KaryoStat™ HD Assay

USER GUIDE

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

Catalog Number 905404
Publication Number MAN0017069
Revision A.0

ThermoFisher SCIENTIFIC

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1 Before you begin

Introduction

IMPORTANT! The CytoScan™ Assay protocol is optimized for processing from 8 to 24 samples at a time to obtain whole genome copy number and SNP information from CytoScan™ Arrays. This protocol is not intended for Genome Wide Association studies.

Cytogenetics studies are performed to identify structural changes in DNA, such as copy number changes. Individuals typically have two copies of the genome in each of their cells: one inherited from the mother, and one inherited from the father. Chromosomal abnormalities are common in several disease states such as:

- Deletions-When one or both copies of a particular chromosome region are lost.
- Gains-When a chromosome or chromosomal region is duplicated or multiplied.
- Uniparental Disomies (UPDs)-When two copies of a chromosome or chromosomal region are present, but both have been inherited from a single parent.

Traditional cytogenetics techniques, such as karyotyping and fluorescent *in situ* hybridization (FISH) have been used to study chromosomal abnormalities for decades. However, karyotyping only detects abnormalities at low resolutions (larger than ~5 Mb), and FISH is a more focused and targeted approach without the benefit of genome-wide analysis. Further, these techniques are limited to only providing copy number information so that UPDs cannot be identified.

Together, the CytoScan™ Arrays and the CytoScan™ Assay, along with the Command Console™ and Chromosome Analysis Suite software, enable you to perform high resolution genome-wide DNA copy number analysis. The solution for cytogenetics also provides genotyping information, enabling detection of loss of heterozygosity (LOH), which can be used to detect UPDs. The combined high resolution DNA copy number data and the ability to detect gains, losses, and UPDs on a single array makes the CytoScan™ Solution a great tool for next generation cytogenetics studies.

Assay warnings and precautions

Follow universal precautions for laboratory and assay procedures, and waste disposal. Follow federal, state, local, and within-country regulations. See Appendix G, "Safety" on page 87.

Before you begin, go to Appendix A, "Workflow and practices" on page 57 for specific setup instructions, and equipment and technique illustrations and Appendix C, "Thermal cycler protocols" for specific thermal cycler protocol set ups.

For additional specific warnings, precautions, and procedures, refer to "Documentation and support" on page 90:

Precautions

The physical and toxicological properties of the products in this kit(s) have not been thoroughly investigated. Follow prudent laboratory practices and use general laboratory safety equipment (eye protection, lab coat, 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 re- enter 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. 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.
- Mix and match reagents from other reagent kits.
- Use reagents after more than four freeze-thaw cycles.
- Use any water other than nuclease-free water supplied with the CytoScan Reagent 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 (A_{260}/A_{280} ratio) of input DNA must be between 1.7-2.1.
- Use proper gowning procedures.
- 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 –25 to –15°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 well- controlled. Enzyme activity is a function of temperature.
- Enzyme handling: Maintain tube in –25 to –15°C cooler.
- 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 (room temperature) 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 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 the GeneChip™ Hybridization Oven 645 only. Hybridization ovens should be serviced at least once a year to ensure that they are operating within specification.
- After scanning the arrays, be sure to select the appropriate reference file according to the array type you used.

Genomic DNA general requirements and recommendations

The general requirements for genomic DNA sources and extraction methods are described in this chapter. The success of this assay requires the amplification of PCR fragments between 150 to 2000 bp in size throughout the genome. To achieve this, the genomic DNA must be of high quality, and must be free of contaminants that would affect the enzymatic reactions carried out.

For this protocol, you will use the CytoScan™ Assay Kit (24 sample, Cat. No. 901808). This kit contains the control Genomic DNA. This control meets the requirements outlined below. The size of the starting genomic DNA can be compared with the control Genomic DNA to assess the quality. The control Genomic DNA should also be used as a routine experimental positive control for troubleshooting.

Assay performance may vary for genomic DNA samples that do not meet the general requirements described below. However, the reliability of any given result should be assessed in the context of overall experimental design and goals.

- DNA must be double-stranded (not single-stranded). This can be verified using PicoGreen® quantitation. This requirement relates to the restriction enzyme digestion step in the protocol.
- DNA must be free of PCR inhibitors.

  Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (i.e., EDTA). The genomic DNA extraction/purification method should render DNA that is generally salt-free because high concentrations of certain salts can also inhibit PCR and other enzyme reactions. DNA should be prepared as described in Chapter 2, "CytoScan™ Assay procedure".
- DNA must not be contaminated with other human genomic DNA sources, or with genomic DNA from other organisms.

  PCR amplification of the ligated genomic DNA is not human specific, so sufficient quantities of non-human DNA may also be amplified and could potentially result in compromised genotype calls. Contaminated or mixed DNA may manifest as high detection rates and low call rates.
- DNA must not be degraded.

