

KaryoStat™ Assay

USER GUIDE

KaryoStat™ Assay is equivalent to and comprised of CytoScan™ Optima arrays and reagents

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Introduction

KaryoStat™ Assay is intended to be used with genomic DNA in conjunction with CytoScan™ Optima array, GeneChip™ System 3000, and Chromosome Analysis Suite (ChAS).

KaryoStat™ Assay is equivalent to and comprised of CytoScan™ Optima arrays and reagents. CytoScan™ Optima array consists of features covering control, copy number (CN), and single-nucleotide polymorphism (SNP) probes. There are a total of 18,018 CN markers and 148,450 SNP markers uniformly spaced over the genome with enhanced interrogation of 396 regions of prenatal interest. Cumulatively, through the collection of SNPs and non-polymorphic probes the application provides the ability to support detection of CNVs, enable the elucidation of allelic imbalance (i.e., the pattern of inheritance deviates from expected Mendelian inheritance patterns), identify copy number neutral abnormalities such as absence (AOH) or loss of heterozygosity (LOH), and characterize unbalanced translocation events in the samples of interest.

Assay warnings and precautions

Follow universal precautions for laboratory and assay procedures, and waste disposal. Follow federal, state, local, and within-country regulations.

Before you begin, go to Appendix A, “Workflow and practices” for specific setup instructions, and equipment and technique illustrations, and Appendix B, “Thermal cycler programs” for specific thermal cycler program set ups.

For additional specific warnings, precautions, and procedures, see:

- *GeneChip™ Command Console™ 3.2 User Guide* (Cat. No. 702569)
- *GeneChip™ Fluidics Station 450 User Guide for AGCC* (Cat. No. 08-0295)
- *GeneChip™ Hybridization Oven 645 User Guide* (Cat. No. 08-0255)

Visit our website for all user guides (*CytoScan™ Optima Assay User Guide*, *Chromosome Analysis Suite (ChAS) User Guide*, *GeneChip™ Command Console™ User Guide*, *GeneChip™ Fluidics Station 450 User Guide for AGCC*, *GeneChip™ Hybridization Oven 645 User Guide*), and training materials (containing training kit and positive control DNA), SDS for all hazards in CytoScan™ Optima Assay reagents.

Precautions

The physical and toxicological properties of the products in this kit have not been thoroughly investigated. Follow prudent laboratory practices and use general laboratory safety equipment (eye protection, labcoat, and lab gloves) and good personal hygiene when working with these or any laboratory reagents. Refer to the Safety Data Sheet for more information.

Proper laboratory practice is necessary as previously amplified PCR product is the most likely potential source of contamination. We strongly recommend two separate work areas be used to minimize the risk of cross-contamination during the assay procedure. It is essential to adhere to workflow recommendations. PCR reactions should be set up in the Pre-PCR Area only. Personnel should not reenter the Pre-PCR Clean Area once exposed to PCR products without first showering and changing into clean clothes.

Carefully reading and following the protocol as written is essential. The KaryoStat™ Assay has been validated using the reagents and suppliers listed. Substitution of reagents and not following detailed procedures are not recommended as your results could be suboptimal.

Pre/post-PCR

- Follow standard procedures and single-direction workflow for the Pre-PCR laboratory area.
- Use dedicated equipment for each area (e.g., thermal cyclers, microfuges, pipettes and tips, ice buckets, etc.)
- Place all reagents and master stocks in use area. Do not move equipment between Pre- and Post-PCR Rooms, e.g., ice buckets, pipettes, etc.
- Use separate copy of assay procedure in Pre- and Post-PCR areas.
- Follow procedures for re-entry of Pre-PCR Clean Room from post-PCR.
- If pre-PCR work is done in a laminar flow hood or PCR cabinet, then additionally ensure:
 - laminar flow hood is always on
 - UV lamp is on when not in use

Do not

- Use kit after its expiration date.
- Use reagents after more than four freeze-thaw cycles.
- Use any water other than Nuclease-free Water supplied with the CytoScan™ Optima Kit.
- Reuse a plate seal.

Do

- Use 250 ng of double-stranded genomic DNA (gDNA) that is not degraded (size >10 kb by gel analysis), not contaminated, and free of PCR inhibitors; verify concentration using quantitation method specific to dsDNA. The purity ratio (A260/A280 ratio) of input DNA must be between 1.7–2.1.
- Follow procedures for gowning, etc.
- Use nuclease-free pipette tips with aerosol barriers for all pipetting steps.
- Use extraction methods that yield DNA compatible with DNA quality as specified above.
- Chill essential equipment such as cooling blocks and reagent coolers before use.
- Keep all tubes, master mixes, and working solutions in chilled cooling blocks on ice.
- Keep enzymes at –25 to –15°C until needed, then immediately place in reagent cooler chilled to –15 to –25°C. Do not store enzymes at –80°C.
- Where indicated, keep reagents chilled at 2 to 8°C and place on ice during use. After thawing, immediately place on ice and use within 1.5 hours.
- Maintain sample consistency; ensure all transitions to incubation temperatures are rapid and wellcontrolled. Enzyme activity is a function of temperature.
- Enzyme handling:
 - Maintain tube in –15 to –25°C cooler
 - Quick spin and vortex 1 to 3 seconds
 - Place back in cooler for use
- Because Fragmentation Reagent activity can decline over time after dilution on ice, add it to the samples as quickly as possible.
- Maintain 15 to 30°C (RT) throughout the procedure.
- Always use freshly prepared master mixes.
- Use only Nuclease-free Water supplied with the kit.
- Follow instructions for sealing, vortexing, and centrifuging. Ensure that plates are tightly sealed to prevent sample loss and cross-well contamination. Always use a new seal.
- Pipet accurately using calibrated pipettors.
- Use equipment calibrated according to manufacturer instructions.
- Use only specified assay stopping points.
- Check that your spectrophotometer or NanoDrop is accurately calibrated, and be sure the OD measurement is within the linear range of the instrument as per the manufacturer's recommendations.
- Hybridization oven temperature is critical to the performance of the assay. Use only the GeneChip™ Hybridization Oven 645. Hybridization ovens should be serviced at least once a year to ensure that they are operating within specification.

Controls





- Use of a pre-qualified sample [e.g., Ref 103 (Cat. No. 900421)] as a positive control is highly recommended. These controls are effective troubleshooting tools that will help you confirm the successful completion of each stage of the assay.
- Use a negative control. Use a blank well with Low EDTA TE Buffer (Cat. No. 902424). The absence of bands on your PCR gel for the negative control confirms no previously amplified PCR product has contaminated your samples.
- Oligonucleotide controls are included in the reagent kit. These controls are added to the target samples prior to hybridization.






GeneChip™ System 3000

- *GeneChip™ Fluidics Station 450 User Guide for AGCC* (Cat. No. 08-0295).
- *GeneChip™ Hybridization Oven 645 User Guide* (Cat. No. 08-0255).

Safety symbols and information

Table 1

Reagent	Contains	Warnings/precautions
Hyb Buffer Part 1 	Tetramethylamm onium chloride	DANGER. Toxic if swallowed and in contact with skin; causes skin irritation, serious eye irritation; may cause respiratory irritation. Avoid breathing and avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
TdT Buffer 	Cacodylic acid	DANGER. Harmful if swallowed, may cause cancer. Toxic if swallowed and in contact with skin; causes skin irritation, serious eye irritation; may cause respiratory irritation. Avoid breathing and avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
BSA 	Glycerol	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
DNA Ligase 	Glycerol	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.

Reagent	Contains	Warnings/precautions
Fragmentation Buffer 	Tris [Tris(hydroxymethyl) aminomethane]	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
Fragmentation Reagent 	Glycerol	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
Nsp I 	Glycerol	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
CytoScan DNA Polymerase 	Glycerol	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.
TdT 	Glycerol	WARNING. Causes minor skin irritation and eye irritation. Avoid contact with skin or eyes; if contact occurs wash immediately. If skin irritation or rash occurs, seek medical advice.

These materials contain $\leq 0.1\%$ sodium azide: Array Holding Buffer, Stain Buffer 1, Stain Buffer 2, and Purification Beads. Exercise caution in disposal: buildup of sodium azide in lead and copper plumbing can form highly explosive metal azides.



KaryoStat™ Assay procedure

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Before you begin

Read all warnings and precautions and refer to “CytoScan™ Optima Assay reagents, required.” on page 67 for module and component part number and labeling.

Prepare gDNA plate

Dilute stock gDNA to working concentration

1. Place a 96-well plate in the upper half of the cooling block on ice (Figure 1).

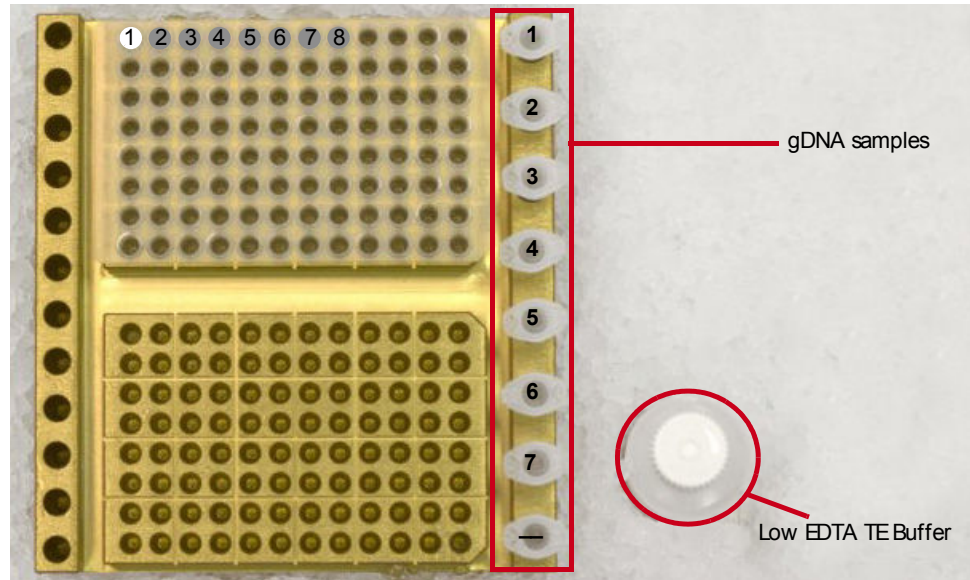


Figure 1 96-well plate in cooling block, on ice.

2. Place the gDNA at 15 to 30°C (RT) until thawed (≤ 30 minutes); then place in the cooling block on ice.

IMPORTANT! Use within 1 hour.

3. Vortex the gDNA samples for 3 seconds.
4. Centrifuge $650 \times g$ for 1 minute; then place in the cooling block.
5. Dilute each sample to $50 \text{ ng}/\mu\text{L}$ with Low EDTA TE in separate wells of the 96-well plate or in 1.5 mL Eppendorf™ tubes.

Note: Use double-strand-specific quantitation method to determine sample concentration.

6. Tightly seal the plate.
7. Centrifuge $650 \times g$ for 1 minute.
8. Place on cooling block.

Aliquot prepared gDNA into assay plate

Set up work area

1. Mark 96-well plate (Table 2, Figure 2).

Table 2 Temperature range definitions.

Temperature	Range
Freeze	-15 to -25°C
Ice	2 to 8°C
RT (Room Temperature)	15 to 30°C

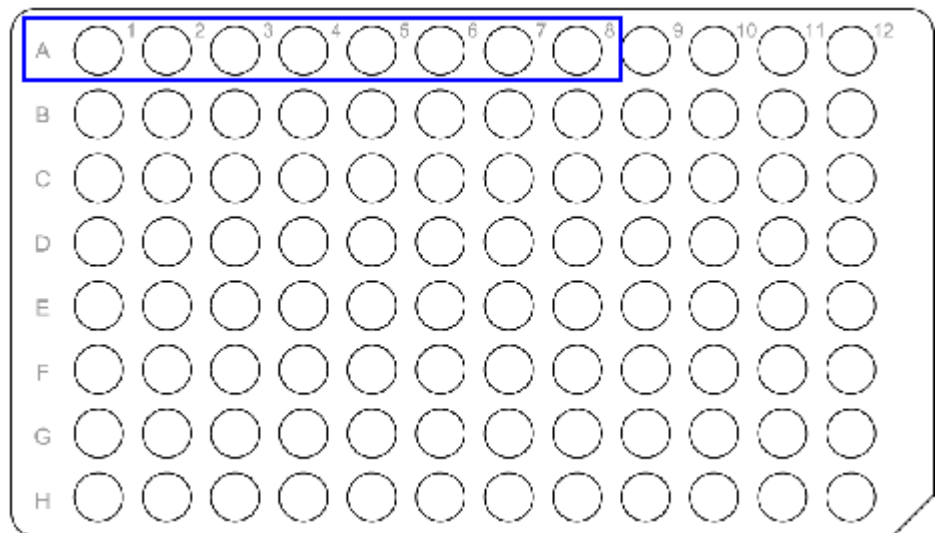


Figure 2 Plate marking.

2. Place the plate on the lower half of the cooling block.
3. Vortex gDNA 3 seconds; centrifuge.

- Transfer 5 μL of first sample from gDNA stock plate to well A1 of DIG-LIG plate; transfer all samples in same way (Figure 3).

Diluted gDNA samples
(at 50 ng/ μL concentration)

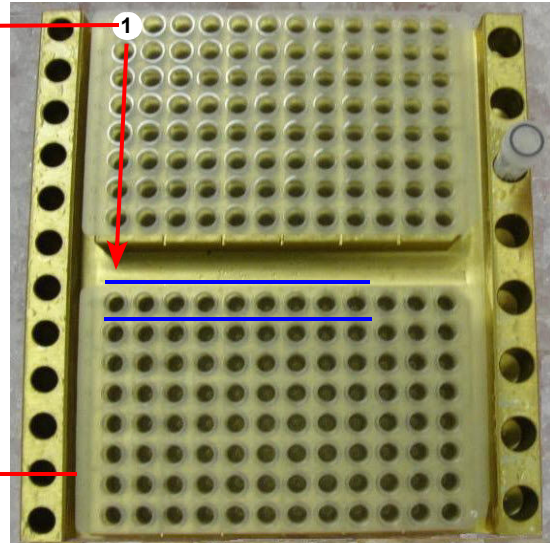


Figure 3 Transfer samples from gDNA plate to DIG-LIG plate.

- For the controls, aliquot 5 μL of:
 - Positive Control gDNA to well A7.
 - Low EDTA TE buffer [negative control] to well A8.
- Tightly seal DIG-LIG plate with new seal; centrifuge $650 \times g$ for 1 minute.

Restriction enzyme digestion

Prepare reagents_ equipment_ and consumables

Turn on the thermal cycler to preheat lid. Leave block at RT.

Set up work area

- Place the cooling block and water on ice.

Note: For more than 8 samples, place 8-12 tube strip on upper half of cooling block.
- Label a 1.5 mL tube “Dig” and place it in the cooling block.
- Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes.

Prepare reagents

- Thaw the following reagents at RT (≤ 30 minutes), immediately place on ice:
 - Nsp I Buffer
 - 100X BSA

IMPORTANT! Set up reaction within 1 hour.

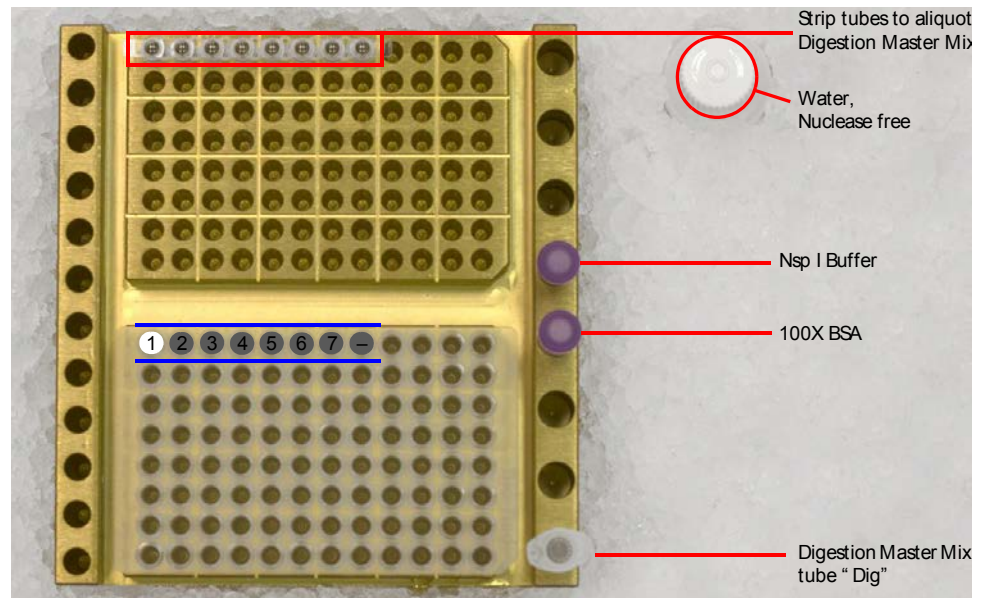


Figure 4 Digestion setup (Nsp I enzyme not pictured, still at -15 to -25°C).

2. Prepare Nsp I Buffer and 100X BSA:
 - a. Vortex 3 times for 1 second each.
 - b. Quick spin 3 seconds.
 - c. Place in cooling block on ice.
3. Place Nuclease-free Water on ice.

Prepare digestion master mix

Prepare and store all reagents, tubes, and cooling block on ice. Dispense within 10 minutes.

1. To the 1.5 mL Eppendorf™ tube (“Dig”) add per Table 3:
 - Water, Nuclease free, chilled (2 to 8°C)
 - Nsp I Buffer
 - 100X BSA

Table 3 Digestion master mix (≥ 8 samples, 20% overage).

Reagent	1 sample	8 samples	16 samples	24 samples
Water, Nuclease free	11.55 µL	110.9 µL	221.8 µL	332.6 µL
Nsp I Buffer	2.00 µL	19.2 µL	38.4 µL	57.6 µL
100X BSA	0.20 µL	1.9 µL	3.8 µL	5.8 µL
Nsp I	1.00 µL	9.6 µL	19.2 µL	28.8 µL
Total	14.75 µL	141.6 µL	283.2 µL	424.8 µL

2. Vortex 3 times for 1 second each.
3. Quick spin 3 seconds.
4. Place Digestion Master Mix in chilled cooling block (2 to 8°C).
5. Remove Nsp I enzyme from the freezer and immediately place in a chilled cooler (–15 to –25°C).
6. Mix Nsp I.
 1. Quick spin 1 second.
 2. Vortex 1 second.
 3. Quick spin 3 seconds.
7. Return to –15 to –25°C cooler while preparing Digestion Master Mix.
8. Immediately add Nsp I to Digestion Master Mix.
9. Return enzyme to –15 to –25°C cooler.
10. Vortex Digestion Master Mix 3 times for 1 seconds each.
11. Quick spin 3 seconds.
12. Place in chilled cooling block (2 to 8°C).

