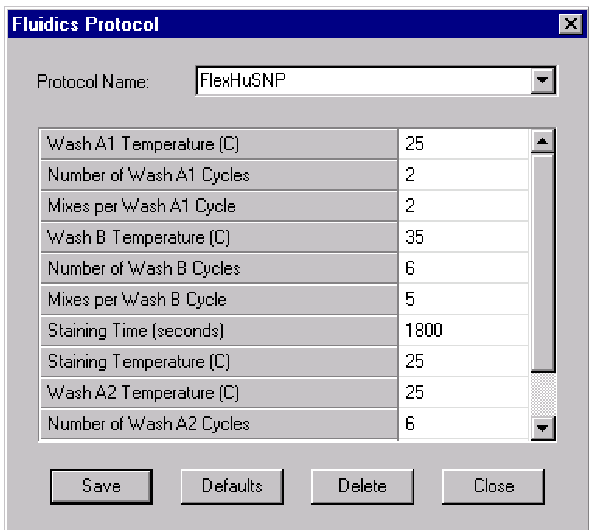


Figure 4.10 Fluidics Protocol dialog box

2. In the **Protocol Name** drop-down list, select FlexHuSNP.

Note Only the protocols in this list may be edited. All others are defined for specific applications and cannot be customized.

3. Highlight the parameter value to be changed and enter the new value. (Parameters values must be within the ranges in Table 4.2.) Enter a Hybridization Time of zero if only a wash is desired. To omit Wash A or B, enter zero for the Number of Wash A or Wash B Cycles.

Table 4.2 Valid ranges for hybridization or stain parameters

Parameter	Valid Range
Wash A1 Temperature (°C)	15 to 50
Number of Wash A1 Cycles	0 to 99
Mixes per Wash A1 Cycle	1 to 99
Wash B Temperature (°C)	15 to 50
Number of Wash B Cycles	0 to 99
Mixes per Wash B Cycle	1 to 99
Stain Time (seconds)	0 to 86399
Stain Temperature (°C)	15 to 50
Wash A2 Temperature (°C)	15 to 50
Number of Wash A2 Cycles	0 to 99
Mixes per Wash A2 Cycle	1 to 99
Holding Temperature (°C)	15 to 50

4. Click **Save** to save the parameters under the same protocol name (the old protocol will be overwritten) or enter a new protocol name in the **Protocol Name** field and then click **Save**. The new protocol name is added to the drop-down list.

5. Click **Defaults** to return the parameter settings to the default values.

6. Click **Delete** to delete the currently selected protocol from the system.

If Bubbles are Present

Return the probe array to the probe array holder. Latch the probe array holder by gently pushing it up until a light click is heard. Engage the washblock by firmly pushing up on the probe array lever to the Engage position.

The fluidics station will drain the probe array and then fill it with a fresh volume of the last wash buffer used. When it is finished, if the LCD window displays EJECT CARTRIDGE again, remove the probe array and inspect it again for bubbles. If no bubbles are present, it is ready to scan. Proceed to the section, *Scanning a GeneChip® Probe Array,* on page 49.

If several attempts to fill the probe array without bubbles were unsuccessful, then the array should be filled with Wash Buffer A manually. Excessive washing will result in a loss of signal intensity. Contact Affymetrix® Technical Support for details on the procedure. Remove the probe array and run the CLEAN procedure on the particular fluidics station module before next use.

THE AGILENT GENEARRAY® SCANNER

Affymetrix® Microarray Suite controls the Agilent GeneArray Scanner and enables the user to initiate a scan, view the intensity data as they are collected during scanning, and analyze the data after the scan is completed. Refer to the *Agilent GeneArray Scanner User's Guide* for a description of the instrument, its components, and set up.

WARNING

Always turn on the scanner first before starting the computer workstation and launching Microarray Suite. Allow the scanner laser 15 minutes of warm up time before scanning a probe array.

Scanning a GeneChip® Probe Array

1. Click the **Start Scan** button in the Instrument Control shortcut bar or in the main toolbar. Alternatively, select **Run** → **Scanner** from the main menu. The default for HuSNP™ is set for a dual scan at 570 and 530 nm.
   ⇒ The Scanner dialog box opens (Figure 4.11).

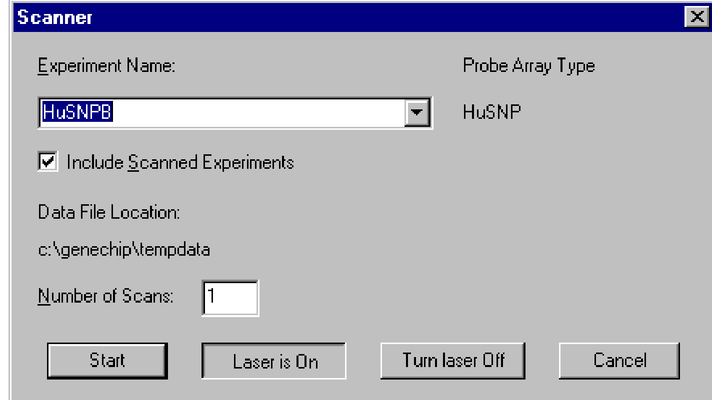


Figure 4.11 Scanner dialog box, disk files mode.

The **Experiment Name** drop-down list displays the experiments (*.exp) that have not been scanned in the current directory (no *.dat file containing scan data exists for the experiments).

After a probe array has been scanned at each wavelength, the scan images are stored in image data files (*A.dat and *B.dat). The corresponding experiment name is listed in the **Scanned Experiment** field.

2. To include scanned experiments in the **Experiment Name** drop-down list, select the **Include Scanned Experiments** option.

3. Select the experiment name of the probe array to be scanned from the **Experiment Name** drop-down list.

   The **Probe Array Type** field automatically displays the probe array type that was entered during experiment setup. The Number and type of Scans is set per probe array type.

Note Increasing the number of scans increases the scan time as well as the amount of fluorophore bleaching and may result in lower fluorescence intensities.

4. If it is necessary to rescan a probe array, select the experiment name from the **Experiment Name** drop-down list.

IMPORTANT

The existing *.dat file will be overwritten when the probe array is rescanned. To retain the original scan data, use the **File** → **Save As** commands from the main menu to save the original *.dat file under a different name.

In disk files mode, an associated *.exp file with the new name is automatically created.

5. Click **Start** in the Scanner dialog box.
   ⇒ The Scanner dialog box opens (Figure 4.12).

Figure 4.12 Scanner dialog box

6. Load the probe array in the scanner and close the scanner door.

7. To view the scanner options, click the **Options** button.
   ⇒ The Scanner Options dialog box opens (Figure 4.13).

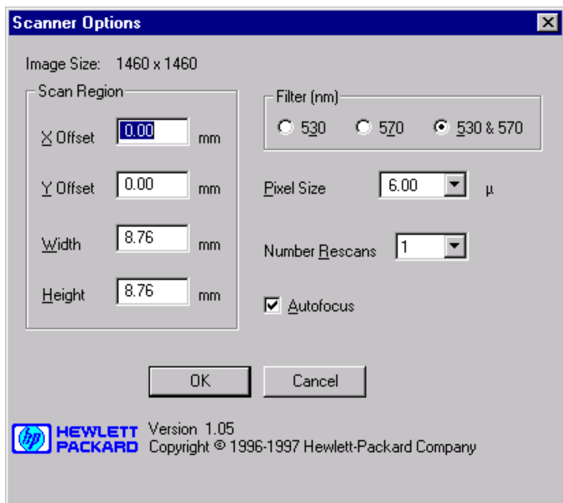


Figure 4.13 Scanner Options dialog box

IMPORTANT

Do not modify the scanner settings. Microarray Suite automatically selects the appropriate settings based on the probe array type specified during experiment setup.

8. Click **OK** to close the Scanner Options dialog box.

9. Click **OK** in the Scanner dialog box or press **Enter** to start scanning the probe array.

   If the **Scan in Progress** feature is enabled, the Image window automatically opens in the main display area when a scan is initiated and displays the fluorescence intensity of the probe array line by line as the scan progresses. To enable (or disable) this option, select **View** from the main menu and place (or remove) a check mark next to **Scan in Progress**.

During a scan, the status log, found at the bottom of the Microarray Suite window, displays messages regarding scanner activity or status. After the scan is completed, the image is stored in an image data file (*.dat) and displayed in the main display area. Additionally, a grid is aligned and a cell intensity file (*.cel) is generated. The *.dat file may be closed and analyzed later.

Stopping a Scan

1. Click the **STOP** button STOP in the main toolbar or select **Run** → **Stop scanner** from the main menu.

   ⇒ A question box appears (Figure 4.14).

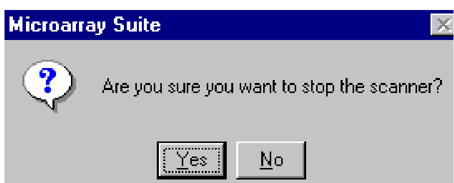


Figure 4.14 Stop scanner prompt

2. At the prompt, click **Yes** to stop the scanner or **No** to resume scanning.

3. After the scan is stopped, the data from a partial scan may be saved. At the prompt (Figure 4.15), click **Yes** to save the data in the *.dat file.