  The genomic DNA fragment must have Nsp I restriction sites intact so that ligation can occur on both ends of the fragment and PCR can be successful. The approximate average size of genomic DNA may be assessed on a 0.8% or 1% agarose gel using an appropriate size standard control. Control Genomic DNA can be run on the same gel for side-by-side comparison. High quality genomic DNA will run as a major band at approximately 10-20 kb on the gel.

  Pre-amplification methods or pre-digestion with restriction enzymes other than Nsp I have not been tested by Thermo Fisher Scientific. If other methods are desired, we recommend conducting experiments to evaluate their performance with this assay.

Sources of human genomic DNA

The following sources of human genomic DNA have been successfully tested in our laboratories for DNA that meets the requirements described in the section "Genomic DNA general requirements and recommendations".

- Blood
- Cell line

Blood collection methods

The two blood collection methods that have been shown to be compatible with the assay are EDTA and Heparin.

Genomic DNA extraction/ purification methods

Genomic DNA extraction and purification methods that meet the general requirements outlined above should yield successful results. Methods that include boiling or strong denaturants are not acceptable, because the DNA would be rendered single-stranded. Genomic DNA extracted using the following methods have been tested at Thermo Fisher Scientific:

- QIAGEN – Gentra® Puregene® Kit
- 5 PRIME – PerfectPure DNA Blood Kit

IMPORTANT! The CytoScan™ Assay requires genomic DNA concentration ≥ 50 ng/uL. Therefore, the elution volumes for each of the kits will need to be adjusted accordingly to achieve the desired concentration.

RNase treatment

The presence of RNA and free nucleotides can interfere with some quantitation methods using specrophotometer or a NanoDrop instrument. To eliminate RNA contamination, perform RNase treatment during extraction as follows:

- QIAGEN – Gentra Puregene Kit

  Perform RNase treatment as recommended in the extraction kit manual prior to elution of genomic DNA.
- 5 PRIME – PerfectPure DNA Blood Kit

  Use only RNase-treated purification columns for extraction of genomic DNA.

The purified genomic DNA extracted using the two methods above should meet the DNA quality specifications per the manufacturer's kit extraction manual.

Controls

- Use of a pre-qualified sample [e.g., Applied Biosystems 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 and act to confirm successful hybridization, washing, staining, and scanning of the array.

Data analysis

- Once you have scanned the arrays, use the ChAS software to perform .CEL file analysis.
- Be sure to use the appropriate reference file when performing data analysis. Files may vary according to the Genome build used to create them.

  For example, the following is the file you should use if using NA33:
  - CytoScanHD_Array Single Sample Analysis NA33
  - CytoScan750K Array Single Sample Analysis NA33

2 CytoScan™ Assay procedure

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

Temperature definitions

Read all warnings and precautions for reagent kit modules, component catalog numbers, and labeling.

Table 1 Temperature range definitions

Temperature	Range
Freeze	–25 to –15°C
Ice	2 to 8°C
Room temperature	15 to 30°C

Section 1: 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).

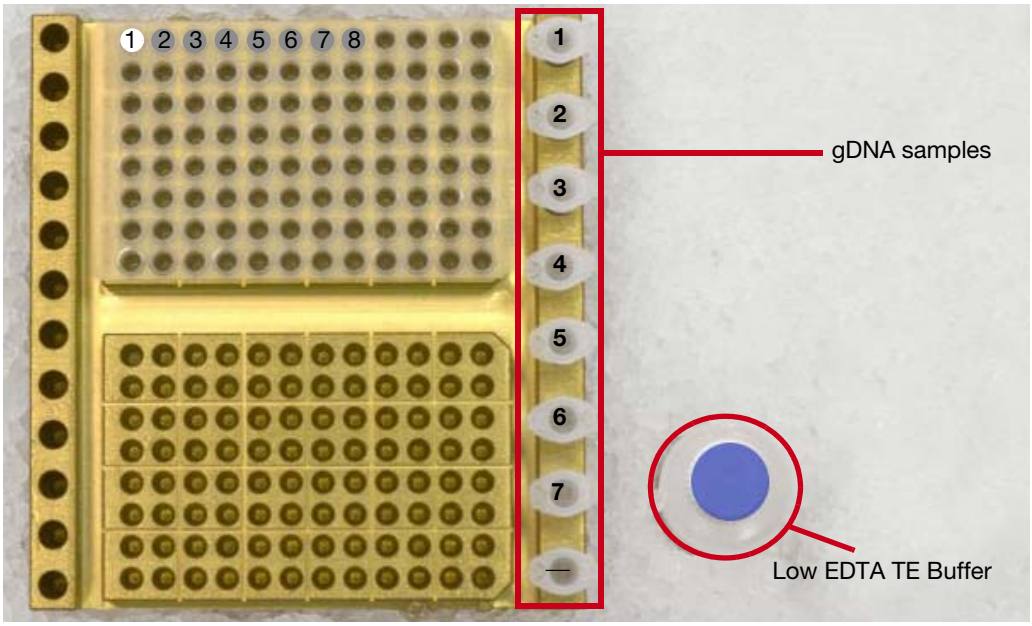


Figure 1 96-well plate in cooling block, on ice

2. Place the gDNA at 15 to 30°C (room temperature) 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 650x*g* for 1 minute; then place in the cooling block.
5. Dilute each sample to 50 ng/µL with Low EDTA TE in separate wells of the 96-well plate or in 1.5 mL eppendorf tubes.
6. Tightly seal the plate.
7. Centrifuge 650x*g* for 1 minute.
8. Place on cooling block.