Add digestion master mix to samples

1. Divide the Digestion Master Mix equally into 8- or 12-strip tubes on cooling block on ice.
2. Seal strip tube with adhesive film strip (or strip caps).
3. Centrifuge $650 \times g$ for 1 minute and place on cooling block on ice.
4. Remove and discard seal.
5. Unseal the plate and discard seal.
6. Use multichannel P20 pipette and add $14.75 \mu\text{L}$ of the Digestion Master Mix to each sample in row A (Figure 5).

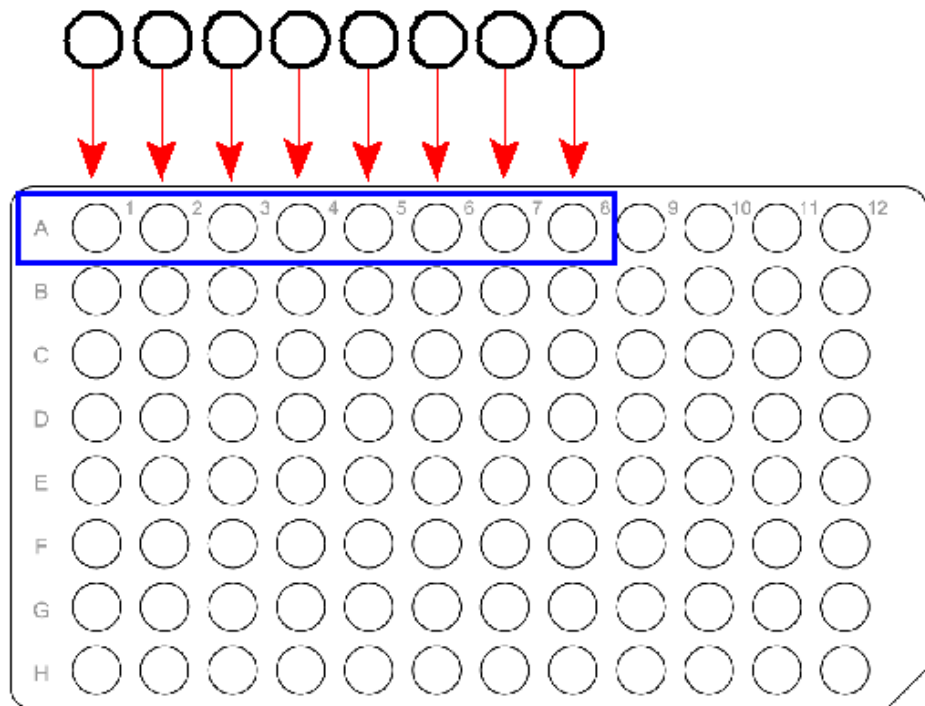


Figure 5 Addition of digestion master mix to samples.

7. Seal the plate tightly with a new seal.
 1. Vortex the plate 1 second all corners and center.
 2. Centrifuge $650 \times g$ for 1 minute.

Load samples on thermal cycler

3. Load the plate on thermal cycler, ensure thermal cycler lid is preheated, and run CytoScan™ Optima Digest program.

Table 4 CytoScan™ optima digest.

Temperature	Time
37°C	2 hours
65°C	20 minutes
4°C	5 minutes
4°C	∞

4. Discard the remaining Digestion Master Mix and return reagents to the freezer. When done, leave the plate in thermal cycler. Process within 2.5 hours.
5. Ensure the plate is sealed tightly and centrifuge $650 \times g$ for 1 minute.
6. Place the plate in cooling block on ice and immediately go to “Ligation” on page 18.

Ligation

Turn on the thermal cycler to preheat lid. Leave block at RT.

Set up work area

1. Place cooling block on ice.
2. Label a 1.5 mL tube “Lig” and place it in cooling block.
3. Place 8-tube strip on upper half of cooling block.
4. Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes.

Thaw reagents and digested samples

1. Allow the following to thaw at RT (≤ 30 minutes):
 - Adaptor, Nsp I
 - DNA Ligase Bfr

2. Immediately place on ice and use within 1 hour.

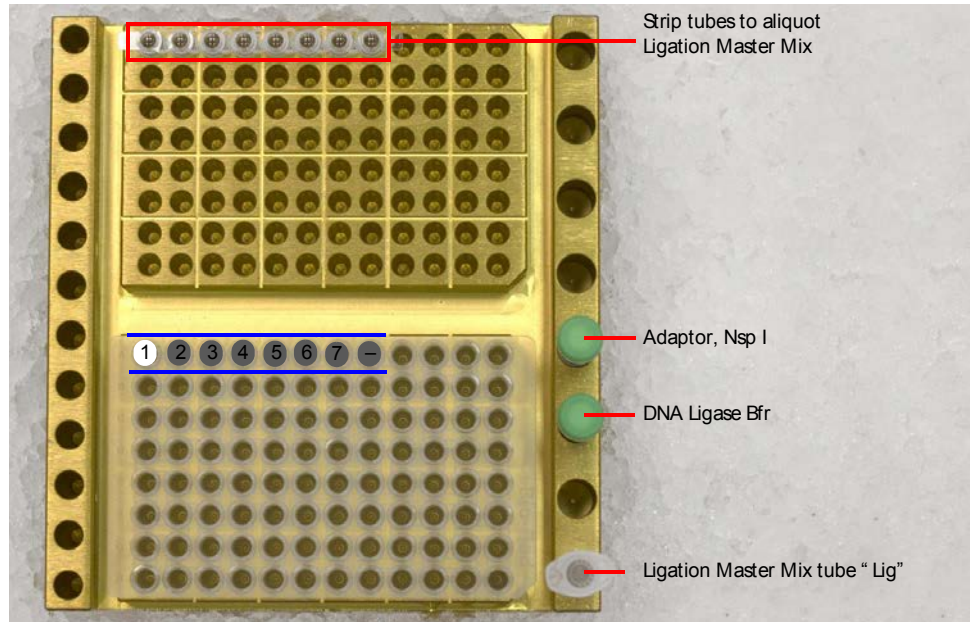


Figure 6 Ligation setup (DNA Ligase not pictured, still at -15 to -25°C).

Prepare digested samples and reagents

1. Prepare samples:
 - a. Centrifuge $650\times g$ for 1 minute.
 - b. Place in lower half of cooling block on ice.
2. Prepare reagents:
 - a. Vortex DNA Ligase Bfr and Adaptor, Nsp I 3 times for 1 second each.
Note: If precipitate is present in DNA Ligase Bfr, vortex until precipitate is dissolved.
 - b. Quick spin 3 seconds.
 - c. Place in chilled cooling block (2 to 8°C).

Prepare ligation master mix

Prepare and store all reagents, tubes, and cooling block on ice. Dispense within 10 minutes.

1. Label a 1.5 mL tube "Lig" and add per Table 5:
 - DNA Ligase Buffer
 - Adaptor, Nsp I

Table 5 Ligation master mix (≥8 samples, 25% overage).

Reagent	1 sample	8 samples	16 samples	24 samples
DNA Ligase Bfr	2.50 µL	25.0 µL	50.0 µL	75.0 µL
Adaptor, Nsp I	0.75 µL	7.5 µL	15.0 µL	22.5 µL
DNA Ligase	2.00 µL	20.0 µL	40.0 µL	60.0 µL
Total	5.25 µL	52.5 µL	105.0 µL	157.5 µL

2. Vortex 3 times for 1 second each.
3. Quick spin 3 seconds.
4. Remove the DNA Ligase from freezer and immediately place in chilled cooler (-15 to -25°C).
5. Mix DNA Ligase.
 - a. Quick spin 1 second.
 - b. Vortex 1 second.
 - c. Quick spin 3 seconds.
6. Place in -15 to -25°C cooler.
7. Immediately add the DNA Ligase to the Ligation Master Mix; place back in -15 to -25°C cooler.
8. Vortex the Ligation Master Mix 3 times for 1 second each.
9. Quick spin 3 seconds.
10. Place the Ligation Master Mix in cooling block on ice.
11. Immediately go to next step.

Add ligation master mix

1. Divide the Ligation Master Mix equally into 8 or 12 strip tubes on ice.
2. Seal strip tube with adhesive film strip (or strip caps) and quick spin.
3. Place back in cooling block on ice.
4. Remove and discard the strip tube seal.
5. Unseal the digested sample plate and discard seal.

- Use a multi-channel P20 pipette to add 5.25 μL of the Ligation Master Mix to each digested sample (Figure 7).

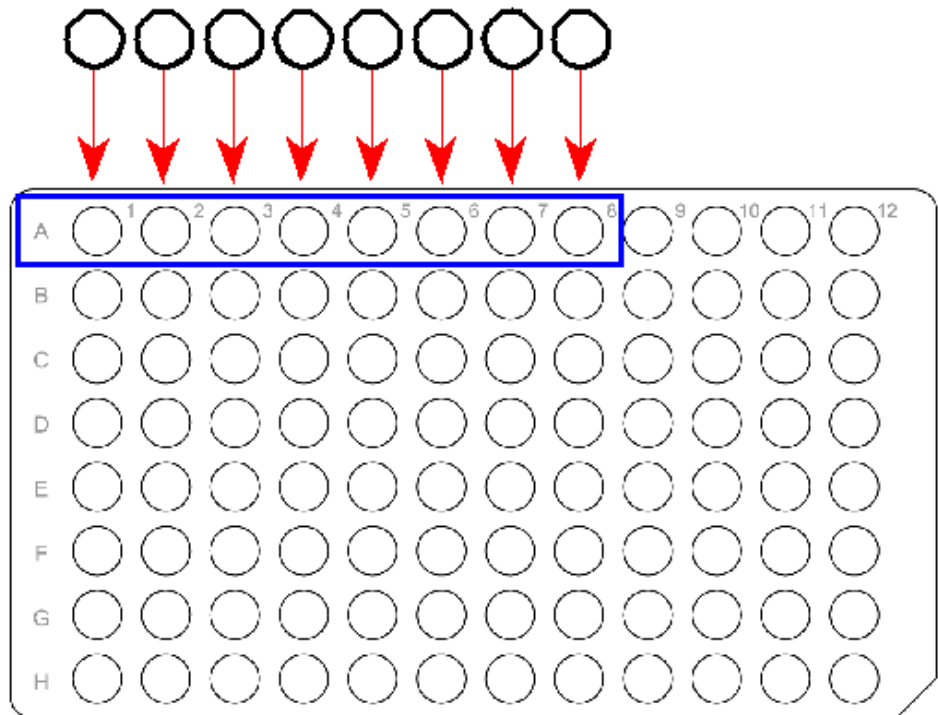


Figure 7 Addition ligation master mix.

Load samples on thermal cycler

Ensure thermal cycler lid is preheated.

- Seal the plate tightly with new seal.
- Vortex for 1 second in all corners and center.
- Centrifuge $650 \times g$ for 1 minute.
- Load the plate on the thermal cycler and run CytoScan™ Optima Ligate program (Table 6).

Table 6 CytoScan™ optima ligate thermal cycler program.

Temperature	Time
16°C	3 hours
70°C	20 minutes
4°C	5 minutes
4°C	∞

- Discard the remaining Ligation Master Mix and return reagents to the freezer.

6. When program finishes, leave the plate in thermal cycler.

Note: You can hold plate in thermal cycler at 4°C for up to 16-20 hours (overnight).
7. After removing from thermal cycler, ensure the plate is sealed tightly.
8. Centrifuge 650 × g for 1 minute.
9. Store at –15 to –25°C.

STOPPING POINT Optional: The plate may be frozen at –15 to –25°C for up to 1 week.

PCR

Turn on the thermal cycler in the Post-PCR Room to preheat lid.

Dilute ligated samples

Twenty minutes before use, place Nuclease-free Water on ice.

1. Place cooling block on ice.
2. Place reagent reservoir on upper half of cooling block on ice (Figure 8).

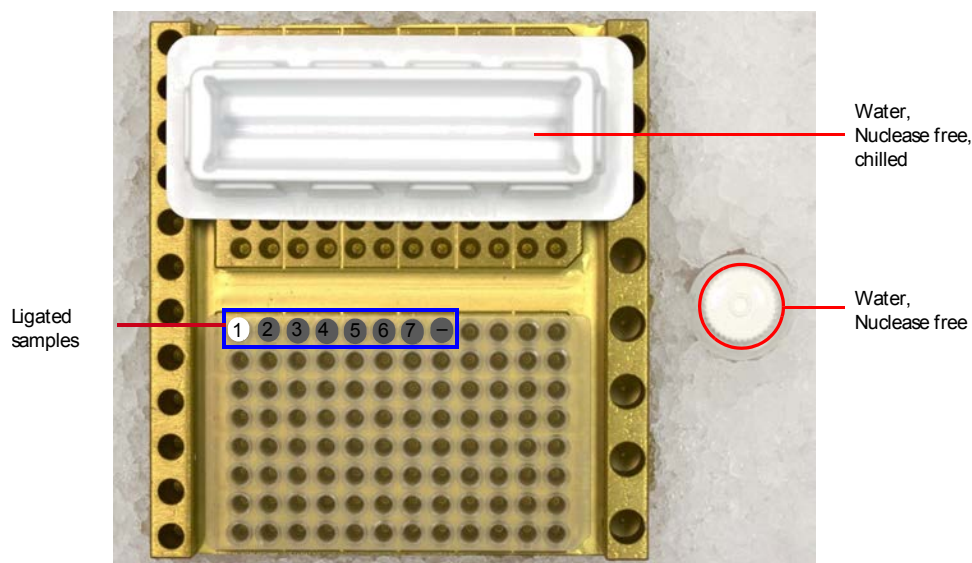


Figure 8 Reagent reservoir on upper half of cooling block on ice.

3. Pour the chilled Nuclease-free Water into reagent reservoir.
4. Place the plate in lower half of the cooling block on ice.

Note: If samples are frozen, thaw at RT (≤ 30 minutes). Centrifuge 650 × g for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.
5. Unseal the ligated sample plate and discard seal.

- Use a P200 pipette to add 75 μL of Nuclease-free Water to each reaction.

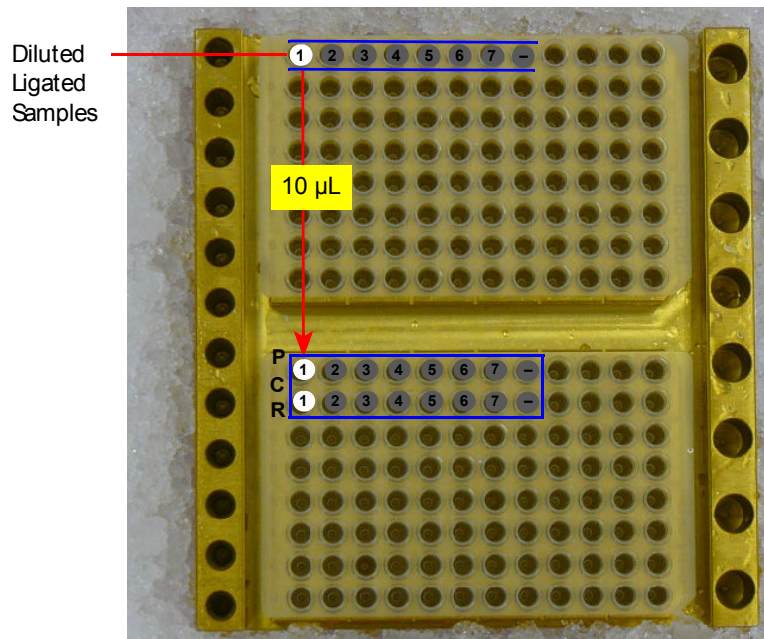
Sample	Volume/sample
Ligated DNA	25 μL
Water, Nuclease free (chilled)	75 μL
Total	100 μL

- Tightly seal plate with new seal.
- Vortex 1 second in all corners and center.
- Centrifuge $650 \times g$ for 1 minute.
- Go to “Run PCR” on page 23.

Run PCR

Transfer to PCR plate

- Place cooling block on ice.
- Keep the diluted ligated sample plate on the upper half of the cooling block.
- Label a new plate “PCR” and then place in the lower half of the cooling block.
- Unseal the ligated and diluted sample plate and discard seal.
- Use a multi-channel P20 pipette to transfer 10 μL of each sample to the corresponding well of the PCR plate (transfer 2 times). Store leftover samples at -15 to -25°C .



- Seal the plate tightly with a new seal and centrifuge $650 \times g$ for 1 minute.

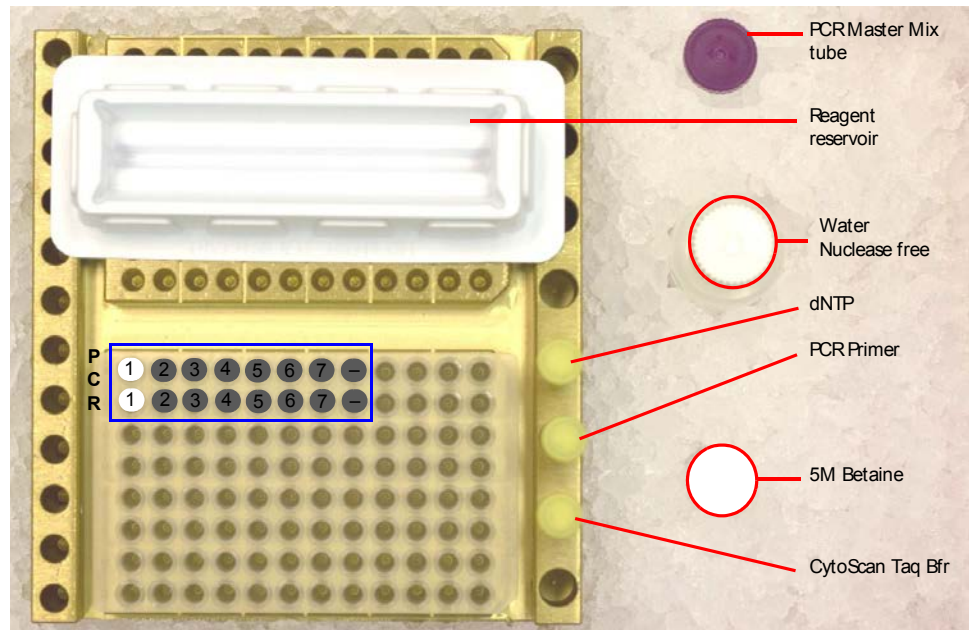
Thaw reagents and samples

Thaw at RT (≤ 30 minutes); immediately place on ice and use within 1 hour:

- CytoScan™ Taq Buffer
- dNTP
- PCR Primer

Prepare reagents and ligated samples

1. Label 15 mL centrifuge tube "PCR".
2. Place on ice:
 - Water, Nuclease free (chilled on ice for 10 minutes)
 - 5M Betaine (If precipitate is present, vortex until precipitate is dissolved)
 - Reagent reservoir on upper half of cooling block on ice



3. Immediately place in the lower half of the cooling block on ice.
4. Prepare reagents (not enzyme):
 - a. Vortex 3 times for 1 second each.
 - b. Quick spin 3 seconds.
 - c. Place in chilled cooling block (2 to 8°C).