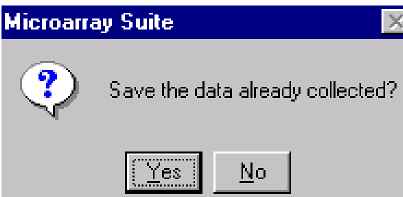


Figure 4.15 Save data prompt

Note

Selecting the same experiment name and repeating a scan of the probe array will overwrite the *.dat file of partial scan data that was saved. To retain the original scan data, use the **File** → **Save As** command from the main menu to save the *.dat file under a new name prior to initiating a new scan of the probe array.

Shutting Down the Scanner

1. Click the **Start Scan** button in the main toolbar or select **Run** → **Scanner** from the main menu.

   ⇒ The Scanner dialog box opens (Figure 4.16).

2. Click **Turn laser Off** in the Scanner dialog box.

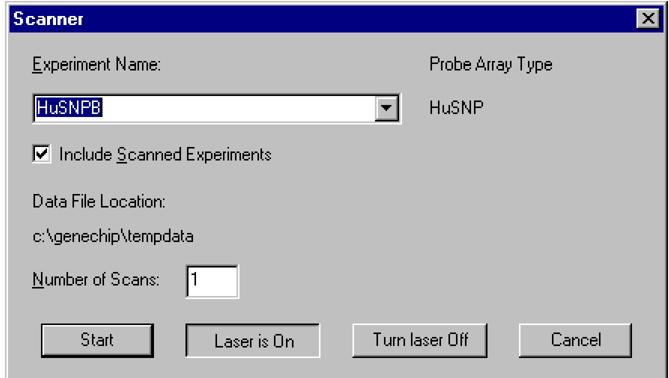


Figure 4.16 Scanner dialog box, disk files mode.

3. At the stop scanner prompt, click **Yes** to turn off the laser power immediately and initiate the laser cool down routine.

   The laser cool down routine lasts for several minutes and is finished when the cooling fan in the laser power supply shuts down. The scanner power switch may now be safely turned off.

TROUBLESHOOTING

Fluidics Station Troubleshooting

Problem	Possible Cause	Solution
"Fluidics Station X does not respond" error message on workstation.	Computer communicated error. Power not switched on the fluidics station prior to attempt to connect with fluidics station from the workstation.	Restart computer, turn fluidics station power on, then try to connect again.
	Incorrect fluidics station designated for communication.	Designate correct fluidics station on workstation. Refer to the *GeneChip® Fluidics Station 400 User's Guide* or GeneChip® Software On-line Help.
	Loose module connection.	Call Affymetrix® Technical Support for assistance.

TROUBLESHOOTING

Fluidics Station Troubleshooting

Problem	Possible Cause	Solution
Cartridge needles of the fluidics station not engaging with the probe array.	Probe array not engaged properly.	Place the probe array in the probe array holder. Push up on the probe array holder to engage the latch. Push up on the probe array lever until the Engage position is reached.
	Salt build-up on the cartridge needles.	Run the CLEAN script with fresh deionized water to flush out salt blockage. Clean cartridge needles with wet cotton swab.
	Extra flashing on the probe array.	Use another probe array. Call Affymetrix® Technical Support to report problem.
	Possible defective septa on probe array.	Use another probe array.
	Probe array holder aligned and attached to the fluidics station improperly.	Call Affymetrix Technical Support for service.
“Temperature Timeout” error message on workstation.	Fluidics station did not reach the designated temperature in time.	Call Affymetrix Technical Support for service.

TROUBLESHOOTING

Fluidics Station Troubleshooting

Problem	Possible Cause	Solution
"Missing Fluid Error" appears in the LCD window. Probe array not filling completely with sample or stain solution during initial stages of hybridization or stain script.	Possible defective probe array or holes in the septa of the probe array.	Check integrity of the probe array and for large holes in the septa. If defective, use another probe array.
	Sample or stain solution not in place properly.	Make sure that the sample or stain vial is in the sample holder and that the metal sampling tube reaches the bottom of the vial.
	Not enough volume of sample or stain solution.	Add more sample or stain solution to the sample vial and make sure that the metal sampling tube reaches the bottom of the vial.
	Blocked sampling tube or line of the fluidics station.	Run the CLEAN or PRIME scripts with fresh deionized water to flush out salt blockage.
	Excessive bubbling or foaming in probe array.	Make sure detergent concentration is not too high.
	Failure of one of the fluidics sensors.	Call Affymetrix Technical Support for service.

TROUBLESHOOTING

Fluidics Station Troubleshooting

Problem	Possible Cause	Solution
"Missing Fluid Error" appears in LCD window. Probe array not filling completely with buffer during wash script.	Buffer bottle is empty.	Fill buffer bottles. Reprime fluidics station before reinitiating wash script.
	Deionized water was used instead of buffer.	Replace water with buffer. Reprime fluidics station before reinitiating wash script.
	Module was not primed.	Prime module.
"Error while Draining" or "Error while Filling" message on workstation.	Insufficient sample volume.	Run recover script. Discard sample and repeat experiment.
	Excessive bubbling or foaming in probe array.	Run recover script. Change the buffer making sure that the detergent concentration is not too high.
	Buffer conductivity is too low.	Run recover script. Change the buffer making sure that the salt concentration is appropriate.
	Failure of one of the fluid sensors.	Run recover script. Call Affymetrix® Technical Support for service.
	Defective septa in probe array.	Run recover script. Use a new probe array.
Incomplete drain of the probe array during hybridization/stain/wash script. "Error while Draining"	Fluid caught in the lines of the fluidics station above the probe array.	Run recover script. If full sample volume is not recovered, discard sample and repeat experiment.

TROUBLESHOOTING

Fluidics Station Troubleshooting

Problem	Possible Cause	Solution
Recovered more sample than initially input during RECOVER script.	Fluid caught in the lines of the fluidics station above the probe array.	Discard sample and repeat experiment.
Air bubbles left in probe array at the end of a hybridization/stain/wash script.	Air bubble in washline.	See *If Bubbles are Present* on page 48.
“Sensor Timeout” error message on workstation.	User did not respond to “Remove vial” prompt or other prompt.	If uncertain whether instrument is primed, repeat prime script. Otherwise, proceed to next script.
Buffer leaking inside the fluidics station.	Washblock may need to be replaced.	Refer to the *GeneChip® Fluidics Station 400 User’s Guide* to replace a washblock.
	Salt buildup in the lines of the fluidics station.	Run the CLEAN or SHUTDOWN scripts with fresh deionized water to flush out salt blockage.
	Bad tubing attachments inside the fluidics station.	Call Affymetrix® Technical Support for service.

TROUBLESHOOTING

Scanner Troubleshooting

Problem	Possible Cause	Solution
The scan is blank.	The correct *.CIF file may not have been used.	Specify correct library file for the scan and verify that the correct parameter settings are specified in the *.CIF file.
	The scale of the image is set incorrectly.	In the Tools menu under Defaults, select the Image Settings tab. Select the Autoscale feature or set the intensity range from 0-10,000.
If after turning on the scanner and initializing Affymetrix® Microarray Suite software, the scanner progress bar does not progress beyond the “Warming” state.	The laser interlock system may not be intact.	Ensure that the power module key is turned horizontally and that all data and electrical connections to the power module are properly attached.
	Laser or laser power supply has failed, or useful life has been expended.	Call Affymetrix Technical Support for service.
Scan image at wrong pixel size. (This may be confirmed by looking at the size of the *.DAT file.)	Incorrect library file selected or incorrect setting specified in the *.CIF file.	Specify correct library file for the scan and verify that the correct parameter settings are specified in the *.CIF file.