Aliquot prepared gDNA into assay plate

Set up work area

1. Mark 96-well plate (Figure 2).
2. Place the plate on the lower half of the cooling block.
3. Vortex gDNA 3 seconds; centrifuge.
4. Transfer **5 µL** of first sample from gDNA stock plate to well A1 of DIG-LIG plate; transfer all samples in same way (Figure 2).
5. For the controls, aliquot 5 µL of:
   a. Positive Control gDNA to well A7.

b. Low EDTA TE buffer [negative control] to well A8.

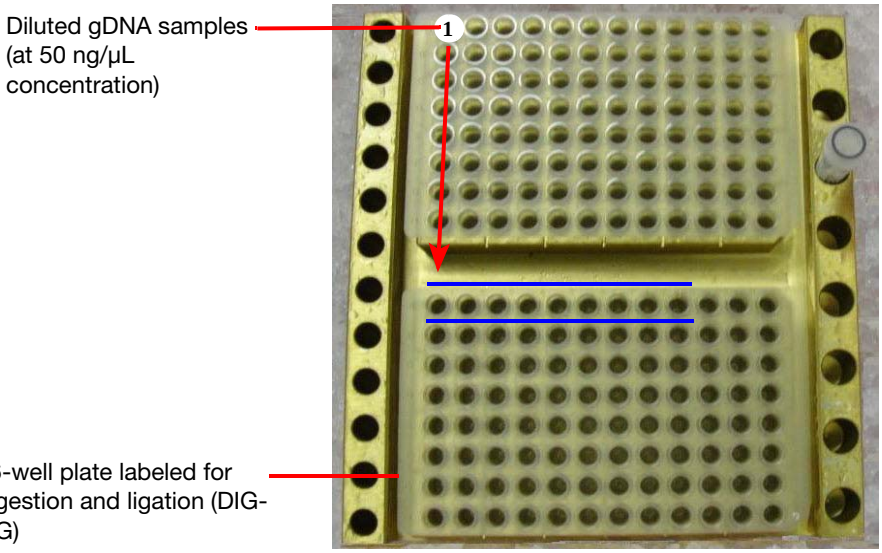


Figure 2 Transfer samples from gDNA Plate to DIG-LIG plate

6. Tightly seal DIG-LIG plate with new seal; centrifuge 650x*g* for 1 minute.

Section 2: Restriction enzyme digestion

Prepare reagents, equipment, and consumables

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

Set up work area

1. 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.
2. Label a 1.5 mL tube "Dig" and place it in the cooling block.
3. Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes (if needed).

Prepare reagents

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

IMPORTANT! Set up reaction within 1 hour.