Prepare PCR master mix

Prepare and store all reagents, tubes, and cooling block on ice. Dispense within 20 minutes.

1. Keep 15 mL centrifuge tube on ice, add reagents in order shown in Table 7.

Table 7 PCR master mix (≥8 samples, 20% overage).

Reagent	1 reaction	8 samples	16 samples	24 samples
Water, Nuclease free (chilled)	50.3 µL	965.8 µL	1,931.5 µL	2,897.3 µL
CytoScan™ Taq Bfr	10.0 µL	192.0 µL	384.0 µL	576.0 µL
5M Betaine	20.0 µL	384.0 µL	768.0 µL	1,152.0 µL
dNTP	3.5 µL	67.2 µL	134.4 µL	201.6 µL
PCR Primer	4.2 µL	80.6 µL	161.3 µL	241.9 µL
CytoScan™ Taq DNA Polymerase	2.0 µL	38.4 µL	76.8 µL	115.2 µL
Total	90 µL	1,728 µL	3,456 µL	5,184 µL

IMPORTANT! Leave the CytoScan™ Taq DNA Polymerase at –20°C until ready to use.

2. Vortex 3 seconds.
3. Take the CytoScan™ Taq DNA Polymerase from the freezer and immediately place in a chilled cooler (–15 to –25°C).
4. Mix CytoScan™ Taq DNA Polymerase.
 - a. Quick spin 1 second.
 - b. Vortex 1 second.
 - c. Quick spin 3 seconds.
5. Immediately add to the PCR Master Mix, then return to –15 to –25°C cooler.
6. Vortex the PCR Master Mix 3 times for 1 second each.
7. Pour the PCR Master Mix into the reagent reservoir. Keep cooling block on ice.

Add PCR master mix to samples

1. Unseal the PCR sample plate and discard seal.
2. Use a multi-channel P200 pipette to add 90 µL of the PCR Master Mix to each sample on the PCR plate.
 - Avoid contamination: Change pipette tips after each transfer.
 - 8 samples: Tilt reagent reservoir so each pipette tip picks up 90 µL.

Sample	Volume/sample
Ligated and diluted DNA	10 µL
PCR Master Mix	90 µL
Total	100 µL

3. Tightly seal the plate with a new seal.
4. Vortex for 1 second in all corners and center.
5. Repeat vortex 1 time.
6. Centrifuge at 650 × g for 1 minute.
7. Immediately load the plate on the thermal cycler.

Load plate and run PCR program

Ensure thermal cycler lid is preheated. Ensure block is RT (15 to 30°C).

1. Move the plate on ice to Post-PCR Room.
2. Load the plate on the thermal cycler.
3. Run the CytoScan™ Optima PCR program.

Note: The plate maybe held 16-20 hours (overnight) in the thermal cycler at 4°C.

Table 8 CytoScan™ optima PCR thermal cycler program.

Temperature	Time	Cycles
95°C	1 minute	1X
95°C	30 seconds	30X
60°C	45 seconds	
68°C	60 seconds	
68°C	7 minutes	1X
4°C	5 minutes	
4°C	Hold (can be held overnight)	
Volume: 100 µL Specify Maximum Mode		

4. When done, leave the plate in the thermal cycler. If proceeding to purification, the plate must be processed within 2.5 hours.
5. After removing from thermal cycler, keep the plate on a 96-well plate rack.
6. Ensure that the plate is tightly sealed.
7. Centrifuge $650 \times g$ for 1 minute.

STOPPING POINT Optional: If not proceeding immediately to the next step, the plate may be stored at -20°C for up to 1 week.

Check PCR reaction

1. Unseal the PCR plate and discard seal.
2. Transfer 3 μL of the PCR product from each well of row A to corresponding wells of the strip tube (Figure 9) for in-process QC.

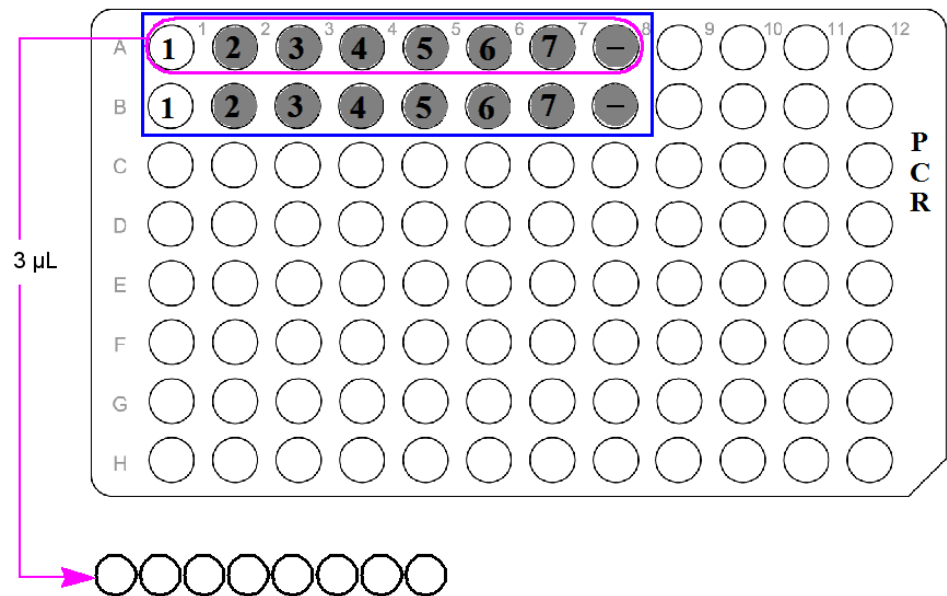


Figure 9 Transfer of PCR products to gel plate.

IMPORTANT! For >12 samples, transfer 3 μL PCR product from first row for every 2 rows of same samples.

3. Seal the PCR plate tightly with a new seal.
4. Seal the gel strip tubes tightly with an adhesive film strip or strip tube caps.
5. Vortex gel strip tubes at maximum setting for 1-3 seconds.
6. Centrifuge in microfuge.

7. Prepare the QC samples by adding 17 µL of diluted loading buffer (see “In-Process quality control” on page 54) and run samples on 2% agarose gel to resolve bands.
8. Inspect gel and follow “In-Process quality control” on page 54. The majority of PCR target distribution is 150 to 2200 bp (note higher intensities at 300 to 2200 bp).

PCR product purification

Prepare purification wash buffer

1. Add 45 mL absolute ethanol to the Purification Wash Buffer bottle.
2. Cap the bottle tightly and mix by inverting unopened Purification Wash Buffer bottle 10 times.
3. Write the date of the ethanol addition on the bottle.

Prepare PCR products

Pool PCR products

Note:

- Requires transfer of assay intermediate from plate to independent tubes.
- Perform these steps at room temperature.

1. Mark each 2.0 mL tube with the sample number using a permanent marker.
2. Ensure that the plate is tightly sealed.
3. Centrifuge $650 \times g$ for 1 minute.
4. Remove and discard plate seal.

- Use a P200 pipette to transfer all 2 well aliquots of each sample to marked 2.0 mL tube.

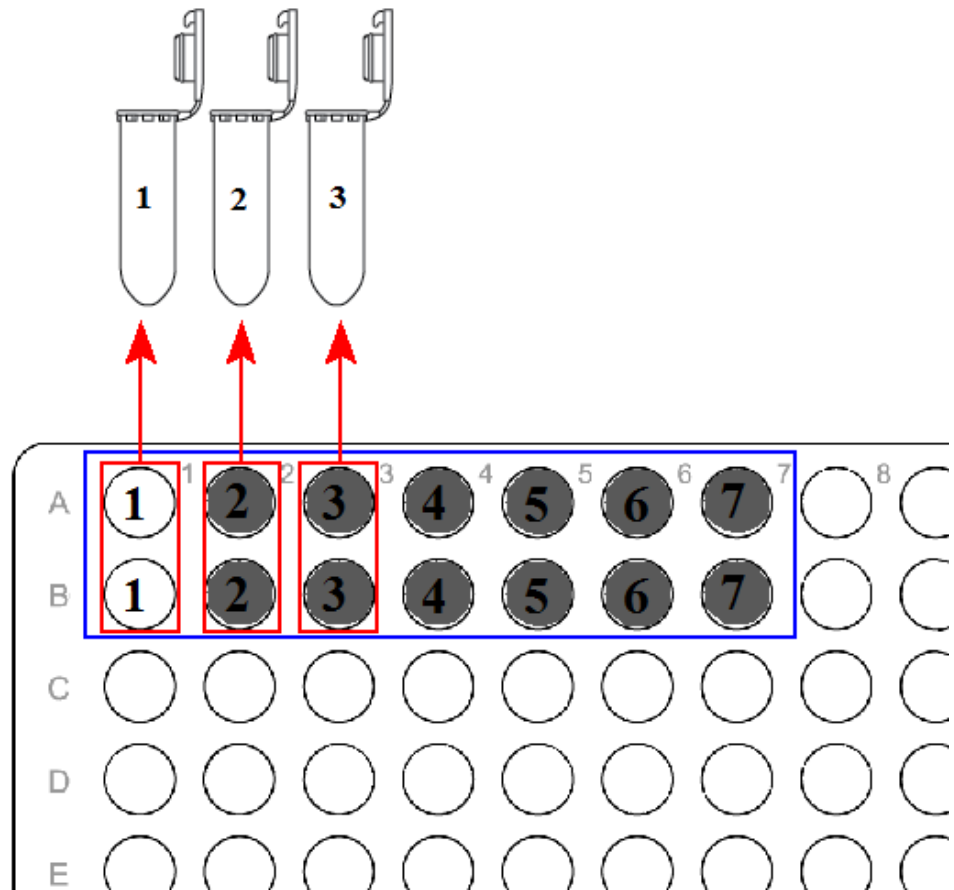


Figure 10 Pool PCR products.

Note: If using a multi-channel P200 pipette, organize tips to ensure multi-channel pipetting into wells of plate and tubes to match well/tube and pipette.

IMPORTANT! To avoid cross-contamination, use new pipette for each transfer and take care with pipette tips when pooling samples for purification.

- Ensure that the total volume in each well is transferred and pooled.

Volume in each PCR well	= 100 μ L
Total volume in 2 PCR wells	= 200 μ L
Total Volume in Each 2.0 mL Tube	= 200 μ L/tube -3 μ L aliquoted for PCR gel

Purify PCR products

Change tips between pipetting steps.

1. Thoroughly mix the Purification Beads stock by inverting the bottle 10 times. Examine the bottom of the bottle to ensure that the solution appears homogeneous.
2. Slowly open tube caps to prevent spill.
3. Add Purification Beads to each pooled sample:
 - 8 Samples: Use a single-channel P1000 pipette to add 360 μ L Purification Beads per sample directly from bottle.
 - 16 and 24 Samples: Use a multi-channel P1000 pipette to add 360 μ L Purification Beads per sample.
 - 16 samples: Add 8 mL Purification Beads to reagent reservoir.
 - 24 samples: Add 12.5 mL Purification Beads to reagent reservoir.
 - Add 360 μ L Purification Beads to each pooled sample, 3 to 4 samples at a time. Organize tips to enable multi-channel pipetting into tubes to match tube and pipette.
4. Securely cap each tube and invert 10 times.
5. Incubate at RT for 10 minutes.
6. Load tubes onto centrifuge with cap hinge facing out and centrifuge 16,000 \times g for 3 minutes.

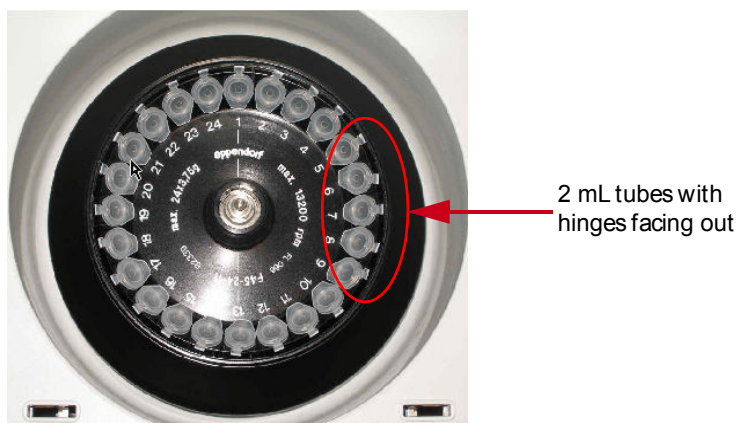


Figure 11 2 mL tubes loaded into centrifuge.

7. Place the tube on magnetic stand so cap hinge is over magnet. Ensure pellet is completely pulled towards magnet.
8. Use a P1000 pipette to remove supernatant without disturbing bead pellet. Discard supernatant.
 - 16 and 24 Samples: Use a multi-channel P1000 pipette. Remove supernatant from 3 to 4 samples at a time.

Add purification wash buffer

1. Use a P1000 pipette to add 800 μ L Purification Wash Buffer to each tube.
 - 16 and 24 Samples: Pour Purification Wash Buffer into reservoir. With multi-channel pipette add 800 μ L Purification Wash Buffer to each tube, 3 to 4 samples at a time.
2. Cap the tubes and load into foam tube adapter. Fully insert the tubes into foam to secure. Space sample tubes evenly. Use balance if necessary.
3. Vortex 2 minutes.

Note: Bead pellet may not be completely resuspended; this is acceptable.
4. Centrifuge tubes, cap hinges facing out, 16,000 $\times g$ for 3 minutes.
5. Place the tubes on magnetic stand and visually verify that beads are completely pulled towards magnet with no beads present in Purification Wash Buffer supernatant at bottom of tubes.
6. Visually verify that bead pellet is adjacent to magnet when removing supernatant.
 - a. Use P1000 pipette to remove supernatant without disturbing bead pellet.
 - b. Discard supernatant.
 - 16 and 24 Samples: Use multi-channel pipette to remove supernatant from 3 to 4 samples at a time.
7. Centrifuge tubes, cap hinges facing out, 16,000 $\times g$ for 30 seconds; place tubes back on magnetic stand so cap hinge is over magnet. Ensure pellet is completely pulled towards magnet.
8. Use a P20 pipette to remove remaining drops of Purification Wash Buffer from each tube bottom, 1 sample at a time. Do not disturb or break off any bead pellet.
9. Take tubes OFF magnetic stand and allow remaining Purification Wash Buffer to evaporate by leaving tubes uncapped at RT for 7 minutes.

Add elution buffer

1. Use a P100 pipette to add 27 μ L Elution Buffer to each tube directly onto beads.
 - 16 and 24 Samples: Use a multi-channel P200 pipette. Add to 3 to 4 samples at a time.
2. Cap tubes and load into foam tube adapter. Balance tubes.
3. Vortex 10 minutes to resuspend beads.
4. Examine each tube to be sure beads are resuspended in homogeneous slurry. (If beads are not fully resuspended, flick tube to dislodge pellet, vortex 2 minutes at maximum speed. Re-examine tubes and dislodge and vortex pellet until beads are resuspended.)

5. With cap hinges facing out, centrifuge tubes $16,000 \times g$ for 3 minutes.
6. Place the tubes on magnetic stand for 10 minutes so beads are pulled to side of tube.
Note: Visually verify that bead pellet is completely pulled towards magnet with no beads present in Elution Buffer at bottom of tubes.
7. Transfer 25 μL of eluted sample to correct well on new 96-well plate (Figure 12).

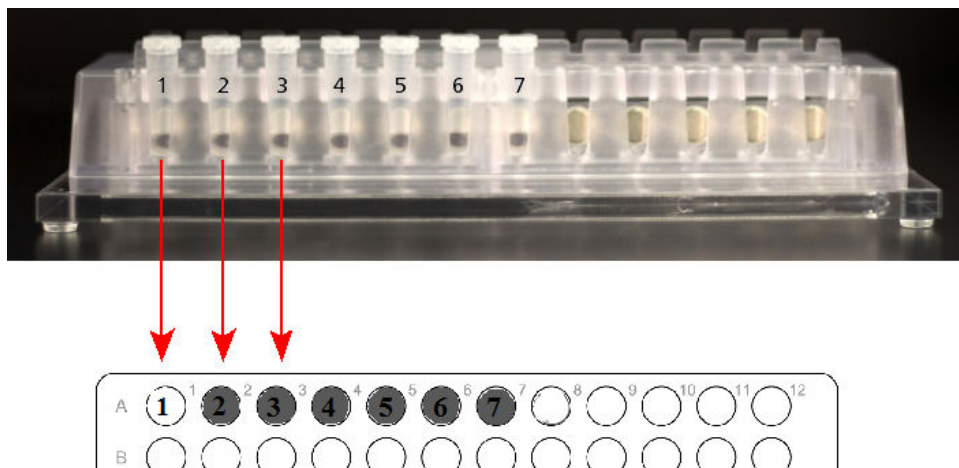


Figure 12 Purified sample transfer to fresh plate.

IMPORTANT! Ensure correct volume is pipetted as fragmentation step is sensitive to input mass.

Note: Eluate may appear brownish. Sometimes a brown residue is seen at pipette tip, but usually remains behind on tip when sample is pipetted out.

8. Tightly seal plate, vortex 1 second in all corners and center.
9. Centrifuge $650 \times g$ for 1 minute.
10. Ensure plate is tightly sealed.
11. Go to “Quantitation” on page 32.

Quantitation

This assay was optimized using a UV spectrophotometer for quantitation. OD measurement accuracy is critical. Be sure OD measurement is within instrument linear range.

Prepare reagents, equipment, and consumables

Turn spectrophotometer on at least 10 minutes before use.

Place on bench top:

- Water, Nuclease free
- UV plate or 96-well plate
- Optional: conical tube or reagent reservoir

Microplate spectrophotometer**Prepare diluted aliquots of purified samples**

1. Use a multi-channel P200 pipette to add 198 μL Nuclease-free Water to sample wells of the UV plate.
2. Make blank: Add 200 μL Nuclease-free Water to each well of empty row (Figure 13).

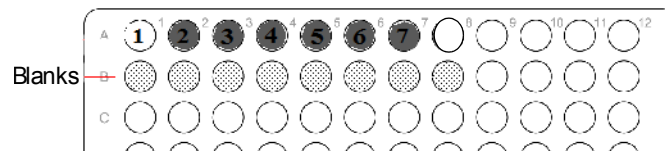


Figure 13 Row for blanks.