TROUBLESHOOTING

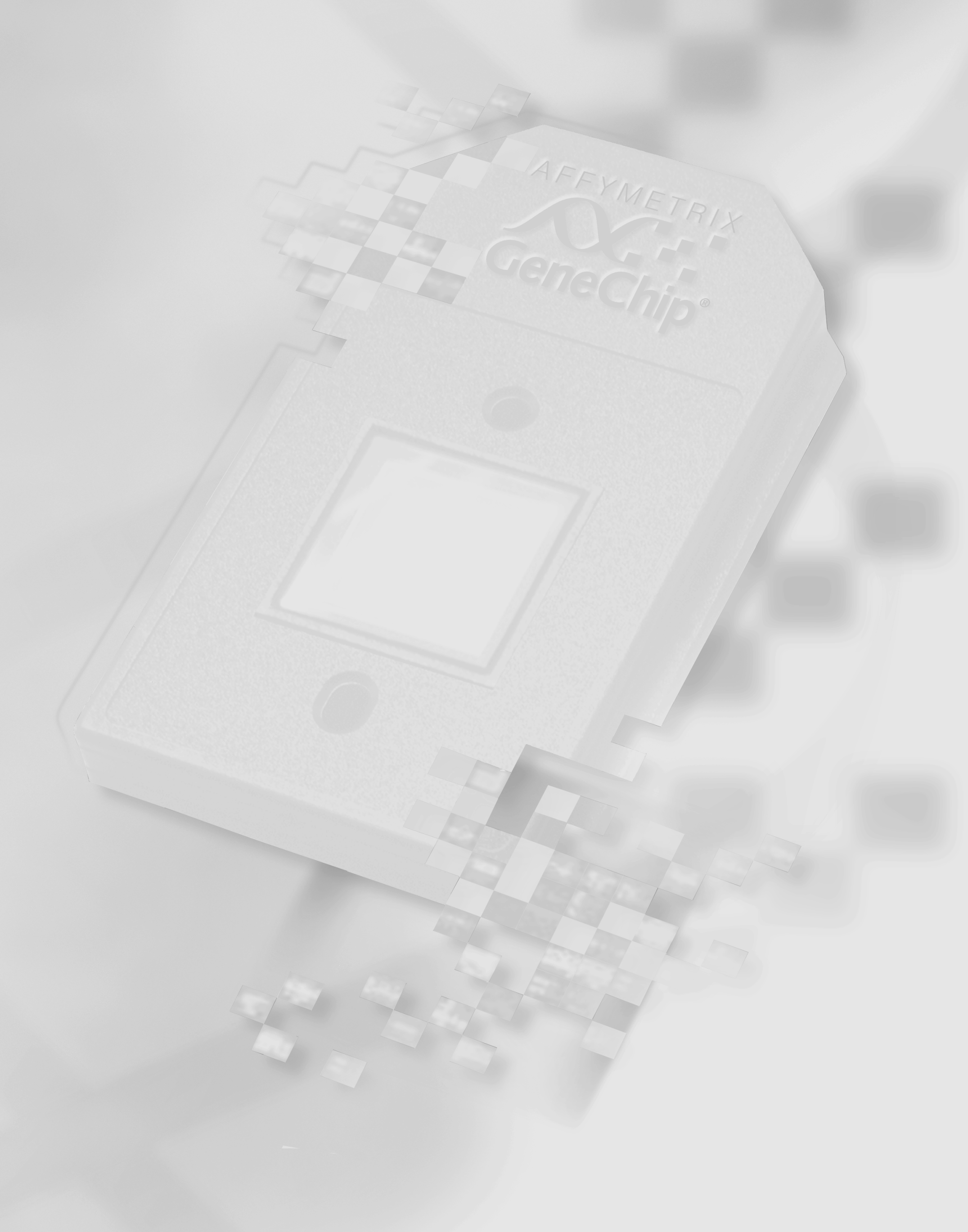
Scanner Troubleshooting

Problem	Possible Cause	Solution
High background or inaccurate readings.	Insufficient wash of probe array during hybridization.	Rewash the probe array. If the background is moderate (greater than 1000 counts), use 2 cycles with 6 mixes per cycle of Wash Buffer A. If the background remains substantially above 1000 counts, call Affymetrix® Technical Support.
	A heavy object may have been placed on the scanner compromising the optical alignment.	Do not place heavy objects on the scanner. Call Affymetrix Technical Support if the scanner optical alignment is in question.
Door does not unlock at the end of a scan.	Too much friction between the door lock solenoid and the door hole into which it fits.	Reinitialize the scanner and Affymetrix Microarray Suite software. Thereafter, avoid attempting to open the door prior to the door unlocking. Avoid leaning on the door or applying downward pressure on the door during a scan.
Probe array gets stuck in the loading port.	Misalignment of the loading port.	Call Affymetrix Technical Support for service.
	Labels present.	Avoid attaching labels to the probe arrays.
Computer fails to communicate with scanner.	SCSI cable connection is not solid.	Reattach the SCSI connection on the back of the scanner; depress locking clips.
Error Message Appears: “Operation not Supported”	In the Tools menu under Defaults and the Configurations tab, the “Turn on Laser at startup” option is selected.	This option should not be selected for Agilent Scanners.

TROUBLESHOOTING

Scanner Troubleshooting

Problem	Possible Cause	Solution
The scan is blank.	The correct *.CIF file may not have been used.	Specify correct library file for the scan and verify that the correct parameter settings are specified in the *.CIF file.
	The scale of the image is set incorrectly.	In the Tools menu under Defaults, select the Image Settings tab. Select the Autoscale feature or set the intensity range from 0-10,000.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5

DATA ANALYSIS

OVERVIEW

This chapter provides a brief overview of Affymetrix® Microarray Suite software capabilities for analyzing the GeneChip® HuSNP™ data. For more detailed descriptions of some of these features, contact Affymetrix Technical support.

GeneChip data analysis begins with assigning an experiment name to a probe array by creating an .exp file. Scanning a probe array creates a .dat file or image file. From this .dat file the software automatically generates a .cel file by demarcating individual cells. A *probe cell* is the area on the surface of the array containing a unique oligonucleotide sequence. The pixel intensities within each probe cell are averaged, producing a .cel file. The user produces the data output file, the .chp file, by running an analysis on a .cel file, using the appropriate GeneChip algorithm for that probe array.

FILE TYPES

Library Files

.cif – chip information file. This file contains grid size and parameters for analysis and scanner settings. **Do not change any information in this file.**

.cdf – chip description file. This file contains names and coordinates for each SNP represented on the probe array. **Do not change any information in this file.**

.std – standard file. This file contains information about the genotypes corresponding to respective hybridization intensity patterns at each SNP-interrogation block.

Test Data Files

.exp – experiment information files. Information about experiment name, sample and probe array are stored in this file. The experiment name then becomes the file name for subsequent files generated in the analysis.

.dat – data file. The image of the scanned probe array is stored in this file.

.cel – cell intensity file. This file is created automatically after grid alignment. The cell averaging analysis calculates the average intensities of each cell and assigns it to an x, y coordinate position. The .cel file can be used to re-analyze data with different algorithm parameters.

.chp – analysis out file (chip file). This is the output generated by the analysis of a .dat or .cel file.

GENOTYPING ALGORITHM

The Genotyping algorithm analyzes hybridization intensity data from the GeneChip® HuSNP™ probe array to call the genotype for 1,494 human SNPs.

Table 5.1 Possible genotype calls for the Affymetrix® HuSNP™ Mapping assay

Genotyping Algorithm	
Possible Call	**Genotype**
A	Homozygous A
B	Homozygous B
AB	Heterozygous AB
AB_A	Heterozygous AB or homozygous A
AB_B	Heterozygous AB or homozygous B
No Signal	Insufficient signal to make a call

Viewing the Genotyping Algorithm Settings

1. Click the **Analysis Settings** shortcut bar in the left pane of the Microarray Suite window, and then click the **Genotyping** algorithm button.
   ⇒ The Genotyping Analysis Settings dialog box opens (Figure 5.1).

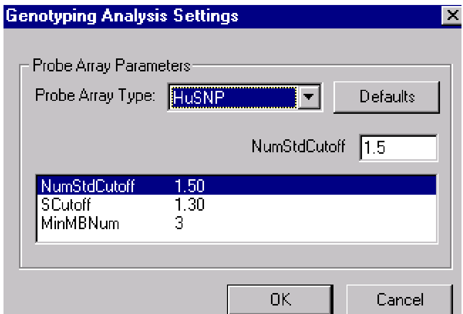


Figure 5.1 Genotyping Analysis Settings

2. Click a tab to view each type of setting. Confirm or change the settings as described in Appendix C.

ANALYZING SAMPLES

After scanning the probe array, two resulting image data files are created, which vary only by the root name ending in either an “A” or a “B” (for example, Experiment1A and Experiment1B, see Figure 5.3). These files are stored on the hard drive of the Microarray Suite workstation as .dat files. In the first step of the analysis, a grid is automatically placed over each .dat file, defining each probe cell. One of the probe array library files, the .cif file, indicates what the grid size should be to the software. You can confirm the alignment of the grid on each .dat file by zooming in on each of the four corners.

Microarray Suite automatically produces a .cel file for each properly gridded .dat file. If an error in grid alignment occurs, adjust its alignment by placing the cursor on an outside edge or corner of the grid. The cursor image will change to a small double-headed arrow. The grid can then be moved using the arrow keys or by clicking and dragging its borders with the mouse.

When the grid is properly aligned, create the .cel file by selecting **Average Cell** from the **Run** menu on the Microarray Suite tool bar. The software calculates the average intensity of each probe cell using the intensities of the pixels contained in the cell. Pixels on the edges of each cell are not included, preventing neighboring cell data from affecting a cell’s calculated average intensity. The calculated average intensity is assigned an (X,Y) coordinate position, corresponding to the cell’s position on the array. The .cel file stores this data using the same name as the .exp and .dat files.

When running a genotype analysis the software applies the selected probe array algorithm to determine the genotype call for each marker. This is done with reference to the information contained in the .cdf file. The resulting analysis is displayed by Microarray Suite as a .chp file in the Nucleotide Analysis window (NAW).