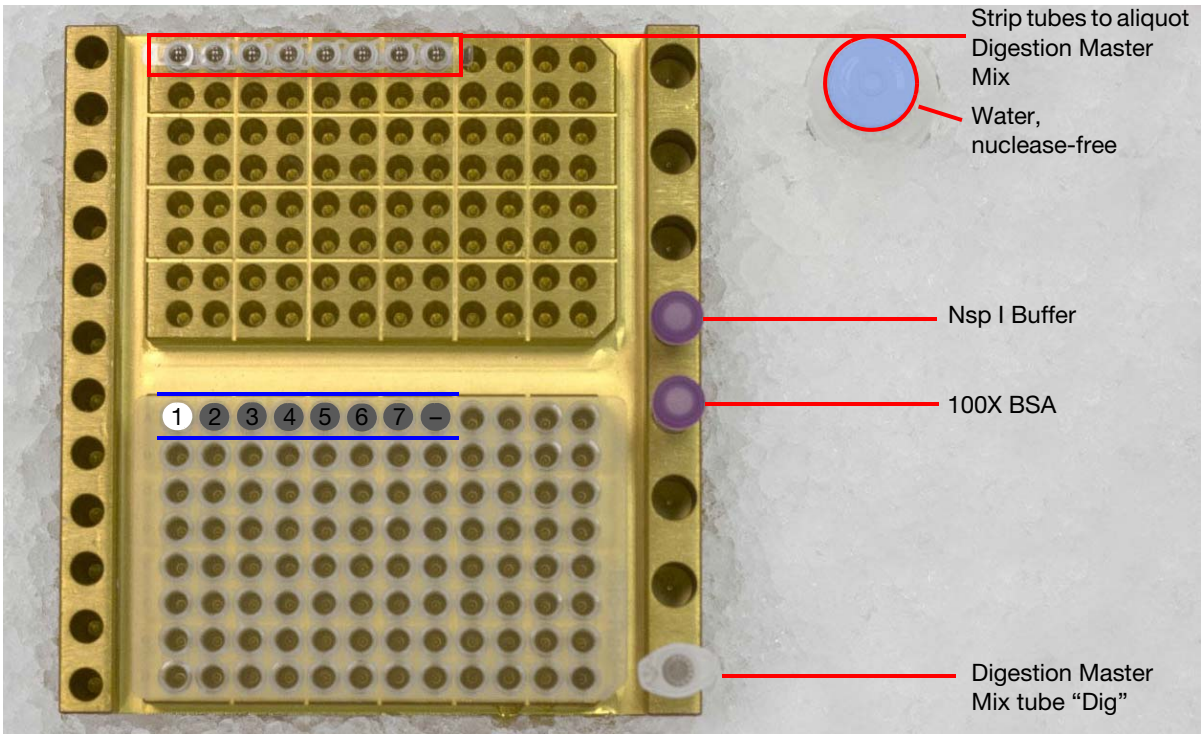


Figure 3 Digestion setup (Nsp I enzyme not pictured, still at –25 to –15°C)

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 2:
   - Water, nuclease-free, chilled (2 to 8°C)
   - Nsp I Buffer
   - 100X BSA

Table 2 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 (–25 to –15°C).
6. Mix Nsp I.
   a. Vortex 1 second.
   b. Quick spin 3 seconds.
7. Return enzyme to –25 to –15°C cooler while preparing Digestion Master Mix.
8. Immediately add Nsp I to Digestion Master Mix.
9. Return enzyme to –25 to –15°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

Note: When working with more than 8 samples, we strongly recommend dividing the master mix into strip tubes and dispensing the master mix from the strip tubes into the samples using a multi-channel pipette. If you are running 16 samples, add 35 µL to each tube. If you are running 24 samples, add 53.5 µL to each tube.

Divide the Digestion Master Mix equally into 8 or 12 strip tubes on cooling block on ice.

1. Seal strip tube with adhesive film strip (or strip caps).
2. Centrifuge 650x*g* for 1 minute and place on cooling block on ice.
3. Remove and discard seal.
4. Unseal the plate and discard seal.
5. Use multichannel P20 pipette and add **14.75 µL** of the Digestion Master Mix to each sample in row A (Figure 4).

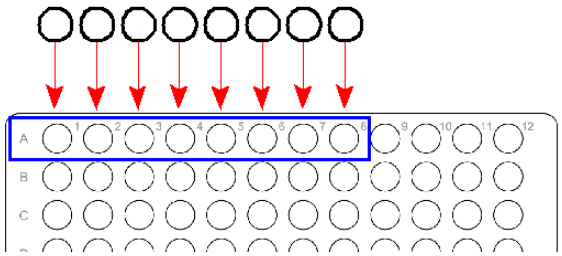


Figure 4 Addition of Digestion Master Mix to samples

6. Seal the plate tightly with a new seal.

Table 3 Digestion final volumes

Sample	Volume/ Sample
Genomic DNA (50 ng/µL)	5.00 µL
Digestion Master Mix	14.75 µL
Total Volume	**19.75 µL**

Load samples on thermal cycler

1. Vortex the plate 1 second all corners and center.
2. Centrifuge 650x*g* for 1 minute.
3. Load the plate on thermal cycler, ensure thermal cycler lid is preheated, and run **CytoScan Digest** protocol.

Table 4 CytoScan Digest protocol

Temperature	Time
37°C	2 hours
65°C	20 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. When the protocol is finished, remove the plate. Ensure the plate is sealed tightly and centrifuge 650x*g* for 1 minute.
6. Place the plate in cooling block on ice and immediately go to *"Section 3: Ligation"*.

Section 3: Ligation

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

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 following to thaw at room temperature (≤30 minutes)
2. Immediately place on ice and use within **1 hour**.
   - Adaptor, Nsp I
   - DNA Ligase Buffer (requires approximately 20 minutes to thaw)