3. Use a multi-channel P20 pipette to:
 - a. Transfer 2 μL of each purified sample to the corresponding well of the UV plate.
 - b. Pipet up and down 2 times so all sample is dispensed (100-fold dilution).
4. Use new seal, tightly seal plate with purified samples, and use lint-free lab wipe on adapter surface.
5. Vortex, then centrifuge $650 \times g$ for 1 minute. Proceed immediately to quantitation or store at -15°C to -25°C .

IMPORTANT! If stored at -15 to -25°C the plate must be removed within 20 hours.

Quantitate diluted purified PCR product

Note: One absorbance unit at 260 nm = 50 $\mu\text{g}/\text{mL}$ (equivalent to 0.05 $\mu\text{g}/\mu\text{L}$) for double-stranded PCR products (for path length = 1 cm).

1. Measure OD of each sample at 260, 280, and 320 nm. Use OD_{280} and OD_{320} as controls.
2. Determine OD_{260} measurement for water blank and calculate average.
3. Determine concentration of each PCR product:
 - a. Calculate one OD_{260} reading for every sample: $\text{OD}_{260} = (\text{sample } \text{OD}_{260}) - (\text{average water blank } \text{OD}_{260})$
 - b. Calculate undiluted concentration for each sample in $\mu\text{g}/\mu\text{L}$:
 $\text{OD}_{260} \times 0.05 \mu\text{g}/\mu\text{L} \times 100$.

4. Determine acceptable DNA yield.

Note: For each sample the DNA yield must be $\geq 2.5 \mu\text{g}/\mu\text{L}$. Average DNA yield for 8 or more samples must be $\geq 3.0 \mu\text{g}/\mu\text{L}$. Do not further process any samples that do not meet this specification.

These OD ranges are based on use of conventional UV spectrophotometer plate readers and assume path length = 1 cm.

- $\text{OD}_{260}/\text{OD}_{280}$ ratio = 1.7 to 2.1.
- OD_{320} measurement very close to zero (≤ 0.1).

5. Immediately go to “Fragmentation” on page 34 or store plate at -15 to -25°C for 16-20 hours.

STOPPING POINT OPTIONAL: The plate maybe stored at -15 to -25°C for up to 1 week.

Fragmentation

Perform all additions, dilutions, and mixing on ice. Ensure all reagents reach equilibrium before use.

Precautions

- Enzyme is temperature sensitive:
 - Handle tube by cap or base only. Do not touch tube sides.
 - Keep at -15 to -25°C except when aspirating enzyme volume.
 - Centrifuge to ensure contents of tube are uniform.
 - Perform all steps rapidly and without interruption.
- Add enzyme to Fragmentation Master Mix last.
- Enzyme is viscous:
 - Pipet slowly.
 - Avoid excess solution outside pipette tip.

Preparation

Turn on thermal cycler to preheat lid. Leave block at RT.

Set up work area

1. Set the plate centrifuge to 2 to 8°C at least 15 to 20 minutes before fragmentation step and close lid.
2. Place cooling block and Nuclease-free Water on ice.

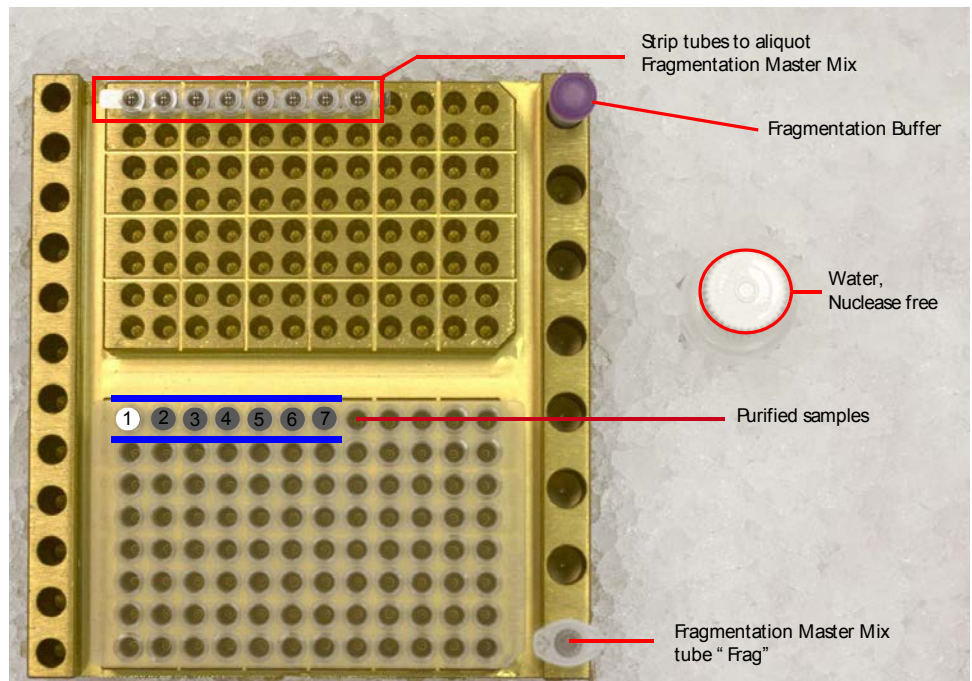


Figure 14 Fragmentation setup (Fragmentation Reagent not pictured, still at -15 to -25°C).

3. Place 8-tube strip in chilled cooling block (2 to 8°C) at least 10 minutes before use.
4. Label a 1.5 mL Eppendorf™ tube “FRAG” and keep chilled in cooling block.
5. Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes.

Thaw and prepare the reagents

1. Thaw the plate of purified, quantitated samples at RT (≤30 minutes).
2. Ensure a tight seal and centrifuge plate 650 × g for 1 minute.
3. Place the plate on the lower half of cooling block on ice for at least 10 minutes. Process within 1 hour.
4. Thaw Fragmentation Buffer at RT (≤30 minutes); place on cooling block on ice.

IMPORTANT! Use within 1 hour.

5. Prepare Fragmentation Buffer:
 - a. Vortex 3 times for 1 second each.
 - b. Quick spin 3 seconds.
 - c. Place in chilled cooling block (2 to 8°C).

Prepare fragmentation master mix

Prepare and store all reagents, tubes, and cooling block on ice. Dispense within 10 minutes. Always prepare Fragmentation Master Mix according to Table 9 even when processing less than 24 samples.

Table 9 Fragmentation master mix.

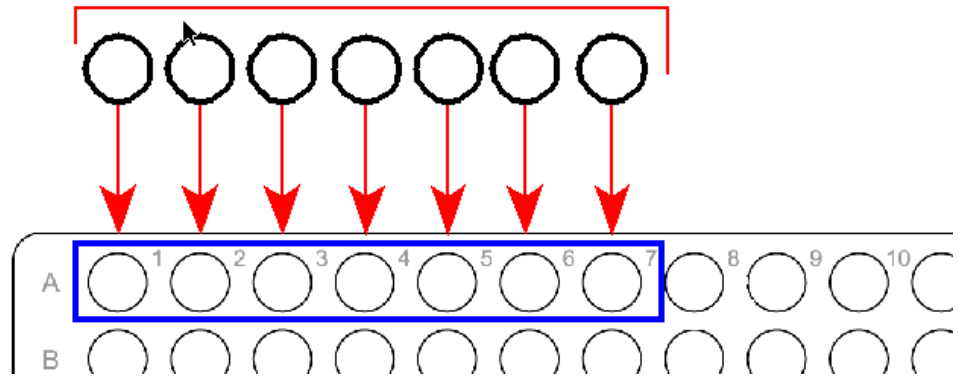
Reagent	Volume
Water, Nuclease free	215 µL
Fragmentation Buffer	275 µL
Fragmentation Reagent	10.0 µL
Total	500 µL

1. Add Nuclease-free Water and Fragmentation Buffer per Table 9.
 - a. Vortex tube 3 times for 1 second.
 - b. Quick spin 3 seconds.
2. Remove the Fragmentation Reagent from the freezer and immediately place in cooler chilled to -15 to -25°C.
 - a. Immediately quick spin 3 seconds to bring down reagent from top of tube.
 - b. Vortex 1 second.
 - c. Quick spin 3 seconds.
 - d. Immediately place in -15 to -25°C cooler.
3. Add Fragmentation Reagent per Table 9.
4. Immediately place back in -15 to -25°C cooler.
5. Vortex the Fragmentation Master Mix 3 times for 1 seconds each.
6. Quick spin 3 seconds and immediately place in cooling block.

Fragmentation

Add fragmentation master mix to samples

1. Quickly add 40 μL of the Fragmentation Master Mix to each well of strip tubes placed in cooling block on ice.
2. Seal the strip tubes with adhesive film strip or strip caps.
3. Centrifuge the strip tubes and place back in cooling block on ice. Remove and discard seal.
4. Remove and discard the plate seal.
5. Add the Fragmentation Master Mix. Use a multi-channel P20 pipette to transfer 5 μL Fragmentation Master Mix to each sample. Do not pipet up and down. Do not introduce air bubbles at bottom of tubes.



STOPPING POINT It is recommended to use a multi-channel pipette to dispense the Fragmentation Master Mix for any number of samples because fragmentation is a time and temperature sensitive step.

Sample	Volume/sample
Purified PCR product	23 μL
Fragmentation Master Mix	5 μL
Total	28 μL

6. Seal the plate tightly with new seal.
7. Vortex 1 second in all corners and center.
8. Bring the sample plate to the centrifuge on cooling block in ice box (2 to 8°C).
9. Centrifuge the plate in prechilled centrifuge 650 $\times g$ for 1 minute. Quickly remove the plate from centrifuge and place in cooling block in ice box.
10. Carry the sample plate on the cooling block and immediately load the Fragmentation plate on thermal cycler with preheated lid.

11. Run the CytoScan™ Optima Fragment thermal cycler program.

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	5 minutes
4°C	∞

12. When done, leave the plate in the thermal cycler. Process within 2.5 hours.
13. After removing from the thermal cycler, ensure that the plate is sealed tightly and centrifuge $650 \times g$ for 1 minute, and transfer to chilled cooling block on ice.
14. Remove and discard any remaining Fragmentation Master Mix. Never reuse Fragmentation Master Mix.

Check fragmentation reaction

1. Ensure that the plate is tightly sealed; centrifuge $650 \times g$ for 1 minute. Place on the lower half of the cooling block on ice.
2. Label an 8-tube strip tube "Dil".
3. Add 14 μ L Nuclease-free Water to each well of strip tube.
4. Unseal the fragmented DNA plate and discard seal.

5. Add 2 μL of fragmented samples from plate to corresponding wells of strip tube “Dil”.

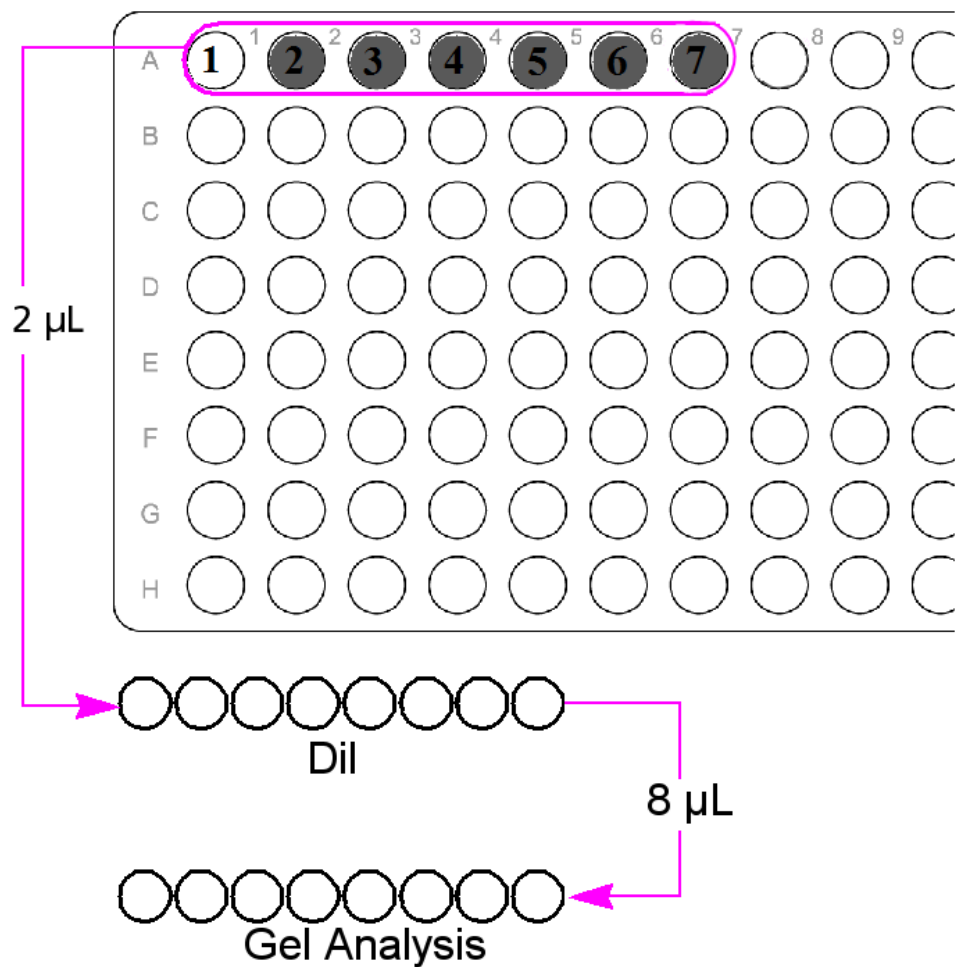


Figure 15 Transfer 2 μL fragmented product to strip tube.

6. Seal fragmented DNA plate with new seal and keep on lower half of cooling block on ice.
7. Label a fresh 8- or 12-well strip tube “Gel Analysis”. Add 12 μL of diluted loading buffer to each well.
 - a. Add 8 μL diluted fragmented product from Step 6 to 12 μL diluted loading buffer in Step 8.
 - b. Pulse vortex.
 - c. Centrifuge $650 \times g$ for 1 minute.
8. Run samples on 4% agarose gel to resolve bands.

9. Inspect gel and follow “In-Process quality control” on page 54. The majority of fragment size distribution must be 25 to 125 bp.
10. Proceed immediately to “Labeling” on page 40.

STOPPING POINT Optional: If not proceeding to the next step the fragmented DNA plate maybe held at -15 to -20°C for up to 60 hours.

Labeling

Preparation

Turn on the thermal cycler to preheat lid. Leave block at RT.

Set up work area

1. Place the cooling block on ice.
2. Place a 8-tube strip in upper half of cooling block on ice.
3. Label a 1.5 mL microfuge tube “LBL” and place in cooling block.

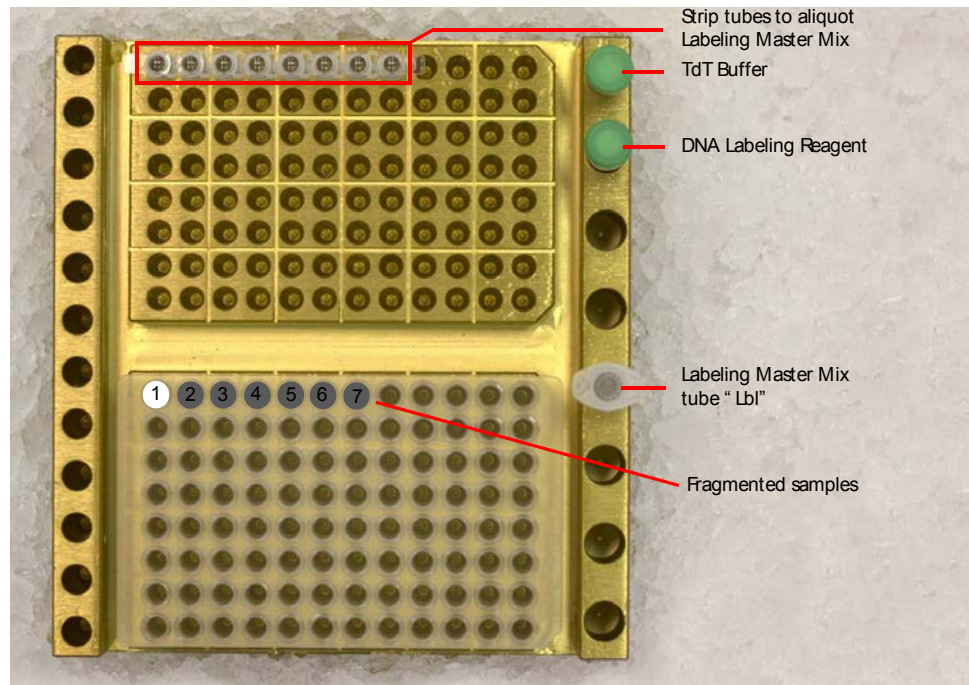


Figure 16 Labeling setup (TdT Enzyme at -15 to -20°C and hence not shown).

4. Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes.

Thaw and prepare the reagents

1. Thaw at RT (≤ 30 minutes), then immediately place on ice and use within 1 hour:
 - TdT Buffer
 - DNA Labeling Reagent

2. Prepare TdT Buffer and DNA Labeling Reagent:
 - a. Vortex both 3 times for 1 second each.
 - b. Quick spin 3 seconds; place in cooling block.

Prepare labeling master mix

Prepare and store all reagents, tubes, and cooling block on ice. Dispense within 10 minutes.

1. Add to the 1.5 mL tube on ice per Table 10:
 - TdT Buffer
 - DNA Labeling Reagent

Table 10 Labeling master mix (≥ 8 samples, 20% overage).

Reagent	1 sample	8 samples	16 samples	24 samples
TdT Buffer	7 μ L	67.2 μ L	134.4 μ L	201.6 μ L
DNA Labeling Reagent	1 μ L	9.6 μ L	19.2 μ L	28.8 μ L
TdT Enzyme	1.8 μ L	16.8 μ L	33.6 μ L	50.4 μ L
Total	9.8 μ L	93.6 μ L	187.2 μ L	280.8 μ L

2. Vortex 3 times for 1 second each.
3. Quick spin 3 seconds.
4. Remove the TdT Enzyme from freezer and immediately place it in a cooler pre-chilled to -15 to -25°C .
5. Mix TdT Enzyme.
 - a. Quick spin 1 second.
 - b. Vortex 1 second.
 - c. Quick spin 3 seconds, place in cooling block.
6. Immediately place in -15 to -25°C cooler.
7. Add the TdT Enzyme to the Labeling Master Mix. Place in -15 to -25°C cooler.
8. Vortex Labeling Master Mix 3 times for 1 second each.
9. Quick spin 3 seconds.

Labeling

Add labeling master mix

Keep samples in cooling block and all tubes on ice when making additions.