The Nucleotide Analysis window displays the tabular and graphic analysis results for genotyping assays and opens when a genotyping analysis is completed or a genotyping analysis output file (*.chp) is opened.

The NAW is divided into two panes. The bottom pane displays genotype calls and related information and the top pane displays intensity graphs of probe set data

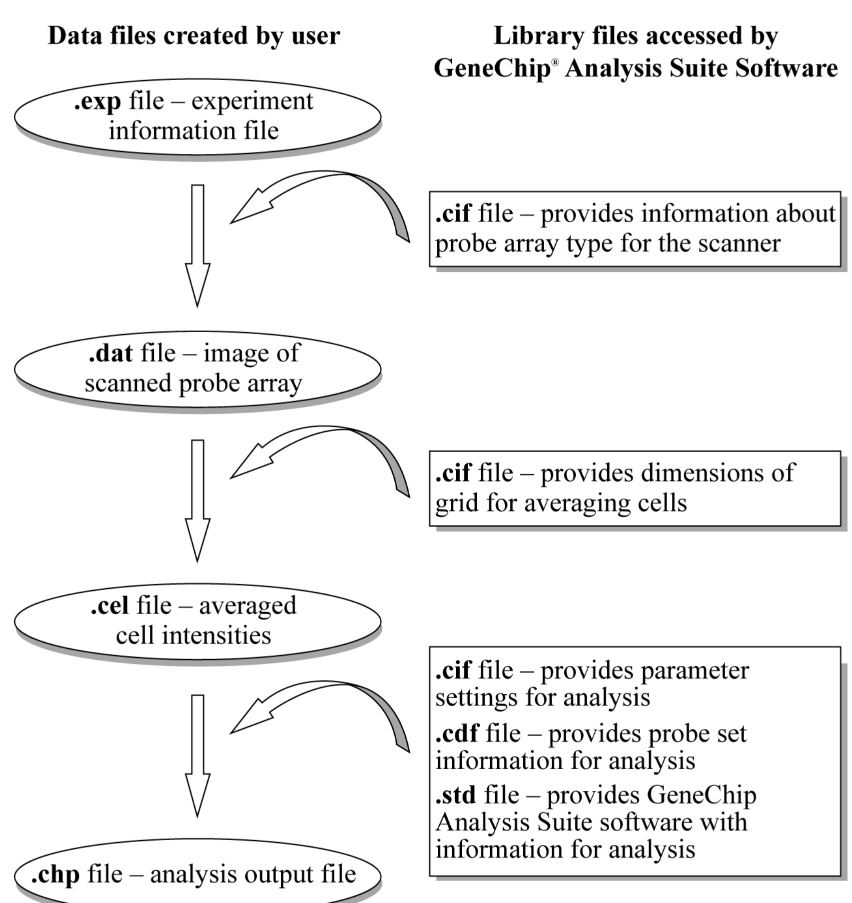


Figure 5.2 Data Analysis Flow Chart

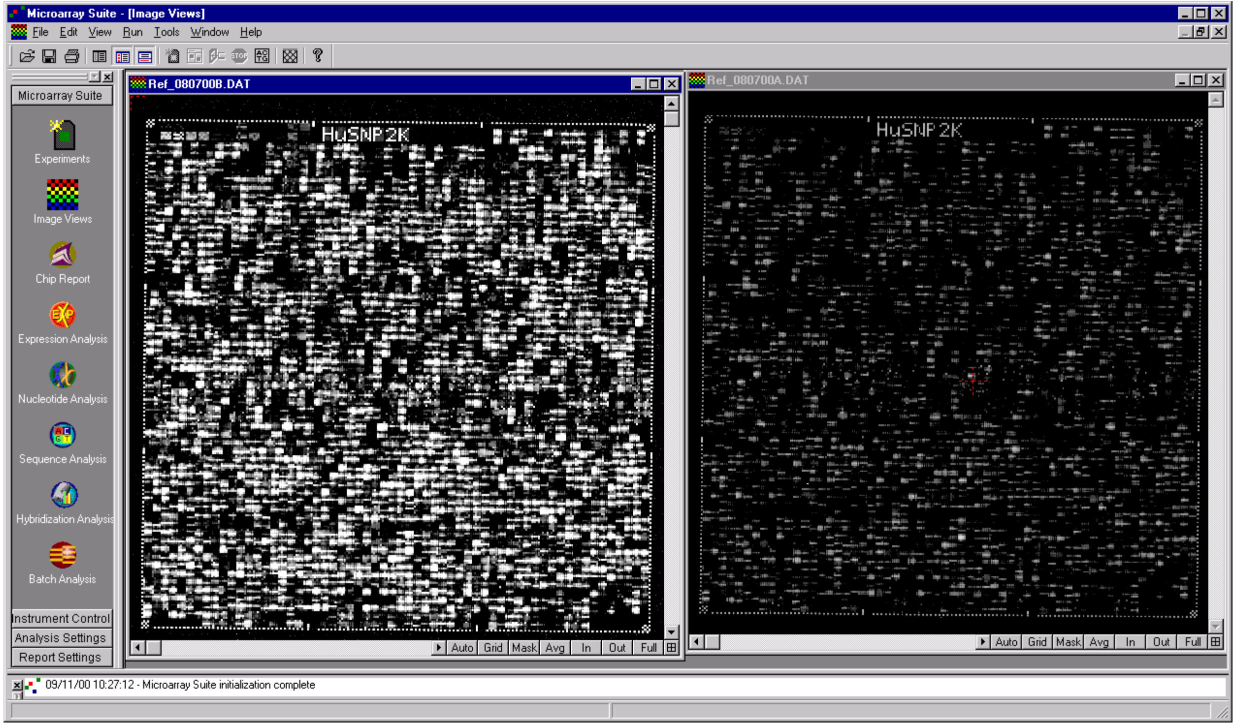


*Figure 5.3 Example of *.DAT files generated from scans at 570 and 530 wavelengths*

RUNNING A GENOTYPE ANALYSIS

1. In the data file tree, right-click the cell intensity file (*.cel) to be analyzed and select **Analyze** from the pop-up menu.

   Alternatively: 1) select **File** → **Analysis** from the main menu and choose the desired *.cel file from the Analyze dialog box that opens, or 2) if the image is displayed, click the **Analyze** button in the main toolbar or select **Run** → **Analysis** from the main menu.

   During the analysis, the status window displays the name of the analysis output file (*.chp) being generated and where it will be stored. The status window also indicates when the analysis is completed. If the status bar is not displayed, click the **Status Log** button in the main toolbar or select **View** → **Status Bar** from the main menu.

   When the analysis is finished, the Nucleotide Analysis window (NAW) opens and displays the output analysis results file (*.chp) (Figure 5.4). If the NAW is already open, the results are added to the open window. It may be necessary to use the scroll bars at the bottom and right side of the NAW to see the newly added results.

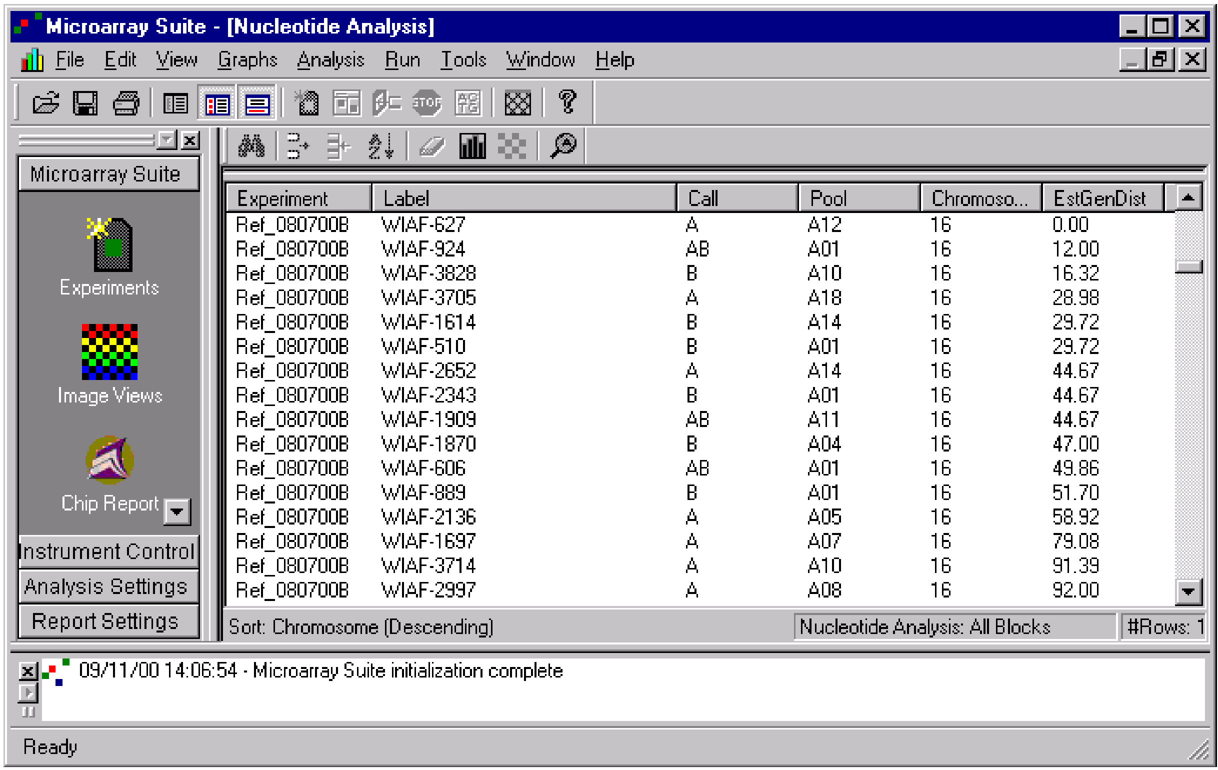


*Figure 5.4 Example of *.CHP Analysis file output*

The .chp file holds the genotype output as well as other information specific to each SNP. The data can be reviewed in this format, or can be exported as a text document, and displayed using other software.