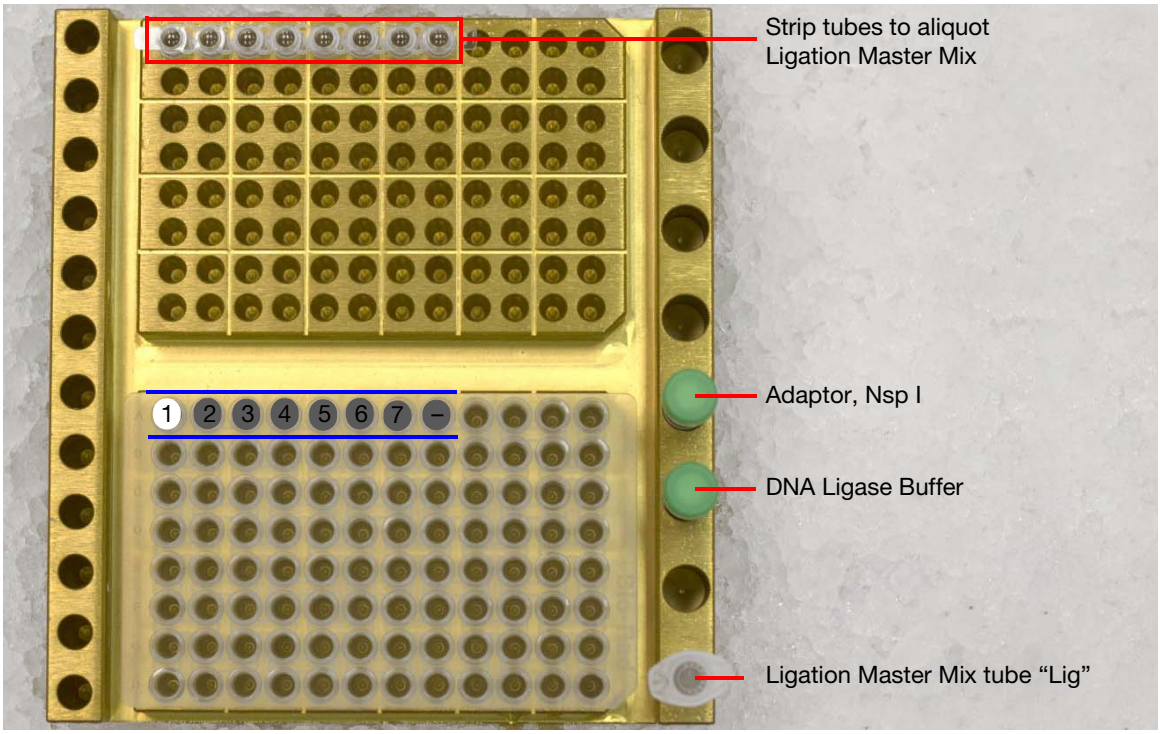


Figure 5 Ligation setup (DNA Ligase not pictured, still at –25 to –15°C)

Prepare digested samples and reagents

1. Prepare samples:
   a. Centrifuge 650x*g* for 1 minute.
   b. Place in lower half of cooling block on ice.

2. Prepare reagents:
   a. Vortex DNA Ligase Buffer and Adaptor, Nsp I, 3 times for 1 second each.

      Note: If precipitate is present in DNA Ligase Buffer, 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 Buffer	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 (–25 to –15°C).
5. Mix DNA Ligase.
   a. Vortex 1 second.
   b. Quick spin 3 seconds.
6. Place in –25 to –15°C cooler.
7. Immediately add the DNA Ligase to the Ligation Master Mix; place back in –25 to –15°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 Master Mix equally into 8 or 12 strip tubes depending on the number of samples.
   - For 16 samples in 8 strip-tubes use 12 µL in each strip tube.
   - For 24 samples in 12 strip-tubes use 12 µL in each tube.
   - For 24 samples in 8 strip-tubes use 19 µL in each tube.
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.
6. Use a multi-channel P20 pipette to add **5.25 µL** of the Ligation Master Mix to each digested sample (Figure 6).

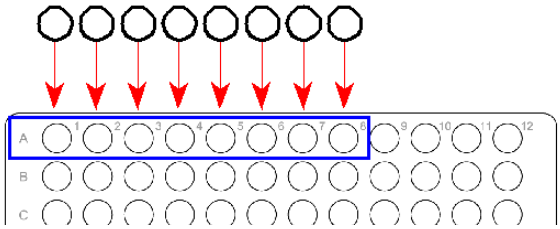


Figure 6 Addition Ligation Master Mix

Table 6 Dig-Lig samples

Sample	Volume/ Sample
Digested DNA	19.75 µL
Ligation Master Mix	5.25 µL
Total	**25.00 µL**

Load samples on thermal cycler

Ensure thermal cycler lid is preheated.

1. Seal the plate tightly with new seal.
2. Vortex for 1 second in all corners and center.
3. Centrifuge 650x*g* for 1 minute.
4. Load the plate on the thermal cycler and run **CytoScan Ligate** protocol (Table 7).

Table 7 CytoScan Ligate thermal cycler protocol

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

5. Discard the remaining Ligation Master Mix and return reagents to the freezer.
6. When protocol finishes, leave the plate in thermal cycler.

   Note: You can hold plate in thermal cycler at 4°C for up to 24 hours (overnight).
7. After removing from thermal cycler, ensure the plate is sealed tightly.
8. Centrifuge 650x*g* for 1 minute.
9. If not proceeding directly to the next step, ensure the plate is sealed tightly and then store the plate at –25 to –15°C.

   Tip: Optional stopping point. The plate may be frozen at –25 to –15°C for up to 10 days.

Section 4: 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 7).