1. Add Labeling Master Mix to pre-chilled strip tubes on cooling block on ice.
2. Seal strip with adhesive film strip or strip caps and quick spin.
3. Place back in cooling block, remove and discard seal.
4. Remove and discard the plate seal.
5. Use a P20 multi-channel pipette to add 9.8 μL Labeling Master Mix to each sample.

Sample	Volume/sample
Fragmented DNA (less 2.0 μL for gel analysis)	26.0 μL
Labeling Master Mix	9.8 μL
Total	35.8 μL

6. Use a new seal and tightly seal the plate.
7. Vortex 1 second each in all corners and center.
8. Centrifuge $650 \times g$ for 1 minute.
9. Place the labeling plate in thermal cycler and run the CytoScan™ Optima Label program.

Table 11 CytoScan™ Optima Label thermal cycler program.

Temperature	Time
37°C	4 hours
95°C	15 minutes
4°C	5 minutes
4°C	∞

10. When program finishes, leave the plate in the thermal cycler or transfer to a chilled cooling block on ice.
11. Ensure that the plate is sealed tightly and centrifuge $650 \times g$ for 1 minute.

Note: If not proceeding to the next step, the plate may be held at -15 to -20°C for up to 1 week.

Target hybridization via AGCC

Important information about this stage

To help ensure the best results, carefully read the information below before you begin this stage of the protocol. Since this user guide is intended as an assay protocol manual, there is no specific section on all of the various features and workflows available in the Command Console™ software. If you would like to learn more about Command Console™, refer to the *GeneChip™ Command Console™ 3.2 User Guide* (Cat. No. 702569).

Prepare the equipment

Turn on the thermal cycler

Power on the thermal cycler to preheat the lid. Leave the block at room temperature.

Preheat the hybridization oven 645

Note: Confirm that the Hybridization Oven 645 is calibrated. The hybridization oven should be serviced at least once per year to ensure operation within specification.

To preheat the hybridization ovens:

1. Turn on the oven at least 1 hour before hybridization with the temperature set to 50°C.
2. Set the rpm to 60.
3. Turn the rotation on and allow to preheat for 1 hour before loading arrays.

Prepare the arrays and create a batch registration file

Note: Remove array from storage 1 hour before hybridization to equilibrate to RT. Do not remove arrays from pouch. During this time you may scan the barcode which will be used in batch registration.

To prepare the arrays:

1. Unwrap the arrays and place on the bench top, septa side up.
2. Mark the front and back of each array with a designation that will identify which sample is loaded onto each array (Figure 17).

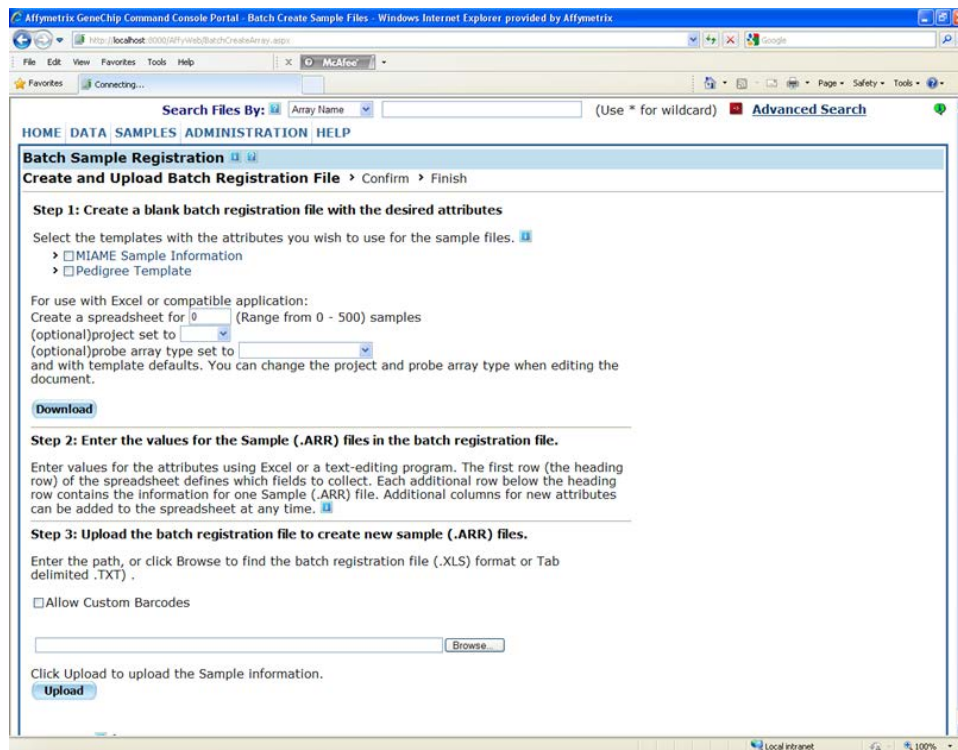
Create a batch registration file

To register a new sample using AGCC:

1. From the Command Console™, launch the AGCC Portal.

IMPORTANT! Confirm that you are running AGCC v.3.2.2 or higher. If not, update your version of AGCC to v.3.2.2 or latest available.

2. Under the **Samples** tab, select **Batch Registration**.
The **Batch Registration** window opens (Figure 2.20).



3. Within Step 1:
 - a. In **Create a Spreadsheet for**, enter the number of samples for which a spreadsheet needs to be created.
 - b. In **project set to**, select Default.
 - c. In **probe Array type set to**, select **CytoScan™ Optima Array**.
 - d. Click **Download**.
An Excel™ spreadsheet opens.
4. Within Step 2:
 - a. Name the experiment file using the following convention:
 SampleName_PlateCoordinate_ExperimentDescriptionString_A
 rrayType_OperatorInitials_yy yymmdd
 The sample file name and the 'Array name' would be identical.
 - b. Scan the corresponding barcodes for each Sample name.
 - c. Save the Excel™ file in "Excel 97-2003 workbook" format.
5. Within Step 3:
 - a. Browse to the location of the batch registration file that was saved.

- b. Upload the batch registration file by clicking the tab to create new sample (.ARR) files. A new window opens.

6. Click **Save** to save the new sample files.

Prepare the arrays

1. Place the arrays on a clean bench top area designated for hybridization.
2. Insert a 200 µL pipette tip into the upper right septum of each array.
3. Paste two 1/2" Tough-Spots™ on the top edge of the array for later use (Figure 17).



Figure 17 Arrays prepared for sample loading.

IMPORTANT! To ensure that the data collected during scanning is associated with the correct sample, mark each array in a meaningful way. It is critical that you know which sample is loaded onto each array.

Prepare the reagents and consumables

Set up the work area

1. Place a double cooling block on ice (Figure 18).

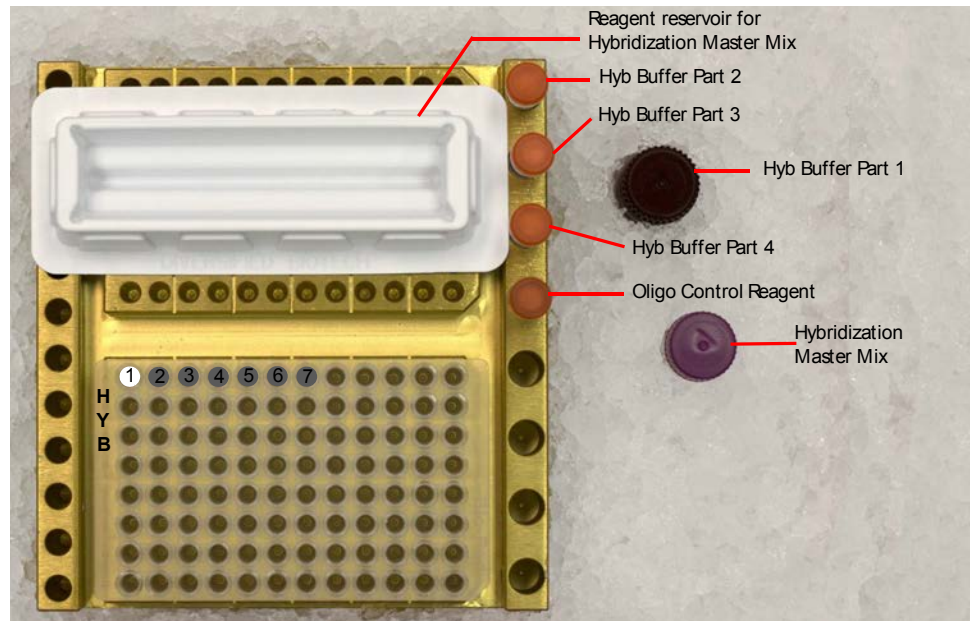


Figure 18 Hybridization setup.

2. Place a reagent reservoir on the upper half of the cooling block on ice.
3. Label the 15 mL centrifuge tube as Hyb Master Mix, and place on the ice.

Prepare the samples

1. If the labeled samples from the previous stage were frozen, allow them to thaw on the bench top to room temperature and spin down at 2000 rpm for 1 minute.
2. Immediately place the plate in the lower half of the cooling block on ice.

Thaw and prepare the reagents

Thaw the following reagents at room temperature. Immediately place on cooling block on ice when thawed.

- Hyb Buffer Part 1
- Hyb Buffer Part 2
- Hyb Buffer Part 3
- Hyb Buffer Part 4
- Oligo Control Reagent

Prepare the reagents as follows:

1. Vortex each reagent at high speed 3 times, 1 second each time.
2. Pulse spin for 3 seconds, then place in the cooling block as shown in Figure 18.
3. Quick spin 3 seconds, then place in cooling block.

Prepare the hybridization master mix

- To the 15 mL Hyb Master Mix centrifuge tube on ice, add the appropriate volume of each reagent in the order shown in Table 12.

Table 12 Hybridization master mix (≥8 Samples, 20% overage).

Reagent	1 sample	8 samples	16 samples	24 samples
Hyb Buffer Part 1	82.5 µL	792 µL	1584 µL	2376.0 µL
Hyb Buffer Part 2	7.5 µL	72 µL	144.0 µL	216.0 µL
Hyb Buffer Part 3	3.5 µL	33.6 µL	67.2 µL	100.8 µL
Hyb Buffer Part 4	0.5 µL	4.8 µL	9.6 µL	14.4 µL
Oligo Control Reagent	1.0 µL	9.6 µL	19.2 µL	28.8 µL
Total	95 µL	912 µL	1824 µL	2736 µL

IMPORTANT! Some of the Hyb Buffer components are viscous; carefully pipet and dispense when preparing the master mix.

- Mix well by vortexing the master mix at high speed 3 times, 3 seconds each time (until the mixture is homogeneous).

IMPORTANT! Make sure the Hybridization Master Mix is adequately vortexed.

Add hybridization master mix and denature

Note: When working with more than 8 samples, we strongly recommend transferring the master mix to a reservoir and dispensing the master mix from the reservoir into the samples using a multi-channel pipette.

To add Hybridization Master Mix and denature the samples:

- Remove and discard the plate seal.
- Pour the Hybridization Master Mix into a reagent reservoir placed on the upper half of the cooling block on ice. Use a multi-channel pipette to add 95 µL of Hybridization mix to the samples.

IMPORTANT! The Hybridization Master Mix is viscous; pipette carefully when dispensing to samples.

- Tightly seal the plate with a new seal and carefully check to confirm that the plate is well sealed.

IMPORTANT! Ensure that the plate is vortexed to mix sample and hybridization buffer well.

- Vortex the plate at high speed for 1 second each in all corners and in the center. REPEAT vortexing to ensure that the plate is well mixed, then spin down at $650 \times g$ for 1 minute.

STOPPING POINT Optional. Seal the plate tightly and store with samples in Hybridization Master Mix at -15 to -25°C for up to 1 week. When ready to use the frozen plate, follow the instruction in the note below.

Note: When ready to use a previously frozen plate:

- Thaw at RT.
 - Ensure plate is sealed tightly.
 - Centrifuge $650 \times g$ for 1 minute.
 - Remove and replace seal with new seal.
 - Seal tightly.
 - Vortex to ensure complete mixing.
 - Centrifuge $650 \times g$ for 1 minute.
 - Place plate on cooling block on ice.
- Place the plate onto the pre-heated thermal cycler and run the CytoScan™ Optima Hyb program.

Table 13 CytoScan™ optima hyb thermal cycler program.

Temperature	Time
95°C	10 minutes
49°C	3 minutes
49°C	∞

Load the samples onto arrays

To load the samples onto arrays:

- When the thermal cycler reaches 49°C , leave the samples at 49°C for at least three minutes, and then open the lid.

IMPORTANT! Load only 4 to 6 arrays at a time. Remove the seal from the hybridization plate for only 4 to 6 samples at a time.

- If you are hybridizing more than eight samples, cut and remove the seal from 4 to 6 samples at a time only.

Leave the remaining wells covered. Keeping these wells covered helps prevent cross-contamination and evaporation.

IMPORTANT! The hybridization mix is very viscous. Pipet slowly to ensure that all of the volume is loaded into the chip.

- Using a P200 pipette, remove 100 μL of the first sample and immediately inject it into an array.

4. Cover the septa on the array with the 1/2" Tough-Spots™ that were previously placed on the top edge of the array (Figure 19).
Press firmly to ensure a tight seal to prevent evaporation and leakage.



Figure 19 Loading samples onto arrays.

5. When 4 to 6 arrays are loaded and the septa are covered:
 - a. Load the arrays into an oven tray evenly spaced.
 - b. Immediately place the tray into the hybridization oven.
6. Repeat this process until all samples are loaded onto arrays and are placed in the hybridization oven.
All samples should be loaded within 30 minutes.
7. Allow the arrays to rotate at 50°C, 60 rpm for 16 to 18 hours.

IMPORTANT! Allow the arrays to rotate in the hybridization oven for 16 to 18 hrs at 50°C and 60 rpm. This temperature is optimized for this product, and should be stringently followed.

Washing, staining, and scanning arrays

Prime the fluidics station

Priming ensures the lines of the fluidics station are filled with the appropriate buffers and the fluidics station is ready to run fluidics station protocols.

Priming should be done:

- When the fluidics station is first started
- When wash solutions are changed
- Before washing, if a shutdown has been performed
- If the LCD window instructs the user to prime

The Fluidics Station 450 is used to wash and stain the arrays; it is operated using AGCC software.

To prime the Fluidics Station:

1. Turn on the Fluidics Station.
2. Prime the Fluidics Station.
 - From Command Console™ application, start the 'Launcher'.
 - From the Launcher, open 'AGCC Fluidics Control' application.
 - From the AGCC Fluidics Control panel, select 'PRIME_450' script for the specific fluidics stations and the modules.
 - Intake buffer reservoir A: use Wash A.
 - Intake buffer reservoir B: use Wash B.
3. To initiate the fluidics script, click the "Run" icon for each module or click the "Run All" icon, for all the selected stations and modules.

Washing and staining arrays

1. Briefly vortex the stain bottles before aliquoting the reagents.
2. Aliquot the following reagents into 1.5 mL microfuge tubes for each array:
 - a. Aliquot 500 µL Stain Buffer 1 into 1.5 mL microfuge tubes (use amber color tubes as Stain Buffer 1 is light sensitive).
 - b. Aliquot 500 µL Stain Buffer 2 into 1.5 mL microfuge tubes (clear/natural tubes).
 - c. Aliquot 800 µL Array Holding Buffer into 1.5 mL microfuge tubes (blue tubes).
3. Select CytoScan™_Optima_Array_450 from the AGCC Fluidics Control Panel:
4. Start the protocol and follow the instructions in the LCD on the Fluidics Station. If you are unfamiliar with inserting and removing arrays from the fluidics station modules, refer to the appropriate Fluidics Station User Guide or Quick Reference Card (Cat. No. 08-0093 for the Fluidics Station 450). Eject the wash block to avoid sensor time out.
5. Remove any previously loaded empty vials.
6. When prompted to "Load vials 1-2-3":
 - a. Place one vial containing 500 µL Stain Buffer 1 in position 1.
 - b. Place one vial containing 500 µL Stain Buffer 2 in position 2.
 - c. Place one vial containing 800 µL Array Holding Buffer in position 3.
7. After 16 to 18 hrs of hybridization, remove no more than 6 arrays at a time from the oven. Remove the Tough-Spots™ from the arrays.

IMPORTANT! Once the arrays are removed from the hybridization oven, quickly load them onto the Fluidics Station. Delays during this step will impact data quality.

8. Immediately insert the arrays into the designated modules of the fluidics station while the cartridge lever is in the Down or Eject position and engage the wash block.
9. Press down on the needle lever to snap needles into position and to start the run. The fluidics protocol begins. The Fluidics Station dialog box at the workstation terminal and the LCD window display the status of the washing and staining steps.
10. When the wash and stain procedure is completed, remove the arrays from the fluidics station by first pressing down the cartridge lever to the Eject position.
11. Check the array window for bubbles or air pockets. If air bubbles are present, return the array to the fluidics station. Follow the instructions on the LCD panel of the fluidics station. Pull the lever up and load to remove bubbles.
12. If air bubbles are still present after repeating the above process a few times, use the manual process.
 - a. Insert a 200 µL pipette tip into the upper right septum of the array.
 - b. Using a pipette, remove half of the solution.
 - c. Manually fill the array with Array Holding Buffer.
13. If the array has no bubble, it is ready for scanning. Proceed to “Scanning arrays” on page 51.

If the arrays cannot be scanned promptly, store them at 4°C in the dark until ready for scanning. Scan must be performed within 24 hours.
14. Pull up on the cartridge lever to engage wash block. Remove the microcentrifuge vials containing stain and replace with three empty vials as prompted.
15. When washing and staining are complete, shut down the fluidics station following the procedure.

Scanning arrays

The GeneChip™ Scanner 3000 7G is controlled by AGCC software.

Prepare the scanner

Turn on the scanner at least 10 minutes before use.



WARNING! The scanner uses a laser and is equipped with a safety interlock system. Defeating the interlock system may result in exposure to hazardous laser light.

Read and be familiar with the operation of the scanner before attempting to scan an array. Refer to the *GeneChip™ Scanner 3000 Quick Reference Card* (Cat. No. 08-0075).

Prepare arrays for scanning

To prepare arrays for scanning:

1. If the arrays were stored at 4°C, allow them to warm to room temperature before scanning.
2. If necessary, clean the glass surface of the array with a non-abrasive towel or tissue before scanning. Do not use alcohol to clean the glass surface.
3. On the back of the array cartridge, clean excess fluid from around the septa.
4. Carefully cover both septa with Tough-Spots™ (Figure 20).