HuSNP™ Tabular Data

The HuSNP tabular data (Figure 5.4) includes information from the analysis output file (*.chp). Each row of the table displays the analysis result for one SNP.

Experiment	The name of the analysis output (*.chp) file.
Label	The identifier for the SNP.
Call	The algorithm generates six possible calls: (1) A (homozygous A allele), (2) B (homozygous B allele), (3) AB (heterozygous), (4) AB_A (two possible genotypes AB or A that could not be distinguished), (5) AB_B (two possible genotypes AB or B that could not be distinguished), or (6) No Signal (insufficient data passed the quality tests to perform an analysis).
Pool	The number of the primer pool containing the primer for the SNP.
Chromosome	The number or name of the chromosome that contains the SNP.
EstGenDist	The Estimated Genetic Distance is the predicted chromosomal location of the SNP expressed in centiMorgans. Genetic distances are measured from the top of the chromosome's short arm. A large set of markers including the SNPs were physically mapped onto radiation hybrid panels. The physical locations of the SNP markers were integrated into the genetic map to obtain the Estimated Genetic Distances.

Exporting Genotype Data

Genotype data may be exported to a tab delimited text file (*.txt).

1. Double-click a genotype analysis output file (*.chp) in the data tree.
   ⇒ The file is opened and displayed in the NAW.
2. Choose **Edit** → **Select All** from the main menu.
3. Select **File** → **Save As** from the main menu.
   ⇒ The Save As dialog box opens (Figure 5.5).

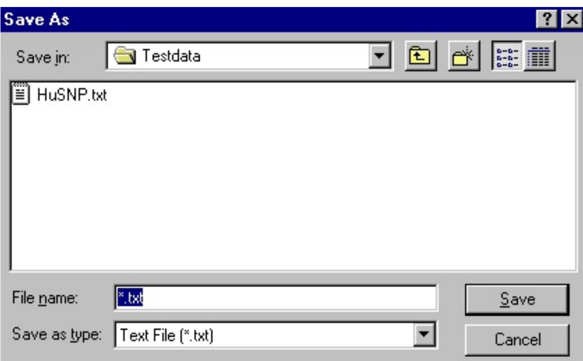


Figure 5.5 Save As dialog box

4. Enter a name for the text file in the **File name** field, then click **Save**.

   The data may be displayed in one (default) or two columns in the text file (Table 5.2).

Note

The two-column display option is not recommended for CYP450 data.

Table 5.2 HuSNP™ genotype calls displayed in separate columns in a text file

GeneChip® HuSNP™ Mapping Assay		
Call	**Calls displayed in separate columns**	
A	A	A
B	B	B
AB	A	B
AB_A	AB_A	
AB_B	AB_B	
No Signal	No Signal	

To display the call column as two separate columns in the text file:

1. Click the **Options** button in the NAW toolbar or select **Analysis** → **Options** from the main menu.
   ⇒ The Analysis Options dialog box opens (Figure 5.6).
2. Click the **Export** tab.
3. Select the **Save calls in separate columns** option, then click **OK**.

Figure 5.6 Analysis Options, Export tab

Mapping Report

The Mapping Report displays the percentage of SNPs assigned to a genotype and the percentage of each genotype call for the array as well as per primer pool (Figure 5.7). This provides both a global and a localized assessment of assay performance.

The Mapping Report also displays the probe array type and the name of the analysis output file (*.chp) examined, the algorithm type and version, and the values specified for the user-modifiable algorithm parameters (NumStdCutoff, Scutoff, and MinMBNum). For further information about these parameters refer to Appendix C.

The **Probe Array Summary** portion of the report displays the correlation coefficient of hybridization intensity data scanned at 530 and 570 nm, the maximum and median intensity for the array, and the genotype calls for the array.

The **Pool Summary** portion of the report displays the percentage of genotype calls for each primer pool (A01 – A21) in the assay.

To generate the report:

1. In the data file tree, right-click the analysis output file (*.chp) for the report.
2. Select **Report** from the pop-up menu.
   ⇒ The report is automatically displayed in the main display area.

%Pass	The percentage of SNPs assigned a genotype.
%A	The percentage of homozygous A genotypes.
%AB	The percentage of heterozygous AB genotypes.
%B	The percentage of homozygous B genotypes.
%AB_A	The percentage of genotypes that could be AB or A (the two possible calls could not be distinguished).
% AB_B	The percentage of genotypes that could be AB or B (the two possible calls could not be distinguished).
% NoSignal	The percentage of genotypes that could not be called.

```
ReportType:   Mapping Report
Date:                        07:38AM 02/14/2000
```

```
Filename:                    HuSNPA.CHP
Probe Array Type:            HuSNP
Algorithm:                   GenoTyping
Alg version:                 1.0
Alg parameters:              NumStdCutoff=1.50 SCutoff=1.30 MinMBNum=3

Probe Array Summary:
CorrelationCoefficient=0.980
MaxIntensity=280603.8
MedianIntensity=7756.0

%Pass          89.2
%A             27.6
%AB            30.7
%B             29.8
%AB_A           0.2
%AB_B           0.8
%NoSignal      10.8

Pool Summary:
```

Pool	%Pass	%A	%AB	%B	%AB_A
A01	98.9	34.0	37.2	27.7	0.0
A02	97.6	30.6	40.0	27.1	0.0
A03	89.2	16.9	48.2	24.1	0.0
A04	85.5	28.9	25.0	31.6	0.0
A05	83.3	22.2	27.8	30.6	0.0
A06	84.5	23.9	23.9	33.8	0.0
A07	74.7	21.3	20.0	33.3	0.0
A08	77.5	28.8	27.5	18.8	0.0
A09	91.5	36.6	26.8	25.6	0.0
A10	95.3	32.9	31.8	30.6	0.0
A11	86.0	33.7	16.3	33.7	1.2
A12	97.4	29.9	36.4	31.2	0.0
A13	95.5	29.5	25.0	38.6	0.0
A14	97.1	27.1	38.6	31.4	0.0
A15	94.5	27.4	39.7	27.4	0.0
A16	89.5	27.6	21.1	40.8	0.0
A17	96.2	26.9	38.5	29.5	1.3
A18	72.0	14.0	32.0	24.0	2.0
A19	98.0	39.2	29.4	29.4	0.0
A20	74.4	15.4	28.2	28.2	0.0
A21	64.1	12.8	25.6	23.1	0.0
Total	89.2	27.6	30.7	29.8	0.2

Figure 5.7 Mapping Report

Obtaining Information from Probe Cells

When viewing an image data (*.dat) or cell intensity (*.cel) file in the Image window, the user may click any probe cell (feature) in the image to obtain more information about the feature and the associated marker (Figure 5.8).

Choose one of the following methods to display the probe cell data:

- Double-click the probe cell of interest
- Click the probe cell of interest; then right-click the image and select **View** → **Probe Cell Data** from the pop-up menu
- Click a probe cell of interest; then select **View** → **Probe Cell Data** from the main menu

Click other probe cells in the image and the probe cell data display is automatically updated.

Probe Cell Data

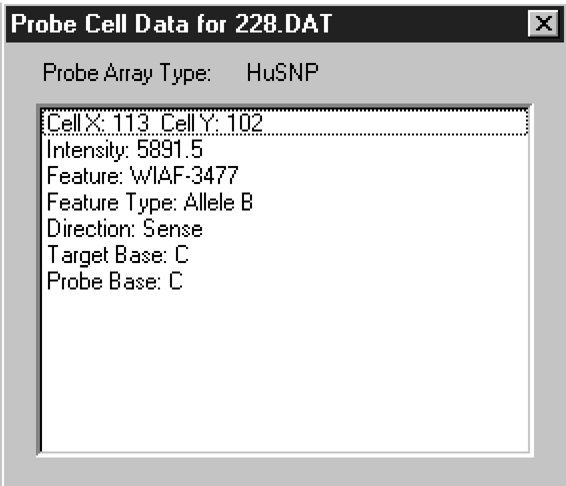


Figure 5.8 Probe cell data

Cell Coordinates	The probe cell x and y coordinates in cell units.
Intensity	The cell intensity calculated by the Cell Analysis algorithm.
Feature	The name of the SNP.
Feature Type	Allele A or allele B.
Direction	The direction (sense or antisense) of the target (sample).
Target Base	The expected nucleotide at the position corresponding to the substitution position of the probe.
Probe Base	The actual nucleotide located at the substitution position of the probe.