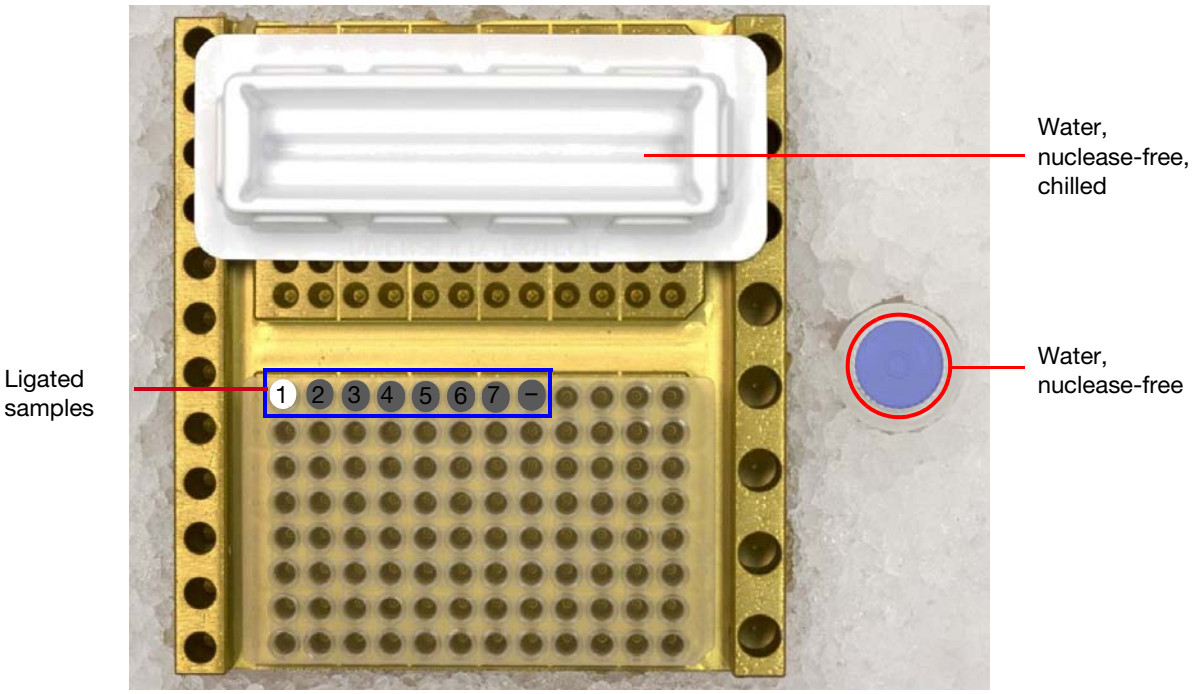


Figure 7 Reagent reservoir on upper half of cooling block on ice

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 room temperature (≤ 30 minutes). Centrifuge 650xg for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.
5. When the **CytoScan Ligate** protocol is finished, take the plate out. Make sure the plate is sealed tightly and spin-down at 2000 rpm for 1 minute.
6. Unseal the ligated sample plate and discard seal.
7. 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**

8. Tightly seal plate with new seal.

9. Vortex 1 second in all corners and center.
10. Centrifuge 650x*g* for 1 minute.
11. Go to "Run PCR" on page 23.
12. If not proceeding with PCR set up, store the plate at –25 to –15°C.

Note: Optional stopping point. The plate may be frozen at –25 to –15°C for up to 10 days.

Run PCR

Transfer to PCR Plate

1. Place cooling block on ice.
2. Keep the diluted ligated sample plate on the upper half of the cooling block.
3. Label a new plate "PCR" and the place in the lower half of the cooling block.
4. Unseal the ligated and diluted sample plate and discard seal.
   Using a multi-channel P20 pipette to transfer **10 µL** of each ligated and diluted sample to the corresponding four wells of the PCR plate. Store leftover samples at –25 to –15°C for up to 10 days.