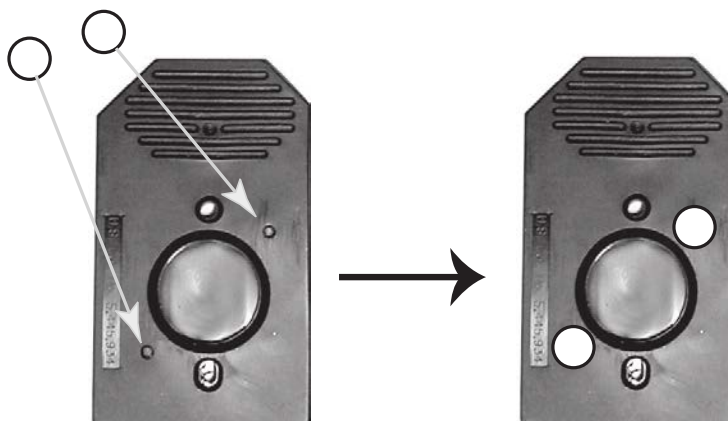


Figure 20 Applying Tough-Spots™ to arrays.

Press to ensure the spots remain flat. If the spots do not apply smoothly (e.g., if you see bumps, bubbles, tears, or curled edges) do not attempt to smooth out the spot. Remove the spot and apply a new spot.

Scanning the array

Note: Customers using the Autoloader should refer to the Autoloader User Guide.

To scan arrays:

1. Open the 'AGCC Scan Control' application from the 'Launcher'.
2. Load the arrays onto the Autoloader of the scanner.
3. Once all the arrays are loaded, click the Start icon to initiate the scan.
4. Select the check box arrays in carousel positions 1-4 at room temperature. If the arrays are not at room temperature, do not select this option. The scanner will wait 10 minutes before scanning begins to allow the arrays to reach room temperature.

Only one scan per array is required. Pixel resolution and wavelength are preset and cannot be changed.



WARNING! The door is locked while the instrument is scanning. Do not attempt to open the door manually.

Adding arrays during an autoloader run

To add arrays while an AutoLoader run is in progress:

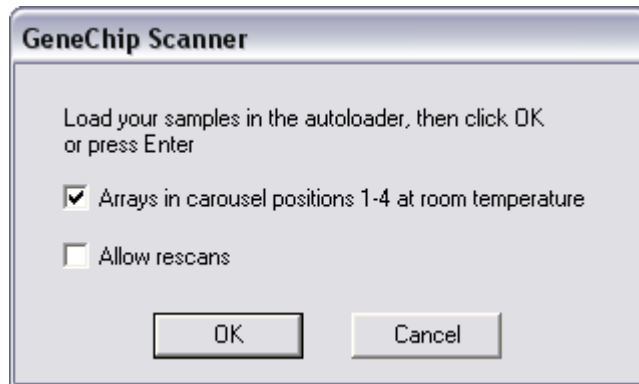
1. Click the **Add Chips** icon.
A **GeneChip Scanner** warning message appears.



2. Click **Add after Scan**.

IMPORTANT! Do not use the **Add Now** feature. Use only the **Add after Scan** feature when working with CytoScan™ Arrays.

3. When the status on the scanner reads Autoloader Door Unlocked, open the scanner and add the arrays.
4. Close the scanner.
5. When the **GeneChip Scanner** dialog box appears, click **OK**.



6. After you click **OK**, click the **Resume** icon.
7. If any arrays in the carousel are to be rescanned, select the **Allow rescans** checkbox.

Shutting down the fluidics station

To shut down the Fluidics Station:

1. Gently lift up the cartridge lever to engage (close) the washblock.
After removing an array from the holder, the LCD window displays the message ENGAGE WASHBLOCK. The instrument automatically performs a Cleanout procedure. The LCD window indicates the progress of this procedure.
2. When REMOVE VIALS is displayed in the LCD, remove the vials.
The REMOVE VIALS message indicates the cleanout procedure is complete.

3. If no other processing is to be performed, place the wash lines into a bottle filled with deionized water.
4. Using AGCC, choose the Shutdown_450 protocol for all modules.
5. Run the protocol for all modules.
The shutdown protocol is critical to instrument reliability. Refer to the instrument user guide for more information.
6. When the protocol is complete, turn the instrument off.
7. Empty the waste bottle.

IMPORTANT! To maintain the cleanliness of the fluidics station and obtain the highest quality image and data possible, a weekly bleach protocol is highly recommended. Follow the procedure outlined in Appendix E, “Fluidics station care and maintenance” for weekly maintenance of the fluidics stations.

In-Process quality control

IMPORTANT! The “In-Process Quality Control” is a critical in-process step and must include PCR and fragmentation gels, and purified PCR DNA yield.

Use strip tubes or 96-well plates.

PCR product

1. Prepare the loading buffer dilution.
2. From the first row, add:
 - 3 μL PCR product
to
 - 17 μL diluted loading buffer or nuclease-free water (total = 20 μL)
3. Vortex.
4. Centrifuge 650 $\times g$ for 1 minute.
5. Load onto gel.
6. Prepare the PCR marker dilutions.

7. Follow manufacturer's instructions to run gel that meets requirements (Figure 21 and Figure 22).

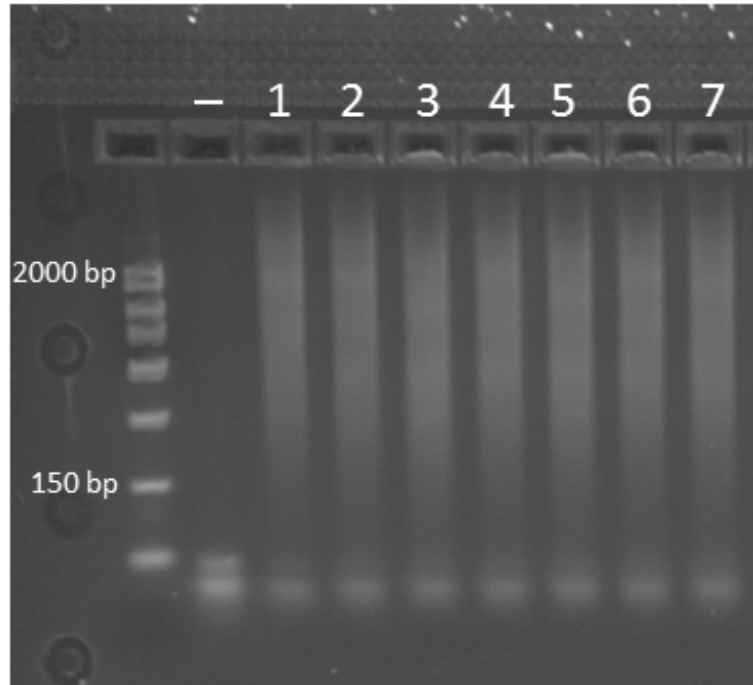
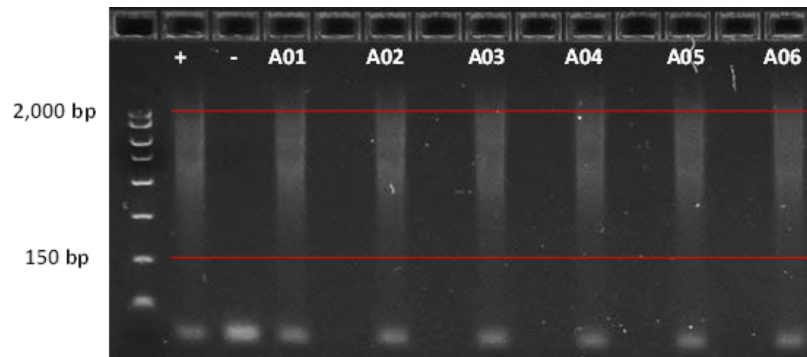


Figure 21 Example PCR gel image on 2% gel with negative control.



Example of PCR products run on 2% TBE agarose gel at 5 v/cm for 45 minutes. Average product distribution is between ~150 to 2000 bp.

Figure 22 PCR size range with positive and negative control.

Requirements – PCR product gel quality control interpretation

- Target distribution of smear covers 150 to 2,200 bp
- Higher intensities at 300 to 2,200 bp

Fragmented product

1. Prepare the loading buffer dilution.
2. Add:
 - 2 μL fragmented product to
 - 14 μL water (1:8 dilution)
3. Add:
 - 8 μL diluted fragmented product to
 - 12 μL diluted loading buffer or nuclease-free water
4. Pulse vortex.
5. Centrifuge $650 \times g$ for 1 minute.
6. Load onto gel.
7. Prepare DNA marker dilutions.
8. Follow manufacturer's instructions and run on 4% agarose gel to meet requirements (Figure 23 and Figure 24).

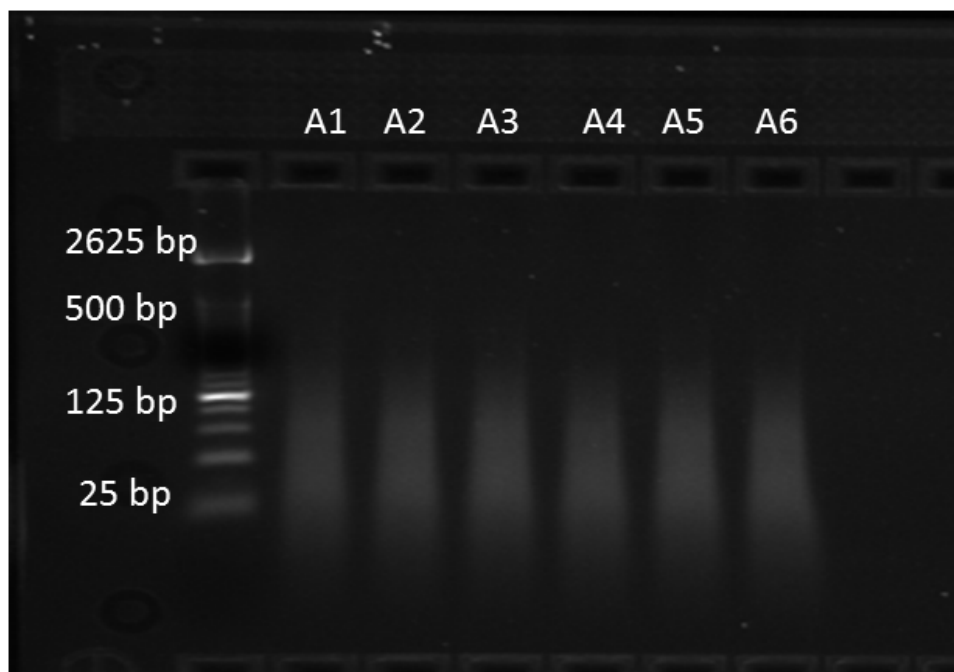


Figure 23 Example fragmentation gel image on 4% gel.

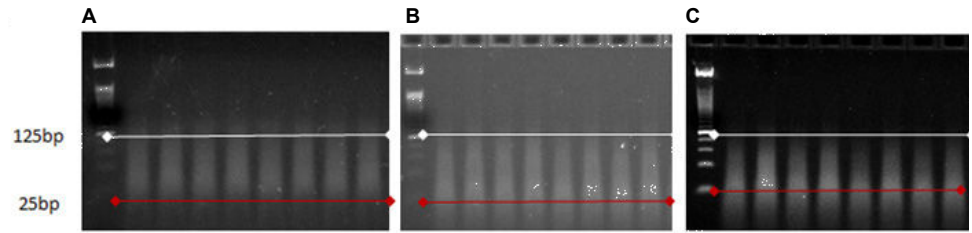


Figure 24 Examples of good fragmentation gels: Passing performance besides variability in smear patterns.

Requirements for fragmentation product gel quality control interpretation

- Target distribution of smear covers 25 to 125 bp
- High intensity at 25 to 125 bp

The three gels in Figure 25 have the majority of high intensity bands above 125 bp (left); at 125 bp (middle); or below 25 bp (right).

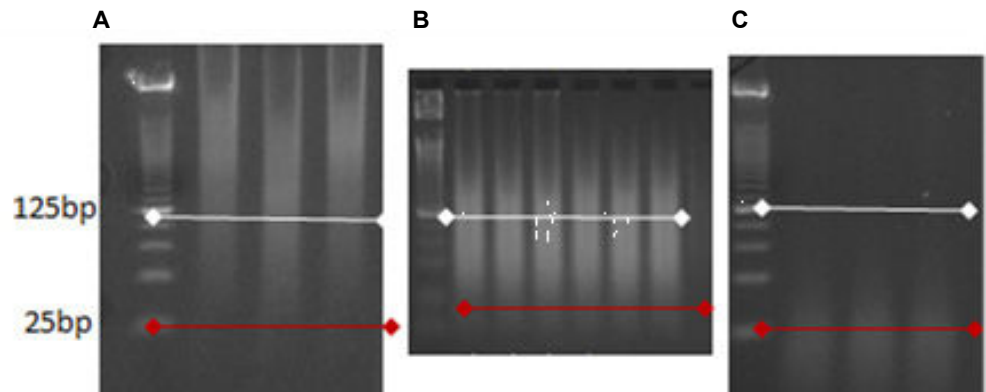


Figure 25 Examples of failing fragmentation gels.

If majority of target distribution is...	Then sample is...
At 125 bp or extends to 2652 bp	Under fragmented (Figure 25, A and B)
Below 25 bp	Over fragmented (Figure 25, C)

Array quality control threshold

After processing the arrays on the Chromosome Analysis Suite, the following thresholds apply:

- MAPD ≤0.29
- SNPQC ≥8.5

For samples not passing these QC thresholds, refer to the troubleshooting section in this user guide (Appendix D, “Troubleshooting the KaryoStat™ Assay”). Other metrics can be used as guidance for sample performance. For more information on how these or other metrics are calculated, refer to the *Chromosome Analysis Suite (ChAS) User Guide* (Cat. No. 702943).



Workflow and practices

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- Specific laboratory practices 61
- PCR practices 62

Workflows

The 3.5-day workflow for one operator and 8 to 24 samples is given in Figure 26. The 2.5-day workflow for one operator and 8 to 24 samples is given in Figure 27.

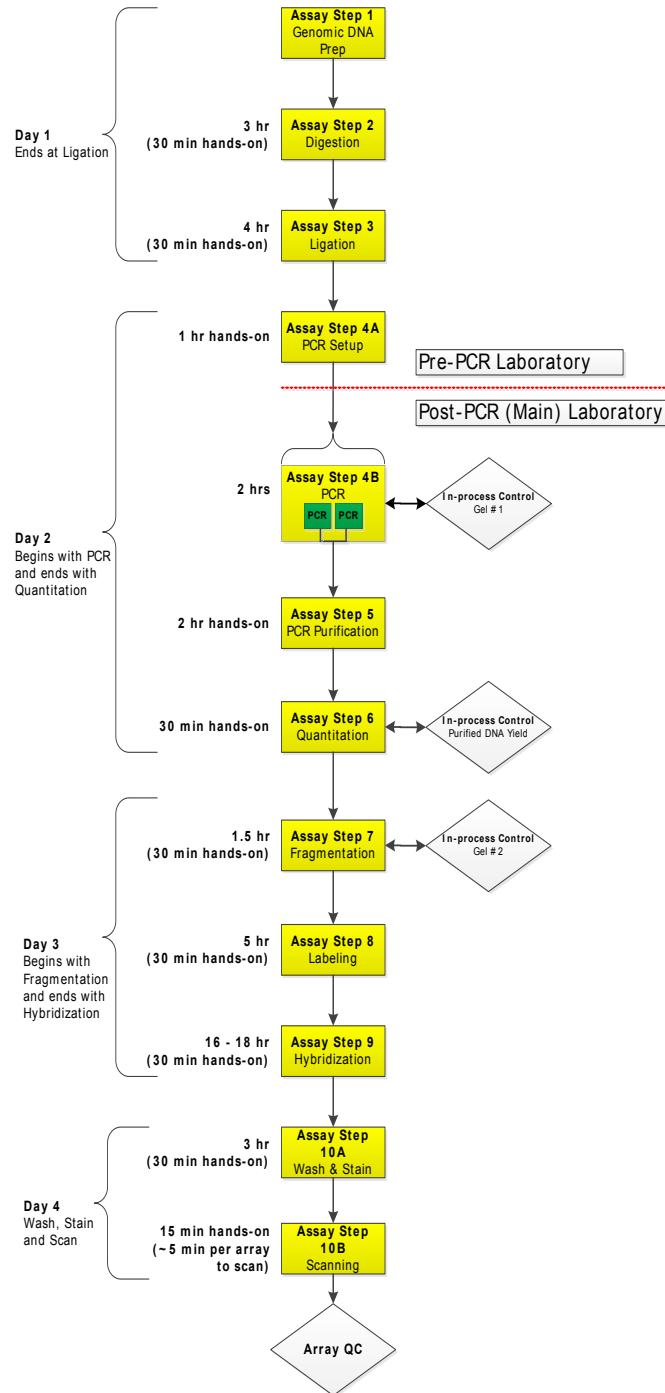


Figure 26 3.5-day workflow

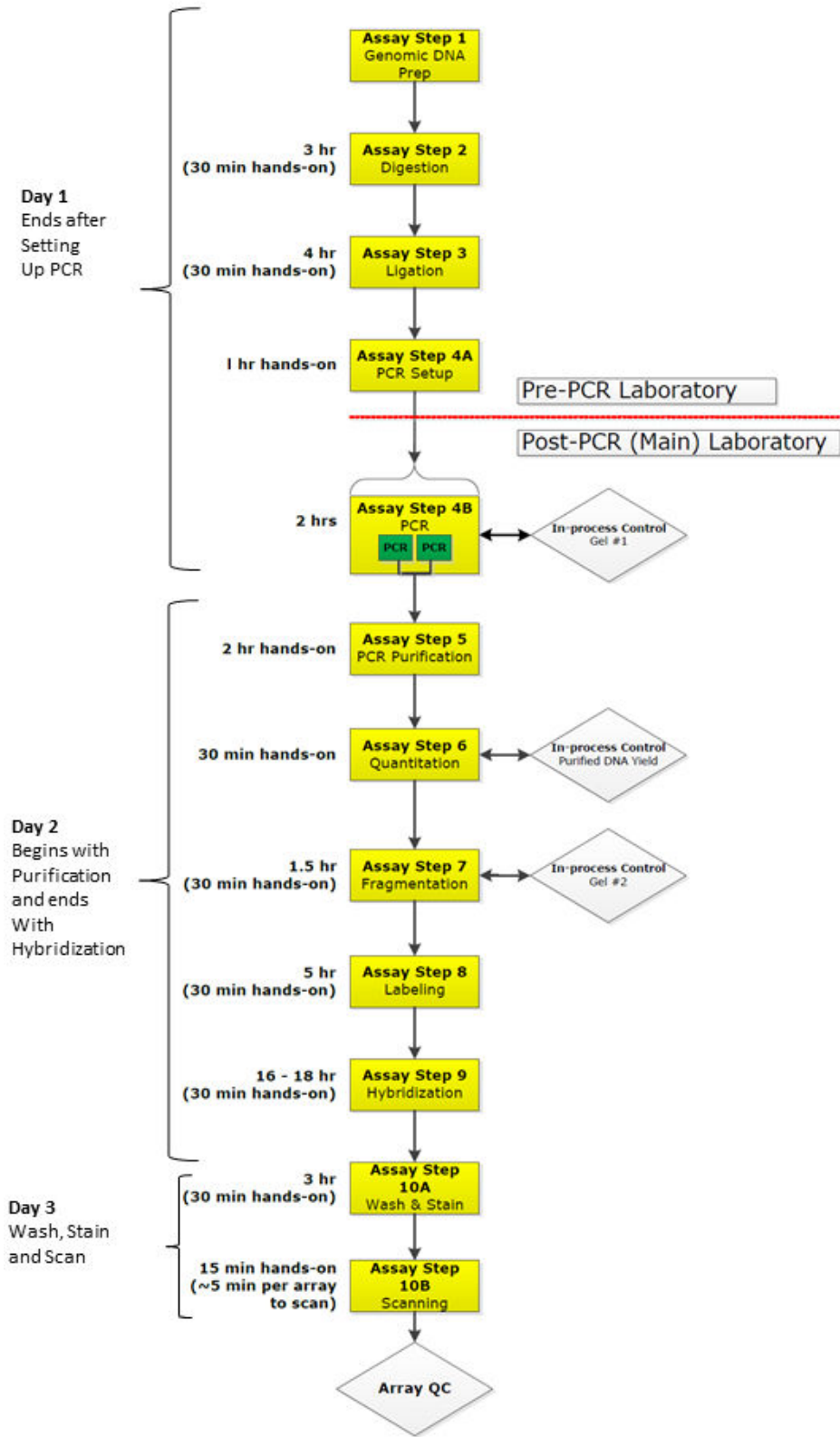


Figure 27 2.5-day workflow

Specific laboratory practices

Seals

Avoid cross-contamination.