Locating a Polymorphism

1. Right-click the data table and choose **Select** from the pop-up menu.
   ⇒ The Select Block dialog box opens (Figure 5.9).
2. Select a polymorphism label (identifier) from the drop-down list and click **Select**.
   ⇒ The corresponding row is highlighted and displayed in the NAW (Figure 5.9).

Experiment	Label	Call	Pool	Chromoso...	EstGenDist
Ref_080700B	WIAF-4527	AB	A12	4	
Ref_080700B	WIAF-603	AB	A15	8	
Ref_080700B	WIAF-700	A	A02	18	87.40
Ref_080700B	WIAF-2576	B	A01	15	68.00
Ref_080700B	WIAF-2665	AB	A12	3	181.95
Ref_080700B	WIAF-303	A	A12		
Ref_080700B	WIAF-512	A	A12	5	98.00
Ref_080700B	WIAF-2295	B	A02	X	
Ref_080700B	WIAF-2313	B	A15	11	19.98
Ref_080700B	WIAF-592	A	A02	6	163.29
Ref_080700B	WIAF-490	A	A12	8	103.49
Ref_080700B	WIAF-623	A	A02	11	0.00
Ref_080700B	WIAF-636	AB	A02	7	81.15
Ref_080700B	WIAF-615	AB	A07		
Ref_080700B	WIAF-638	B	A07	1	105.00
Ref_080700B	WIAF-626	AB	A02	3	181.41
Ref_080700B	WIAF-653	AB	A07	1	113.40

Figure 5.9 NAW, HuSNP™ analysis output file (.chp), Select Block dialog box*

Removing an Experiment from the NAW

1. Highlight any result row of the experiment(s) to be removed.
2. Select **Edit** → **Remove Experiments** from the main menu.

Note

Selecting **Edit** → **Remove Experiment** is equivalent to closing the *.chp file.

Sort Order

The columns of the table that contain text may be sorted alphabetically. Columns that contain numeric data may be sorted numerically in ascending or descending order.

1. Select **Edit** → **Sort** from the main menu.
   ⇒ The Sort dialog box opens (Figure 5.10).
2. Select the first category for the sort from the **Sort By** drop-down list and choose the desired sort order.
3. Select subsequent sort categories from the **Then By** drop-down lists, choose the sort order, and click **OK** when finished.

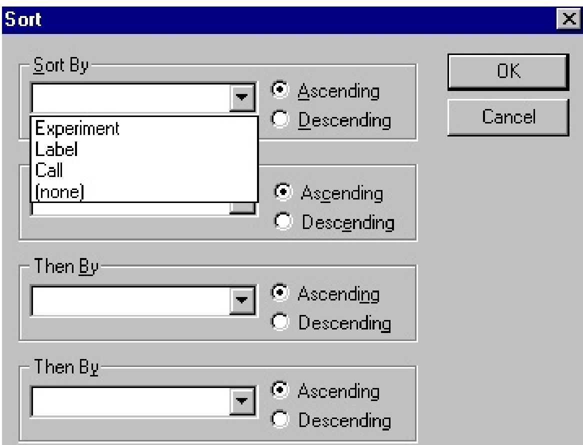


Figure 5.10 Sort dialog box

4. To sort a single column in ascending order, click the column header in the NAW. Click the column header again to sort the information in descending order.

Find Feature

The Find feature performs text searches of the data table.

1. Click the **Find** button in the NAW toolbar or right-click in the data table and select **Find** from the pop-up menu. Alternatively, select **Edit** → **Find** from the main menu.
   ⇒ The Find dialog box opens (Figure 5.11).

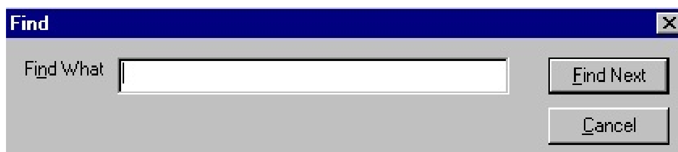


Figure 5.11 Find dialog box

2. Enter the desired text for the search (up to 256 alphanumeric characters including spaces) in the **Find What** field. Then click **Find Next** to view the first search result.

3. Continue to click **Find Next** to view each successive search result.

Note

The **Find** command finds all strings that match the text string for the search. For example, using the **Find** command to search for the text string *4a* would find *G2064A* and *G1934A* as well as other occurrences of *4a*.

APPENDIX A

A

SUPPLIER CONTACT INFORMATION

Supplier	US	Web site
Ambion	(800) 888-8804	www.ambion.com
Amersham/Pharmacia Biotech	(800) 323-9750	www.apbiotech.com
Beckman Coulter	(800) 233-4685	www.beckman.com
BioWhittaker	(800) 638-8174	www.biowhittaker.com
Cole-Parmer	(800) 323-4340	www.coleparmer.com
Eppendorf-5 Prime	(800) 533-5703	www.5prime.com
FMC Bioproducts	US (215) 299-6000	www.fmc.com
Gentra Systems	(800) 866-3039	www.gentra.com
Gibco BRL Life Technologies	(800) 828-6686	www.lifetech.com
Millipore Corp	(800) 645-5476	www.millipore.com
MJ Research	(888) 735-8437	www.mjr.com
Molecular Probes	(541) 465-8300	www.probes.com
Nucleic Acid Purifications	(800) 711-2090	
Perkin Elmer	(800) 345-5224	www.perkinelmer.com
Pierce Chemical	(800) 874-3723	www.piercenet.com

Supplier	US	Web site
Promega Corporation	(800) 356-9526	www.promega.com
QIAGEN	(800) 426-8157	www.qiagen.com
Rainin Instrument Company	(800) 472-4646	www.rainin.com
Sigma Chemical	(800) 325-3010	www.sigma-aldrich.com
USA Scientific	(800) 522-8477	www.usascientific.com
Vector Laboratories	(800) 227-6666	www.vectorlabs.com
VWR Scientific Products	(800) 932-5000	www.vwrsp.com
Whatman BioScience (formerly Cambridge Molecular Technologies)	+44(0) 1223-5-08333	www.whatman.co.uk

REAGENT AND EQUIPMENT LIST

A

Agarose gel (4%), NuSieve® 3:1 Plus (24 well format), FMC Bioproducts, P/N 54928
AmpliTaq® Gold, Perkin Elmer, P/N N9808-0245 (supplied together as AmpliTaq® Gold, $MgCl_2$, and 10X Buffer II)
Antibody (goat), Anti-streptavidin, biotinylated, (0.5 mg), Vector Laboratories, P/N BA-0500

B

Bleach (5.25% Sodium Hypochlorite), VWR, P/N 21899-504
Buffer II (10X), Perkin Elmer, P/N N9808-0245 (supplied together as AmpliTaq® Gold, $MgCl_2$, and 10X Buffer II)

C

Caps for 0.2 mL tubes (strip of 8), MJ Research, Inc., P/N TCS-0801
Centrifuge, swing-bucket

D

Denhardt's Solution (50X), Sigma, P/N D-2532
DNA, Herring Sperm, Promega Corporation, P/N D1811
DNA Ladder (100 bp), Gibco BRL, P/N 15628
DNTPs (stock), Pharmacia Biotech, P/N 27-2035-01
Dye, loading

E

EDTA Disodium Salt, 0.5 M solution, (100mL), Gibco BRL, P/N 15575-038

F

Film, Microseal 'A', MJ Research, Inc., MSA-5001

G

GeneChip® Fluidics Station 400, Affymetrix®, P/N 800101
GeneChip® HuSNP™ Probe Arrays, Affymetrix®, P/N 900194
GeneChip® HuSNP™ Reagent Kit, Affymetrix®, P/N 900193
GeneChip® Hybridization Oven 640, Affymetrix®, P/N 800138

H
Heat block

L
Lids, Seal and Sample aluminum foil, Beckman, P/N 538619

M
Magnesium chloride ($MgCl_2$), Perkin Elmer, P/N N9808-0245 (supplied together as AmpliTaq® Gold, $MgCl_2$, and 10X Buffer II)
Microcentrifuge
Microcon®-10 (microconcentrators), Millipore, P/N 42406
Micropipettors, (P-2, P-20, P-200, P-1000), Rainin Pipetman or equivalent
Microplates, 96-well polypropylene v-bottom, MJ Research, Inc., MLP-9611
Mini agarose gel electrophoresis unit with appropriate buffers and ethidium bromide

O
Oligonucleotide B1 Control (from GeneChip® HUSNP™ Mapping Reagent Kit)