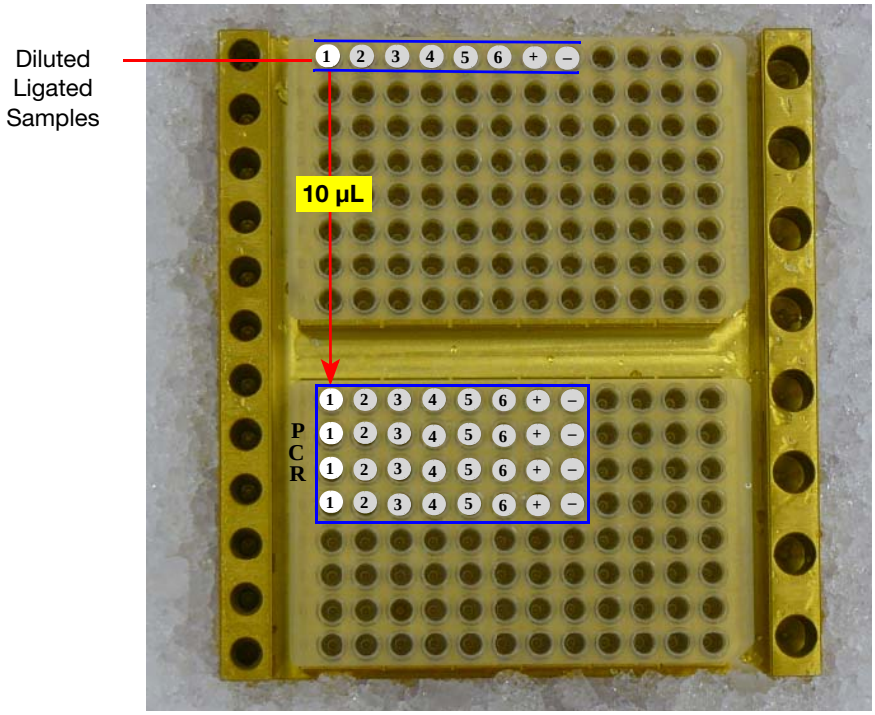


Figure 8 Labeling the 96-well plate for PCR

5. Seal the plate tightly with a new seal and centrifuge 650 x*g* for 1 minute.

Thaw reagents and samples

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

- 10X TITANIUM™ *Taq* PCR Buffer
- dNTP Mixture (2.5 mM each)
- PCR Primer

Prepare reagents and ligated samples

1. Label 15 mL centrifuge tube "PCR". (For more than 8 samples, use a 50 mL tube.)
2. Place on ice (Figure 9):
   - Water, nuclease-free (chilled on ice for 10 minutes)
   - GC-Melt Reagent
   - Reagent reservoir on upper half of cooling block on ice

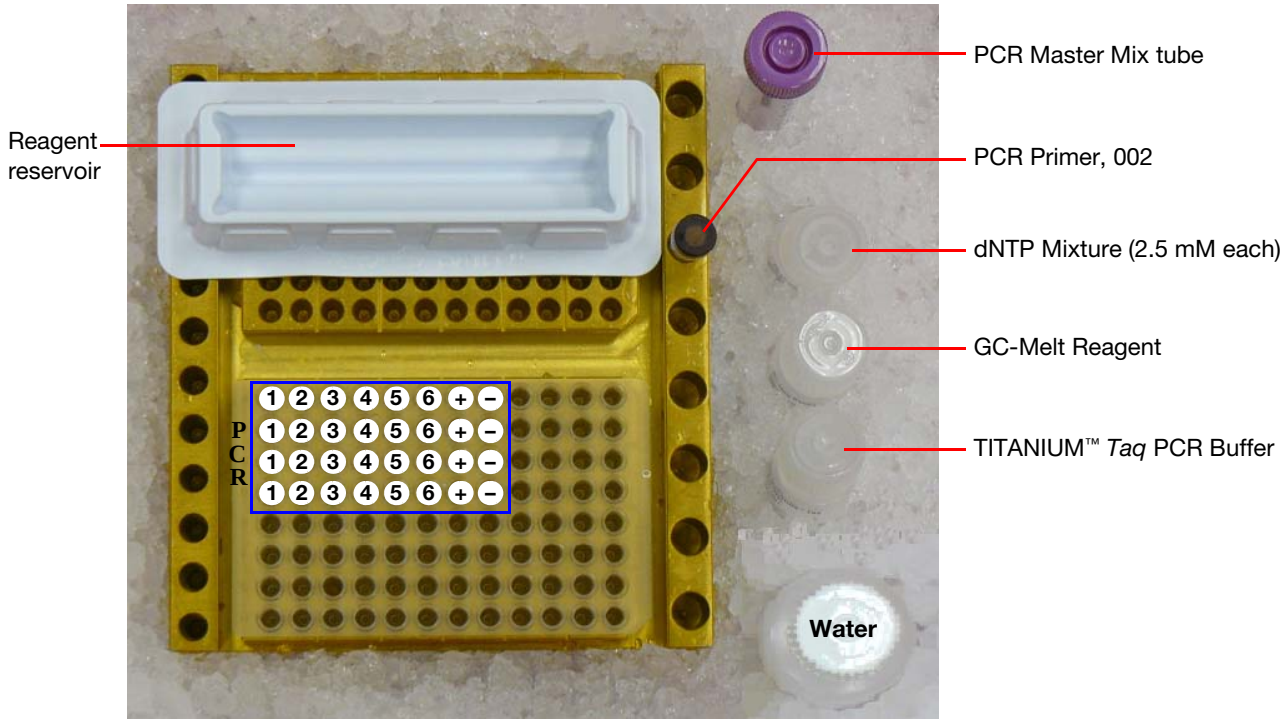


Figure 9

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

   Note: Turn on the thermal cycler (Post-PCR area). Have someone in the Post-PCR Area power on the thermal cycler to preheat the lid. Leave the block at room temperature.

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 8.

IMPORTANT! Leave the 50X Titanium Taq DNAPolymerase at –20°C until ready to use.