Plate

- Seal plate with adhesive film applicator for each well and plate edge surface.
- Ensure tight seals all over the plate and well edges
- Use PCR-certified clear adhesive films for 96-well plates
Use only once and never reuse

Note: The seal on the plate may become loose due to the high temperature in the thermal cycler.

- When removing plate from freezer:
 - a. Thaw plate at RT
 - b. Ensure tight seal
 - c. Centrifuge $650 \times g$ for 1 minute
 - d. Place plate on pre-chilled cooling block (2 to 8°C)

Strip tubes

- Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes
- For master mix, use adhesive film strips or strip caps to seal before centrifuging

Vortex

Always vortex on maximum setting:

- Enzyme: Vortex 1 to 3 seconds once
- Reagents: Vortex 3 times for 1 to 3 seconds each
- Plates: Vortex for 1 to 3 seconds in all corners and in center (Figure 28), repeat once for high volume reagents: PCR (100 μL), hybridization-ready samples (131 μL)

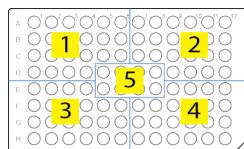


Figure 28 Plate vortexing.

- Master Mix Tubes: Vortex 3 times before and after adding enzyme, 1 to 3 seconds each
- During purification, use vortex with foam tube adapter for 1.5 mL microfuge tubes on maximum vortex setting

Centrifuge

Always centrifuge at maximum setting unless otherwise noted.

- Enzymes: for 1 second
- Reagents: for 1 to 3 seconds
- Plates: 650g for 1 minute
- Master Mix tubes and strips: for 1 to 3 seconds.
- QC strips: for 1 to 3 seconds.

PCR practices

Samples

IMPORTANT! Dilute ligated DNA with chilled Nuclease-free Water before PCR.

- PCR volume = 100 μ L
- Keep PCR plates at 2 to 8°C (ice or chilled cooling block) before purification
- Avoid cross-contamination

PCR product purification

PCR pooling

- Avoid cross-contamination
- Process tubes in same order as samples being pooled
- Change pipette tips after each sample transfer
- Thoroughly mix purification bead solution by shaking and inverting bottle several times so mixture appears homogeneous. Do not vortex bead bottle.

Pellet washing

- Use molecular biology grade ethanol to prepare Purification Wash Buffer
- Allow supernatant to clarify before removal
- Replace vortex foam tube adapters at signs of wear
- Always use:
 - Fresh tips
 - Multi-channel pipette to remove the initial Purification Wash Buffer
 - Single-channel pipette to remove:
 - Residual purification buffer
 - Supernatant
- Remove stand from magnetic rack when drying pellets. Dry for exactly 7 minutes; do not under-or over-dry.

Elution

- Ensure pellets are completely suspended in elution buffer before centrifuging for 3 minutes.
- Final elution step. If beads do not form tight pellet within 10 minutes, verify rack is placed correctly on magnetic stand and extend time until eluant is clear and all beads are pulled towards magnet.



Thermal cycler programs

- Pre-and post-PCR programs 63
- Thermal cycler programs 63

Before you begin processing samples, enter and save these programs into the appropriate thermal cycler(s).

Pre-and post-PCR programs

Table 14 Pre-PCR clean room.

Number of thermal cyclers required	Program name
1	CytoScan™ Optima Digest
	CytoScan™ Optima Ligate

Table 15 Post-PCR room.

Number of thermal cyclers	Required program name
1	CytoScan™ Optima PCR
	CytoScan™ Optima Fragment
	CytoScan™ Optima Label
	CytoScan™ Optima Hyb

Thermal cycler programs

CytoScan™ Optima Digest

Table 16 CytoScan™ Optima Digest thermal cycler program.

Temperature	Time
37°C	2 hours
65°C	20 minutes
4°C	5 minutes
4°C	∞

CytoScan™ Optima
Ligate

Table 17 CytoScan™ Optima Ligate thermal cycler program.

Temperature	Time
16°C	3 hours
70°C	20 minutes
4°C	5 minutes
4°C	∞

CytoScan™ Optima
PCR

You must use thermal cyclers with silver or gold-plated silver blocks. Do not use thermal cyclers with aluminum blocks.

- Ramp speed: Max
- Volume: 100 µL

Table 18 CytoScan™ Optima PCR thermal cycler program.

Temperature	Time	Cycles
95°C	1 minute	1X
95°C	30 seconds	30X
60°C	45 seconds	
68°C	60 seconds	
68°C	7 minutes	1X
4°C	5 minutes	1X
4°C	∞	

CytoScan™ Optima
Fragment

Table 19 CytoScan™ Optima Fragment thermal cycler program.

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	5 minutes
4°C	∞

CytoScan™ Optima Label

Table 20 CytoScan™ Optima Label thermal cycler program.

Temperature	Time
37°C	4 hr
95°C	15 minutes
4°C	5 minutes
4°C	∞

CytoScan™ Optima Hyb

Table 21 CytoScan™ Optima Hyb thermal cycler program

Temperature	Time
95°C	10 minutes
49°C	3 minutes
49°C	∞



Required equipment, consumables, and reagents

- Equipment and software required 66
- CytoScan™ Optima Assay reagents, required. 67
- From other suppliers 68
- Symbols 71

Equipment and software required

Item	Cat. No.
Equipment	
GeneChip™ Fluidics Station 450	00-0079
Tubing, Silicone peristaltic for GeneChip™ Fluidics Station 450	400110
GeneChip™ Hybridization Oven 645	00-0331
GeneChip™ Scanner 30007G	00-0213
Software	
GeneChip™ Command Console™ Software	Version 3.2.2 or higher
Chromosome Analysis Suite (ChAS 2.1 or higher)	901394



CytoScan™ Optima Assay reagents, required.

Module Cat. No.	Module	Item/reagent	Qty	Cat. No.
902527	CytoScan™ Optima – Module 1: Pre-Lab Restriction, Ligation, and Amplification	Nsp I	1	902421
		Nsp I Buffer	1	902422
		100X BSA	1	902423
		DNA Ligase	1	902379
		DNA Ligase Bfr	1	902378
		CytoScan™ Taq Buffer	1	902582
		CytoScan™ Taq DNA Polymerase	1	902581
		dNTP	1	902401
		5M Betaine	1	902402
		Adaptor, Nsp I	1	902426
		PCR Primer	1	902427
902528	CytoScan™ Optima – Module 2: Pre-Lab TE Buffer and Water	Low EDTA TE Buffer	1	902424
		Water, Nuclease free	1	902425
902529	CytoScan™ Optima – Module 3: Main Lab Fragmentation, Labeling, and Hybridization	Fragmentation Reagent	1	902428
		Fragmentation Buffer	1	902301
		TdT Enzyme	1	902429
		TdT Buffer	1	902430
		DNA Labeling Reagent	1	902431
		Oligo Control Reagent	1	902432
		Hyb Buffer Part 1	1	902433
		Hyb Buffer Part 2	1	902434
		Hyb Buffer Part 3	1	902435
Hyb Buffer Part 4	1	902436		
902530	CytoScan™ Optima – Module 4: Main Lab Stain, Holding Buffer, Beads, and Water	Stain Buffer 1	1	902440
		Stain Buffer 2	1	902441
		Array Holding Buffer	1	902442
		Purification Beads	1	902443
		Water, Nuclease free	1	902425
902531	CytoScan™ Optima – Module 5: Main Lab Elution Buffer, and Purification Wash Buffer	Elution Buffer	1	902444
		Purification Wash Buffer	1	902437
		Wash A	4	902438
				1



Module Cat. No.	Module	Item/reagent	Qty	Cat. No.
902531	CytoScan™ Optima – Module 5: Main Lab Elution Buffer, and Purification Wash Buffer	Wash B	4	902439
Arrays required				
902507	CytoScan™ Optima Array Kit, 6 pack			

From other suppliers

Reagents required but not provided

Table 22 Reagents from other suppliers, required.

Item
Bleach, 6.15% sodium hypochlorite
PCR gel DNA ladder: 50 to 2,000 bp
Fragmentation gel DNA ladder: 25 to 2,600 bp
Ethanol, absolute
Gel-loading buffer

Pre-PCR clean room – equipment required but not provided

Table 23 Pre-PCR clean room equipment, required.

Item
If assay is to be performed in one room: <ul style="list-style-type: none"> Laminar Flow Cabinet, 6 foot or PCR Cabinet
Benchtop Cooler, with the capacity to hold 8 to 32 tubes (1.5 mL) and ability to maintain temperature below -15°C for 2 hours.
Centrifuge, plate, multipurpose, 330 µL capacity
Cooling chamber, double-block, with the capacity to hold 96 well plates with a maximum volume capacity of 330 µL
Freezer, -15 to -25°C; deep freeze; manual defrost; 17 cu ft
Microfuge (for tubes and strip tubes)

Item
Pipettors: <ul style="list-style-type: none"> • 12-channel, 2 to 20 μL • 12-channel, 20 to 200 μL • single-channel, 100 to 1,000 μL • single-channel, 2 to 20 μL • single-channel, 20 to 200 μL
Rectangular Ice Tray Large, 9L (16 x 13 in, 41 x 33cm)
Storage Racks, Tube, 96-well
Thermal cycler: capable of holding 200 μ L volume and 96-well plate; heat block capable of holding temperature of 4 to 99.9°C; temperature accuracy of $\pm 0.25^\circ\text{C}$ (at 35 to 99.9°C); heating and cooling block ramp rate of $\leq 4^\circ\text{C}$ per second.
Vortexer, 60 Hz, 75 W, 600 to 3,200 RPM

**Post-PCR room –
equipment
required but not
provided**

Table 24 Post-PCR room equipment required.

Item
Adhesive film applicator (hard plastic)
Anti-vibration pad, used with vortexer to prevent movement during operation
Cooler, benchtop, with capacity to hold 8 to 32 tubes (1.5 mL) and maintain temperature below -15°C for 2 hours
Cooling chamber, double-block, with capacity to hold 96-well plates with a maximum volume capacity of 330 μ L
Electrophoresis supplies
Freezer, -15 to -25°C ; deep freeze; manual defrost; 17 cu ft
Gel imager
Magnetic rack with magnet on the side and capable of holding 8 to 12 tubes of 1.5 to 2 mL capacity
Microcentrifuge, non-refrigerated with capacity to hold 24 tubes and maximum rotation speed of $16,200 \times g$
Microfuge (for tubes and strip tubes)
Microtube foam insert



Item
Pipettors: <ul style="list-style-type: none"> • 12-channel, 100 to 1,200 μL • 12-channel, 2 to 20 μL • 12-channel, 20 to 200 μL • single-channel, 100 to 1,000 μL • single-channel, 2 to 20 μL • single-channel, 20 to 200 μL
Plate centrifuge, refrigerated multipurpose, plate carriers for 4 x 96-well assay plates
Platform Head, 6-inch, for microtube foam insert
Rectangular ice tray, large, 9L (16 x 13in; 41 x 33cm)
Refrigerator, 2 to 8°C, 6 cu ft
Spectrophotometer, UV/VIS, single or multichannel
Storage racks, tube, 96-well with lid, 1.5 mL/2 mL tubes
Thermal cycler: capable of holding 200 μL volume and 96-well plate; heat block capable of holding temperature of 4 to 99.9°C; temperature accuracy of $\pm 0.25^\circ\text{C}$ (at 35 to 99.9°C); heating and cooling block ramp rate of $\leq 4^\circ\text{C}$ per second; thermal uniformity of $\pm 0.5^\circ\text{C}$.
Vortexer, 60 Hz, 75 W, 600 to 3,200 RPM





**Consumables
required but not
provided**

Table 25 Consumables from other suppliers, required.

Item
Adhesive films, clear, PCR-certified, 96-well plates
Adhesive label dot, 1/2-inch and 3/8-inch
Agarose gel, 2%
Agarose gel, 4%
Microcentrifuge tubes, nuclease-free, sterile, 1.5 mL polypropylene
Microcentrifuge tubes, nuclease-free, sterile, 2.0 mL polypropylene
Microcentrifuge tubes, nuclease-free, sterile, non-stick, 1.5 mL amber polypropylene
Microcentrifuge tubes, nuclease-free, sterile, non-stick, 1.5 mL blue polypropylene
Microcentrifuge tubes, nuclease-free, sterile, non-stick, 1.5 mL natural polypropylene
Microcentrifuge tubes, nuclease-free, sterile, non-stick, 50 mL polypropylene microcentrifuge tubes
Pipette tips with aerosol barriers, 20 μL , 200 μL , and 1,000 μL
Plate, OD for UV, 96-well (required only if using microplate spectrophotometer)

Item
Plates, unskirted PCR with a maximum volume of 330 µL
Reagent reservoir, 25 mL
Reference Genomic DNA, Cat. No. 901012 Required for training purposes only.
Tube strips, nuclease-free, sterile, 8-well, 0.2 mL polypropylene

Symbols

Symbol/label	Statement/meaning
	Part/catalog number
	Lot number
	Expiration date YYYY-MM-DD
	Temperature limitation



Troubleshooting the KaryoStat™ Assay

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PCR gel QC step

PCR gel QC step	Likely cause	Solution
Faint or no PCR product visible on gel. Both samples and positive control affected.	Failed restriction digest or adapter ligation.	<ul style="list-style-type: none"> • Repeat the assay from the beginning with Genomic Control DNA after reviewing best practices, ensuring that all equipment is correctly calibrated, and reagents are handled and stored properly. • If available, include ligated material from a previous successful experiment as a positive control for the PCR step. If it fails again, repeat with fresh reagents.
		Ensure that the ligation buffer is thoroughly resuspended before use.
		Ensure that the reaction plates are sealed tightly in all steps.

PCR gel QC step	Likely cause	Solution
Faint or no PCR product visible on gel. Both samples and positive control affected.	Non-optimal PCR conditions.	Use only calibrated thermal cyclers. Double-check PCR programs to ensure that they have been entered correctly.
		Check the PCR reagents. Use only the recommended reagents.
		Verify pipette calibration and function. Repeat PCR from the remaining digestion / ligation material if available, otherwise restart from the beginning.
		Take care with preparation of master mixes. Ensure accurate pipetting and thorough mixing.
		<ul style="list-style-type: none"> • Use the recommended 96-well PCR plates and plate seals. • Ensure that the plates are sealed tightly in all steps.
	Ligation reaction not diluted or diluted ligation reaction not mixed properly prior to PCR.	Be sure to correctly dilute the ligation reaction with the water provided in the kit and mix properly before proceeding with PCR.
Faint or no PCR product visible on the gel. Samples are affected but positive control is OK.	Insufficient or degraded genomic DNA.	<ul style="list-style-type: none"> • Starting amount of 250 ng genomic DNA should be used. • Confirm the concentration using a calibrated spectrophotometer. • Confirm that the genomic DNA sample meets the quality and integrity guidelines. See "Controls" on page 9.

PCR gel QC step	Likely cause	Solution
Faint or no PCR product visible on the gel. Samples are affected but positive control is OK.	Sample DNA contains enzymatic or chemical inhibitors. Nsp I can be inhibited by high concentrations of salts.	Ensure that genomic DNA is extracted using procedures that are compatible with DNA quality defined in "Assay warnings and precautions" on page 6.
Wrong size distribution of PCR product.	Mispipetting of PCR primer volume in the master mix.	<ul style="list-style-type: none"> • Verify pipette calibration and function. • Repeat PCR from the remaining digestion/ligation material if available; otherwise restart from the beginning.
PCR product evident in the negative control.	Reagents or equipment contaminated with ligated product or amplified product.	<ul style="list-style-type: none"> • Always use filter tips. • Clean the pre-PCR lab area and equipment thoroughly using 10% bleach. • Decontaminate the pipettes following manufacturer's recommendation. • Retrain personnel on pre-lab best practices. • Repeat the assay using fresh reagents and sample.

Purification yield QC step

Purification yield QC step	Likely cause	Solution
Low eluate volume (<25 µL)	Insufficient volume due to pipetting error or pipet out of calibration.	Check pipette calibration. Make sure 27 µL of elution buffer is added to the beads for elution and the tubes are centrifuged before placing on the magnet.
	Over drying of beads.	Follow the recommended time for drying.
Low yields (the average purification yield of 7 or more samples is <3.0 µg/µL)	Loss of sample prior to purification.	If the yield is not adequate, repeat the assay.

Purification yield QC step	Likely cause	Solution
or individual yield is <2.5 µg/µL)	Possible problems with input genomic DNA.	<ul style="list-style-type: none"> Use collection and purification procedures that meet DNA quality specifications as described in Chapter 1 to avoid carryover of inhibitors such as heme, EDTA, etc. Starting amount of 250 ng genomic DNA should be used. Confirm the concentration using a calibrated spectrophotometer. Confirm that the genomic DNA sample meets the quality and integrity guidelines provided in Chapter 4.
	Purification Wash Buffer was prepared incorrectly.	Verify that the correct volume of absolute ethanol was added to the Purification Wash Buffer before use.
	Inadequate mixing of Purification Beads and PCR reactions during binding.	Take care to completely mix the PCR reactions and the Purification Beads during sample binding.
	Inadequate bead washing prior to elution.	Repeat purification with attention towards complete removal of the binding eluate before the bead wash.
	Excess Elution Buffer added to beads.	Verify pipette calibration and function.
	Incorrect buffer was used for elution.	Verify that the Elution Buffer was used during the elution step and not the Purification Wash Buffer.
	Purification Beads were over dried.	Do not dry Purification Beads longer than the recommended time.