P
Phycoerythrin-Streptavidin, Molecular Probes, P/N S-866
Pipets, multichannel (1-10 µL, 5-50 µL)
Pipets, transfer (small-bore)
Pipet tips, sterile-barrier, RNase-free
Pipet tips, sterile-barrier and non-barrier (Tips must be pointed, not rounded, for efficient use with the probe arrays)
Plates, Deep Well Titer,1 mL, Beckman, P/N 267004

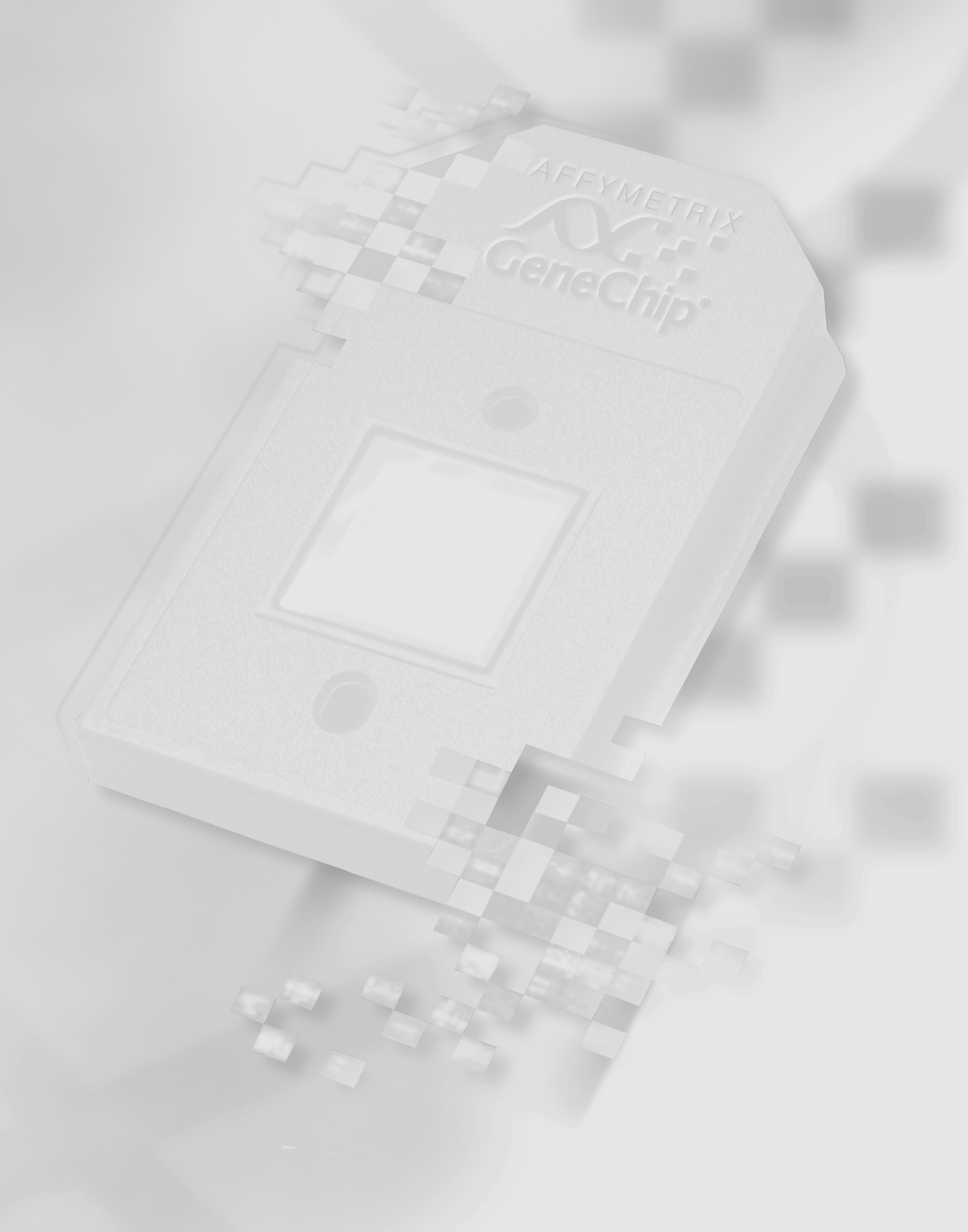
S
Scanner, Agilent GeneArray™
SSPE, 20X, BioWhittaker, P/N 16-010Y

T
TBE, 20X, BioWhittaker, P/N 16-012Y
Thermal cycler, MJ Research DNA Engine or Tetrad; or Perkin Elmer 2400, 9600, or 9700
Thin wall tubes, 0.2 mL, (strip of 8), MJ Research, Inc., P/N TBS-0201
TMAC, Sigma, P/N T3411

Tris-HCl (1M), pH 7.8, Sigma, P/N T-2913
Triton® X-100, Mallinkrodt, P/N H282-01
Tube rack
Tubes, Sterile, RNase-free, microcentrifuge, 1.5 mL, USA Scientific, P/N 1415-2600
Tubing, Tygon®, 0.04″ inner diameter, Cole-Palmer, P/N H-06418-04
Tween® 20, Pierce Chemical, P/N 28320

V
Vacuum filter units 1 liter capacity, (0.20 µm or 0.45 µm), Corning, P/N 25988-1L

W
Water, DEPC-Treated, Ambion, P/N 9902
Water, Distilled, Gibco BRL Life Technologies, P/N 15230-147
Water, Molecular Biology Grade, BioWhittaker, P/N 16-001Y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APPENDIX B

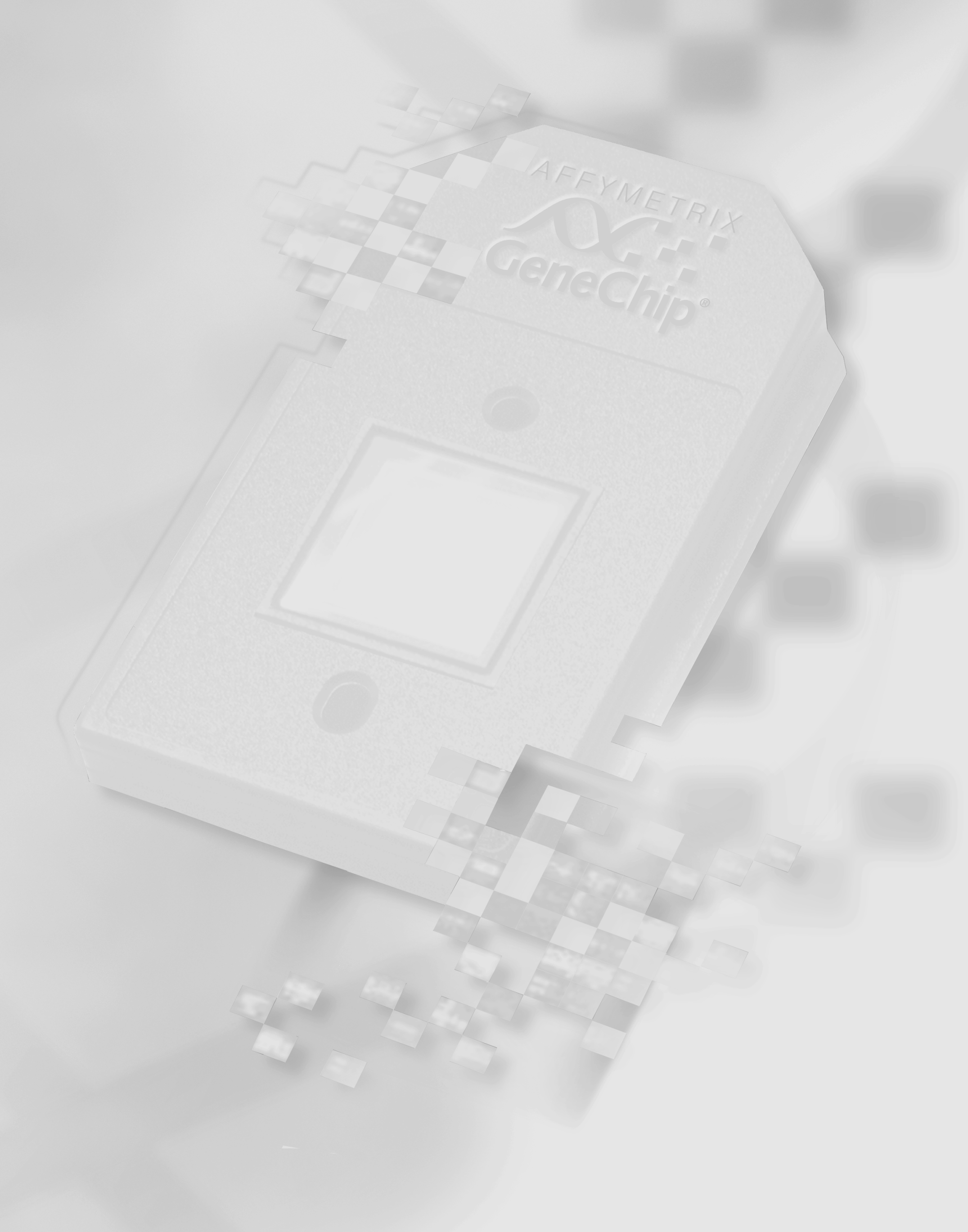
B

CONTACTING TECHNICAL SUPPORT

Affymetrix® provides technical support via phone or E-mail.
To contact Affymetrix Technical Support:

North America	Europe
Affymetrix Inc. 3380 Central Expressway Santa Clara CA 95051 USA	Affymetrix UK Limited 91 Milton Park Abingdon Oxon OX14 4RY United Kingdom
Tel: 888-362-2447 (888-DNA-CHIP) Fax: (408) 731-5441	Tel: +44 (0)7000 785 803 Fax:+44 (0)7000 785 804
E-mail: support@affymetrix.com www.affymetrix.com	
AFFYMETRIX®	

We welcome your comments regarding this manual. Please contact us at:
Technical_Publications@Affymetrix.com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APPENDIX C

C

GENOTYPING ALGORITHM SETTINGS

The Genotyping algorithm evaluates the quality of the hybridization intensity data from each miniblock (a set of four probes: PM_A, MM_A, PM_B, MM_B), which must pass quality controls tests; otherwise, the data from a block (which includes five miniblocks) is not used in the analysis.