Purification yield QC step	Likely cause	Solution
	The eluted DNA plate was inadequately vortexed before taking an aliquot for an OD reading.	Eluted DNA can be heterogeneous. Repeat the dilution followed by an OD reading, making sure to vortex the eluted DNA and the OD plate thoroughly at each step.
	PCR reaction volume was inaccurate.	Repeat the assay and confirm that the PCR reaction is set up correctly.
High yields (>4.5 µg/µL)	Too little Elution Buffer added to the Purification Beads.	Verify pipette calibration and function. Make sure 27 µL of Elution Buffer is added to the Purification Beads for elution.
	Eluted DNA plate inadequately vortexed before OD reading is taken.	Eluted DNA can be heterogeneous. Repeat the dilution followed by OD reading, being sure to vortex the eluted DNA and the OD plate thoroughly at each step.
	Instruments or pipettes may be out of calibration or incorrectly set.	Verify instrument and pipette calibration and settings during operation.
	Yield calculation formula within the software template may be incorrect.	Verify the formula used to calculate the yields from a given O.D.

Fragmentation QC step (gel)

Fragmentation QC step (gel)	Likely cause	Solution
Over fragmentation: Majority of fragmented sample appears <50 bp on a 4% agarose gel.	Excess Fragmentation Reagent was added during preparation of the Fragmentation Master Mix.	<ul style="list-style-type: none"> Carefully observe the pipette tip and the shaft during pipetting of the fragmentation reagent. Touch the tip to the inside of the vial to help remove any droplets of enzyme clinging to the exterior of the tip. Make sure pipettes are calibrated.

Fragmentation QC step (gel)	Likely cause	Solution
<p>Over fragmentation: Majority of fragmented sample appears <50 bp on a 4% agarose gel.</p>	<p>Purified samples or assembled reactions were allowed to warm to room temperature during reaction assembly or prior to incubation.</p>	<p>Ensure that the plate centrifuge is completely chilled to 4°C before spinning the assembled fragmentation plate.</p>
		<p>Keep the master mix, samples, and reaction components on ice or in a cooling block at all times during master mix assembly and dispensing of the master mix to the samples.</p>
		<p>Check that the fragmentation reaction temperature and incubation time are correctly programmed on the thermal cycler and that the fragmentation mix is made correctly as per the guidelines.</p>
		<p>Ensure that the master mix tube and strip tubes are pre-chilled before reaction setup.</p>
<p>Under fragmentation: PCR product is still visible in 150-2200 bp size region on a 4% agarose gel.</p>	<p>Improper storage or handling of the Fragmentation Reagent.</p>	<ul style="list-style-type: none"> • The Fragmentation Reagent should be stored at –15°C to –25°C at all times. • Handle the Fragmentation Reagent as minimally as possible, holding the vial at the cap rather than the center. Return the Fragmentation Reagent to the cooler as soon as the reagent has been dispensed. We recommend storing the Fragmentation Reagent at –15°C to –25°C inside a cooler to preserve its activity. • Do not over vortex the Fragmentation Reagent.

Fragmentation QC step (gel)	Likely cause	Solution
Under fragmentation: PCR product is still visible in 150-2200 bp size region on a 4% agarose gel.	<ul style="list-style-type: none"> Insufficient Fragmentation Reagent or Fragmentation Buffer was added during assembly of the Fragmentation Master Mix. Improper mixing of the Fragmentation Master Mix. 	Verify pipette calibration and function. Take care when preparing the master mix to ensure accurate pipetting and thorough mixing.
	The Fragmentation Master Mix was not made fresh or was allowed to warm to room temperature before use.	<ul style="list-style-type: none"> Keep the Fragmentation Master Mix on ice at all times to preserve activity. Work quickly during reaction assembly. Do not save or reuse a previously assembled Fragmentation Master Mix.
	Samples were frozen during fragmentation reaction assembly or centrifugation.	<ul style="list-style-type: none"> Make sure that cold blocks are not chilled to -15°C to -25°C as sample freezing can occur. Before centrifugation, ensure that the interior of the chilled plate centrifuge is not lower than $2-8^{\circ}\text{C}$.
	Thermal cycler was not programmed correctly or is out of calibration.	<ul style="list-style-type: none"> Confirm that the fragmentation reaction temperature and incubation time are correctly programmed on the thermal cycler. Confirm that the fragmentation mix is made correctly as per the guidelines. Verify that the thermal cycler is within calibration.

.CEL file generation

.CEL file generation	Likely cause	Solution
.CEL file is not generated	Signal from the corner checkerboards is absent.	Verify that the Oligo Control Reagent was added to the Hybridization Master Mix during assembly. The Oligo Control Reagent must be present during hybridization to ensure proper grid alignment.
	Signal from corner checkerboards is dim.	<ul style="list-style-type: none"> • Verify that the correct amount of the Oligo Control Reagent was added to the Hybridization Master Mix during assembly. • Ensure that GeneChip™ Hybridization Oven 645 is calibrated and set to the correct temperature. • Ensure that Hybridization Master Mix was correctly assembled and added at the correct volume to the fragmented samples. • Confirm that Stain Buffer 1 and Stain Buffer 2 are placed in the correct order on the fluidics station. Stain Buffer 1 is light sensitive. Be sure to store Stain Buffer 1 in the dark when not in use. • Use only the recommended staining reagents.
	Bright hybridization artifact(s) obscure gridding oligo locations on the array.	Try to manually align the grid. See the <i>GeneChip™ Command Console™ User Guide</i> (Cat. No. 702569) for instructions. If manual grid alignment fails to produce a .CEL file, repeat the experiment.

DATA QC failures

DATA QC failures	Likely cause	Solution
Low or failing SNPQC	<ul style="list-style-type: none"> • Triploidy or whole genome mosaic samples. • Cross-contamination between samples within a plate. • Contaminated reagents, equipment, or input DNA. 	<ul style="list-style-type: none"> • Analyze the allelic difference track on ChAS, if it shows altered patterns compatible with triploidy or whole genome mosaic, do not discard and continue as usual. • Repeat assay using a control sample of known integrity. • Review and follow best practices. <ul style="list-style-type: none"> – Ensure a tight plate seal at every step. – Use fresh filter tips at each pipetting step. – Use caution when pooling PCR product. • If the problem persists use fresh reagents and fresh input DNA. • Decontaminate the pre-PCR room and equipment if necessary.
		<p>Process only 4 to 6 arrays at a time. When processing arrays for washing it is important to work quickly as delays in this step will impact data quality.</p> <p>Perform all steps after removal of arrays from the oven to the time the washing begins with minimal delays.</p>
	Over or under fragmentation of the PCR product	See above.

DATA QC failures	Likely cause	Solution
Low or failing SNPQC	Hybridization oven out of calibration or oven model is not compatible with this assay.	<ul style="list-style-type: none"> Ensure that only the GeneChip™ Hybridization Oven 645 is used for this assay. Have the oven serviced.
Elevated or failing MAPD	Assay drift due to variation in assay execution.	<ul style="list-style-type: none"> Recalibrate pipettes to ensure accurate delivery of reagent volumes. Consider operator retraining or review by an Field Applications Scientist if the problem persists. Review Best practices under Chapter 1 and Appendix A, "Workflow and practices".
	Over fragmentation	See above.
	Degraded starting material.	<ul style="list-style-type: none"> Perform a QC gel of input DNAs to assess samples for degradation. Ensure that the DNA samples are of high quality (for example, run in a 1 to 2% agarose gel and compare to a Genomic DNA Control).
	Reference is inappropriate for the sample.	Use only the recommended sample types.
High MAPD with low SNPQC	Error during washing the array.	<ul style="list-style-type: none"> Ensure that the Wash A and B lines of the Fluidics Station are placed in the correct wash buffers during priming and array washing. Ensure that the Fluidics Stations are maintained according to the guidelines in the Fluidics Station User's Guide.




Fluidics station care and maintenance

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General fluidics station care

- Use a surge protector on the power line to the fluidics station.
- Always run a Shutdown protocol when the instrument will be off or unused overnight or longer. This will prevent salt crystals from forming within the fluidics system.
- To ensure proper functioning of the instrument, perform periodic maintenance.
- When not using the instrument, leave the sample needles in the lowered position. Each needle should extend into an empty vial. This will protect them from accidental damage.
- Always use deionized water to prevent contamination of the lines. Change buffers with freshly prepared buffer at each system startup.
- The fluidics station should be positioned on a sturdy, level bench away from extremes in temperature and away from moving air.

 **WARNING!** Before performing any maintenance, turn off power to the fluidics station to avoid injury in case of a pump or electrical malfunction.

Fluidics station bleach protocol

We recommend a weekly cleaning protocol for the fluidics station. This protocol uses commonly purchased sodium hypochlorite bleach.

This protocol is designed to eliminate any residual SAPE-antibody complex that may be present in the fluidics station tubing and needles. The protocol runs a bleach solution through the system followed by a rinse cycle with deionized (DI) water. This protocol takes approximately one hour and forty minutes to complete. We recommend running this protocol weekly, regardless of the frequency of use. The current version of the protocol can be found on our website.

The bleach cycle

To avoid carryover, or cross contamination, from the bleach protocol, we recommend the use of dedicated bottles for bleach and DI water. Additional bottles can be obtained from Thermo Fisher Scientific.

Table 26 Recommended bottles.

Cat. No.	Description
400118	Media Bottle, SQ, 500 mL
400119	Media Bottle, SQ, 1000 mL

1. Disengage the washblock for each module by pressing down on the cartridge lever. Remove any probe array cartridge.

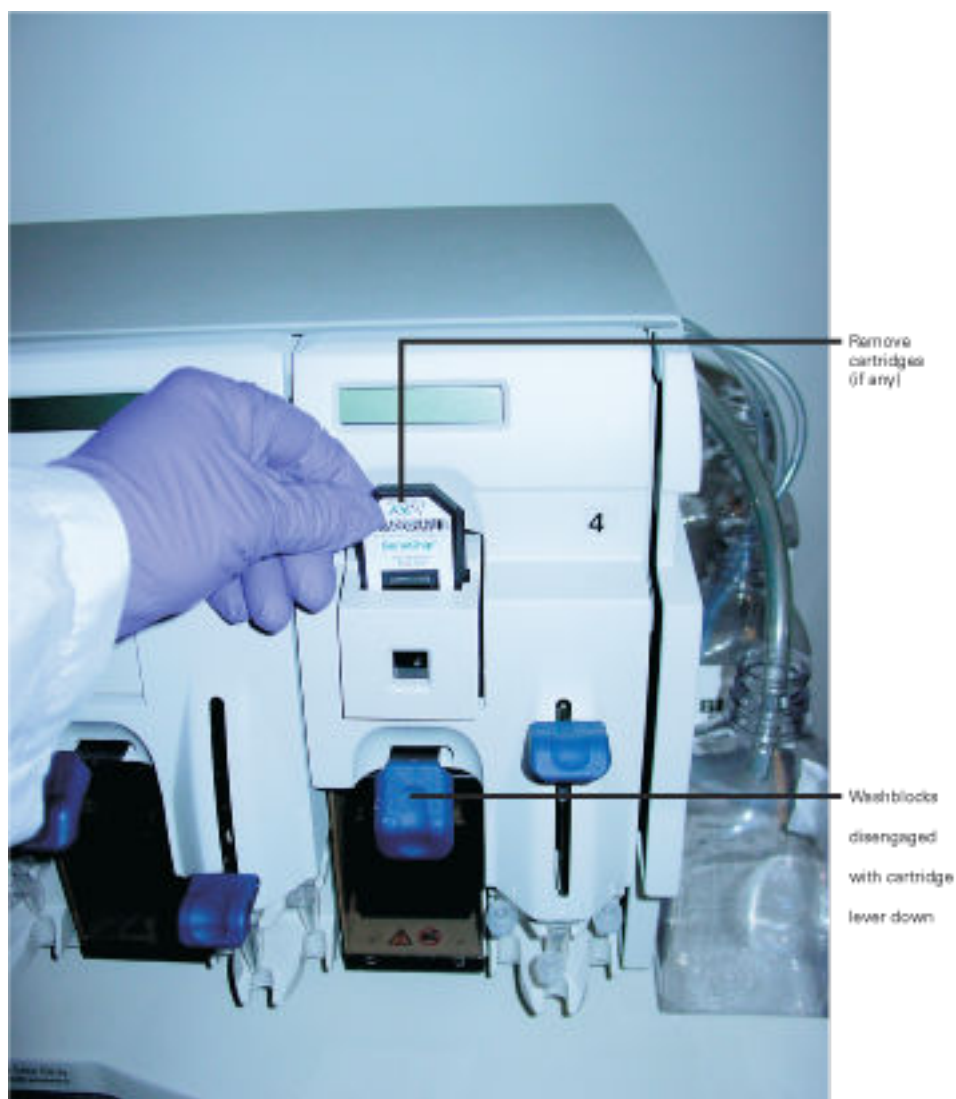


Figure 29 Disengaged washblocks showing cartridge levers in the down position. Remove any cartridges.



2. Prepare 500 mL of 0.525% sodium hypochlorite solution using deionized water.
To make 500 mL of bleach: In a 1 liter plastic or glass graduated cylinder, combine 43.75 mL of commercial bleach (such as Clorox™ bleach, which is 6.15% sodium hypochlorite) with 456.25 mL of DI H₂O, mix well. Pour the solution into a 500 mL plastic bottle, and place the plastic bottle on fluidics station.
-

IMPORTANT!

- Prepare beach solution fresh at the time of use.
 - Each fluidics station with 4 modules requires 500 mL of 0.525% sodium hypochlorite solution.
-
3. Perform the bleach cycle.
 - a. Place on the fluidics station an empty one liter waste bottle, a 500 mL bottle of bleach and a one liter bottle of DI water.
 - b. The Bleach protocol requires approximately one liter of DI water.
 - c. Insert the waste line into the waste bottle.

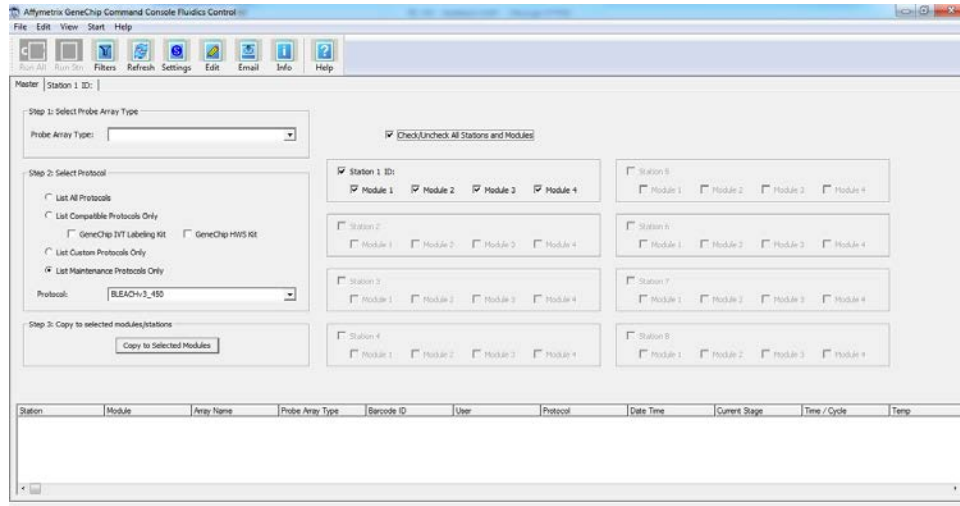
- d. Immerse all three wash and water lines into the bleach solution.

IMPORTANT! Do NOT immerse the waste line into the bleach.



Figure 30 Bleach cycle.

4. Open the instrument control software (AGCC).

5. Choose the current bleach protocol for each module.**Figure 31** Fluidics station protocol window: Select all modules.

6. In AGCC, run the protocol for all modules.

Note: The fluidics station will not start until the needle lever is pressed down (Figure 32). The temperature will ramp up to 50°C.



Figure 32 Press down on the needle levers to start the bleach protocol.

7. Follow the prompts on each LCD. Load empty 1.5 mL vials onto each module if not already done so.
8. Press down on each of the needle levers to start the bleach protocol (Figure 32).



Appendix E Fluidics station care and maintenance

Fluidics station bleach protocol

9. The fluidics station will begin the protocol, emptying the lines and performing the cleaning cycles using bleach solution.
10. After approximately 30 minutes, the LCD will prompt you when the bleach cycle is over and the rinse cycle is about to begin.

The rinse cycle

Once the bleach cycle has finished, the second part of the protocol is a rinse step. This step is essential to remove all traces of bleach from the system. Failure to complete this step can result in damaged arrays.

1. Follow the prompts on the LCD for each module. Lift up on the needle levers and remove the bleach vials. Load clean, empty vials onto each module.
2. Remove the three wash and water lines from the bleach bottle and transfer them to the DI water bottle (Figure 33).



Figure 33 Immerse the three wash and water lines in the DI water bottle. At this step, there is no need to be concerned about the bleach remaining in the lines.



3. Press down on the needle levers to begin the rinse cycle.
 The fluidics station will empty the lines and rinse the needles.
4. When the rinse is completed after approximately one hour, the fluidics station will bring the temperature back to 25°C and drain the lines with air.
 The LCD display will read CLEANING DONE.
5. Discard the vials used for the bleach protocol.
6. After completing the bleach protocol, follow the suggestions for storage of the Fluidics Station 450 in Table 27.

Table 27 Storage suggestions for the fluidics station 450.

If:	Then do this:
Planning to use the system immediately	<p>After running the bleach protocol, remove the DI water supply used in the rinse phase and install the appropriate reagents for use in the next staining and washing protocol (including fresh DI water).</p> <p>Perform a prime protocol without loading your probe arrays.</p> <p>Failure to run a prime protocol will result in irreparable damage to the loaded hybridized probe arrays.</p>
Not planning to use the system immediately	<p>Since the system is already well purged with water, there is no need to run an additional shutdown protocol.</p> <p>Remove the old DI water bottle and replace it with a fresh bottle.</p>
Not planning to use the system for an extended period of time (longer than one week)	<p>Remove the DI water and perform a “dry” protocol shutdown. This will remove most of the water from the system and prevent unwanted microbial growth in the supply lines.</p> <p>Also, remove the pump tubing from the peristaltic pump rollers.</p>

Documentation and support

Customer and technical support

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

- Worldwide contact telephone numbers
- Product support, including:
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation, including:
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