The Genotyping algorithm relies on the analysis settings to derive biologically meaningful results from GeneChip® HuSNP™ Mapping probe array intensity data. Some of the analysis settings are user-modifiable. The default values were empirically optimized through extensive testing at Affymetrix® and should only be modified by expert users.

1. Click **Analysis Settings** in the shortcut bar and then click the **Genotyping** button. Alternatively, select **Tools** → **Analysis Settings** → **Genotyping** from the main menu.

   ⇒ The Genotyping Analysis Settings dialog box opens (Figure C.1).

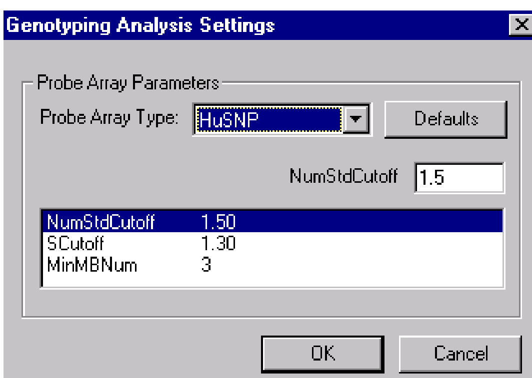
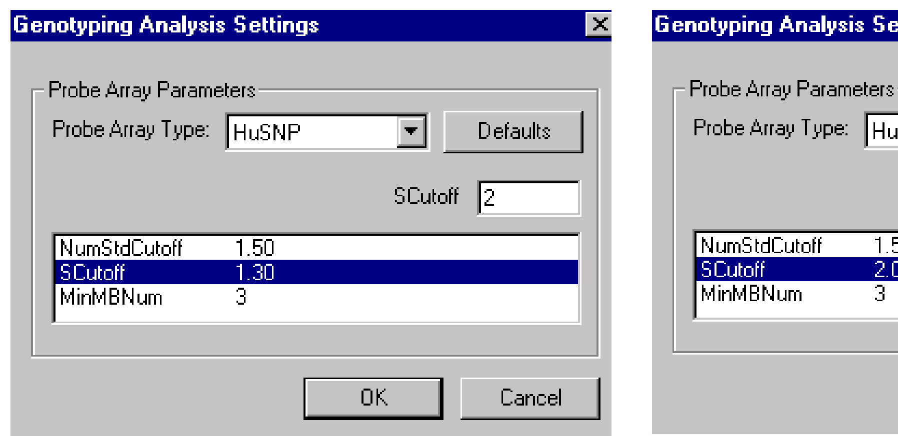


Figure C.1 Genotyping Analysis Settings dialog box

2. Select *HuSNP* from the **Probe Array Type** drop-down list.

   The settings are specific for the selected probe array type and do not affect the settings for other types of probe arrays.

3. To edit a parameter value, highlight the parameter in the lower field and enter the new value in the upper field. Click the lower field to display the new parameter value (Figure C.2).



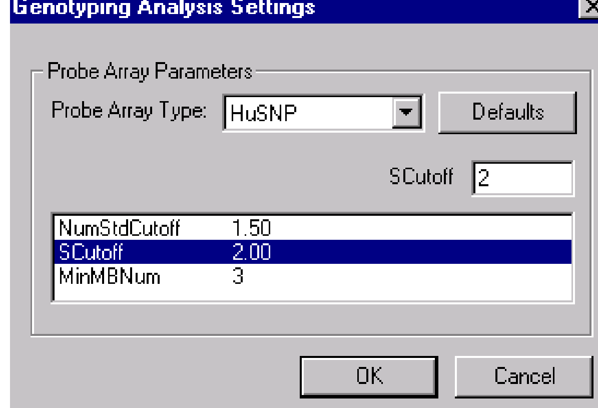


Figure C.2 *Enter a new parameter value in the upper field (left) and click OK to save the new parameter value (right)*

Number of Standard Deviations Cutoff

The number of standard deviations cutoff (NumStdCutoff) is a number of standard deviation units. The intensity data from a miniblock of four probe cells passes one of the quality controls tests if:

$$(PM_A + PM_B - Mean)/Std > NumStdCutoff$$

where:

PM_A = probe designed to be a perfect match to allele A, PM_B = probe designed to be a perfect match to allele B

$$Mean = \frac{\left(\sum_{i=1}^{Nmm} MMi\right)}{Nmm}$$

Nmm = number of mismatch probes (both A and B) for the block

MMi = the intensity of mismatch probe i

$$\text{Std}^2 = \left(\sum_{i=1}^{Nmm} (MMi - Mean)^2 \right) / (Nmm - 1)$$

$$\text{Std} = \sqrt{\text{Std}^2}$$

Increasing the NumStdCutoff threshold increases the stringency of the analysis. To change the threshold value, highlight **NumStdCutoff** in the GenoTyping Analysis Settings dialog box and enter the desired value in the field at the right (Figure C.2).

S Cutoff

The intensity data from a miniblock of four probe cells passes one of the quality control tests if:

$PM_A/MM_A \geq$ S Cutoff OR $PM_B/MM_B \geq$ S Cutoff

where PM_A = perfect match probe designed to be perfectly complementary to allele A, PM_B = perfect match probe designed to be perfectly complementary to allele B, MM_A = mismatch probe to allele A, MM_B = mismatch probe to allele B.

Increasing the S Cutoff threshold increases the stringency of the analysis. To change the threshold value, highlight **S Cutoff** in the GenoTyping Analysis Settings dialog box and enter the desired value in the field at the right (Figure C.2).

Minimum Miniblock Number (MinMBNum)

This specifies the minimum number of miniblocks per block that must pass all of the quality control tests so that the data from that block may be used in the analysis. Increasing the Minimum Miniblock Number increases the stringency of the analysis. To change the threshold value, highlight **MinMBNum** in the Genotyping Analysis Settings dialog box and enter the desired value in the field at the upper right (Figure C.2).

APPENDIX D

D

MICROARRAY SUITE FILE TYPES

Probe Information (Library) Files

The Probe Information or library files contain information about the probe array design characteristics, probe utilization and content, and scanning and analysis parameters. These files are unique for each probe array type. The default path for the Probe Information files is C:\GeneChip\Library. To view the file location, select **Tools** → **Defaults** from the main menu and click the File Locations tab.

Fluidics Protocol Files

The Fluidics Protocol files contain the instrument control instructions used by the GeneChip® Fluidics Station 400. The default path for these files is C:\GeneChip\Protocol.

Experiment Data Files

The experiment information file (*.exp) is created by the user during experiment set up. Affymetrix® Microarray Suite generates the other experiment data files types as data analysis proceeds. The default path for these files is C:\GeneChip\TestData. If desired, the files may be written to another directory. The destination path may be set in the Files Locations tab of the Defaults dialog box. (Select **Tools** → **Defaults** from the main menu and click the Files Locations tab.)

Table D.1 Experiment Data Files

Experiment Data File Name	File Extension	Description
Experiment Information File	*.exp	Contains information about the experiment name, sample, and probe array type. The experiment name also provides the name for subsequent test data files generated during the analysis of the experiment.
Data File	*.dat	The image of the scanned probe array.
Cell Intensity File	*.cel	Microarray Suite derives the *.cel file from a *.dat file and automatically creates it upon opening a *.dat file. It contains a single intensity value for each probe cell delineated by the grid (calculated by the Cell analysis algorithm).
Chip File	*.chp	The output file generated from the analysis of a probe array.
Report File	*.rpt	The report generated from the analysis output file (*.chp).
Graphic Image File	*.tif	A standard file format for graphic images. Microarray Suite exports graphic images in this file format.
Text File	*.txt	A standard format for text files. Microarray Suite exports text in this file format.

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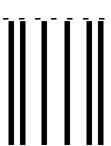
Does the GeneChip® System you use belong to the lab in which you work or is it a shared instrument?: ____________________

If shared, please indicate the location of the instrument that you use: ____________________



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