Table 8 Clontech TITANIUM Taq PCR Master Mix (8 samples, 15% overage)

Reagent	1 Reaction	8 Samples	16 Samples	24 Samples
Water, nuclease-free (chilled)	39.5 µL	1453.6 µL	2907.2 µL	4360.8 µL
10X Titanium™ *Taq* PCR Buffer	10.0 µL	368.0 µL	736.0 µL	1104.0 µL
GC-melt Reagent	20.0 µL	736.0 µL	1472.0 µL	2208.0 µL
dNTP Mixture (2.5mM)	14.0 µL	515.2 µL	1030.4 µL	1545.6 µL
PCR Primer	4.5 µL	165.6 µL	331.2 µL	496.8 µL
50X Titanium™ *Taq* DNA Polymerase	2.0 µL	73.6 µL	147.2 µL	220.8 µL
Total	**90 µL**	**3312 µL**	**6624 µL**	**9936 µL**

2. Vortex 3 seconds.
3. Take the 50X Titanium *Taq* DNA Polymerase from the freezer and immediately place in a chilled cooler (–25 to –15°C).
4. Mix 50X Titanium *Taq* DNA Polymerase.
   a. Vortex at high speed for 1 second.
   a. Pulse spin the enzyme for 3 seconds.
   b. Place it in the –20°C cooler.
5. Immediately add to the PCR Master Mix, then return to –25 to –15°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 to ensure that 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 650x*g* for 1 minute.
7. Immediately load the plate on the thermal cycler.

Load plate and run CytoScan PCR protocol

Ensure thermal cycler lid is preheated. Ensure block is room temperature (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 PCR** protocol.

   Note: The plate maybe held for 24 hours in the thermal cycler at 4°C.

Temperature	Time	Cycles
94°C	3 minute	1X
94°C	30 seconds	} 30X
60°C	45 seconds	
68°C	15 seconds	
68°C	7 minutes	1X
4°C	Hold (can be held overnight)	
Volume: 100 µL		

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 650x*g* for 1 minute.

   Tip: If not proceeding immediately to the next step, the plate maybe stored at –25 to –15°C for up to 10 days.

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 10) for in-process QC.

Transfer 3 µL from only row A of the PCR plate to the corresponding wells of the gel strip tube.

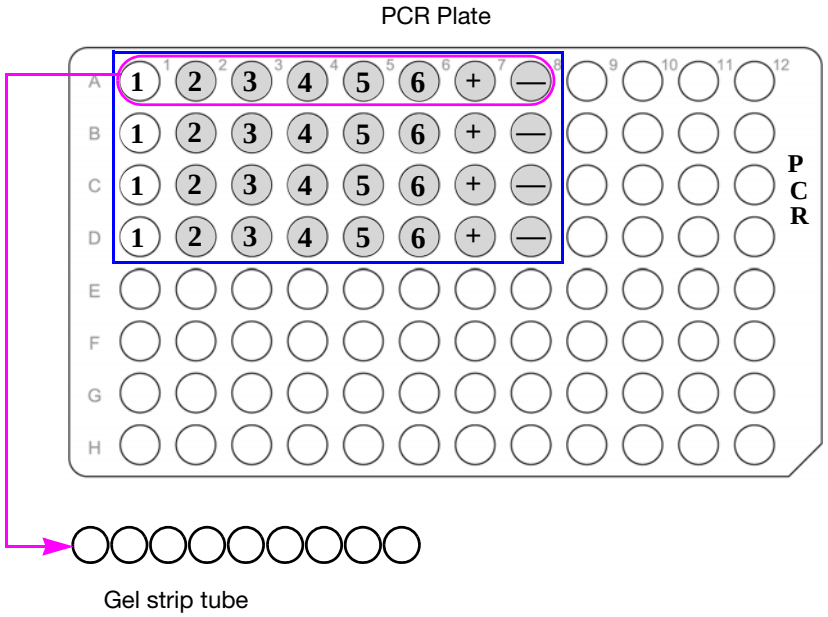


Figure 10 Transfer aliquots of each PCR product to the gel plate.

3. Seal the PCR plate tightly with a new seal.
4. Prepare the QC samples by adding 17 µL of diluted loading buffer to strip tubes (see "Section 11: In-process quality control").
   a. Add 3 µL of amplified sample.
   b. Seal the strip tubes, vortex and centrifuge.
   c. Add to gel plate and run samples on 2% agarose gel to resolve bands.
5. Inspect gel and follow "Section 11: In-process quality control". PCR Product target size distribution is 150 to 2000 bp.

Section 5: 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 the Purification Wash Buffer bottle 10 times.
3. Write the date of the ethanol addition on the bottle.

IMPORTANT! Ensure that absolute ethanol has been added to Purification Wash buffer 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 1.5 mL tube with the sample number using a permanent marker.
2. Ensure that the plate is tightly sealed.
3. Centrifuge 650x*g* for 1 minute.
4. Remove and discard plate seal.
5. Using a P200 single or multi-channel pipette, transfer all 4 aliquots of each sample to the appropriately marked 1.5 mL tube (Figure 11).

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.

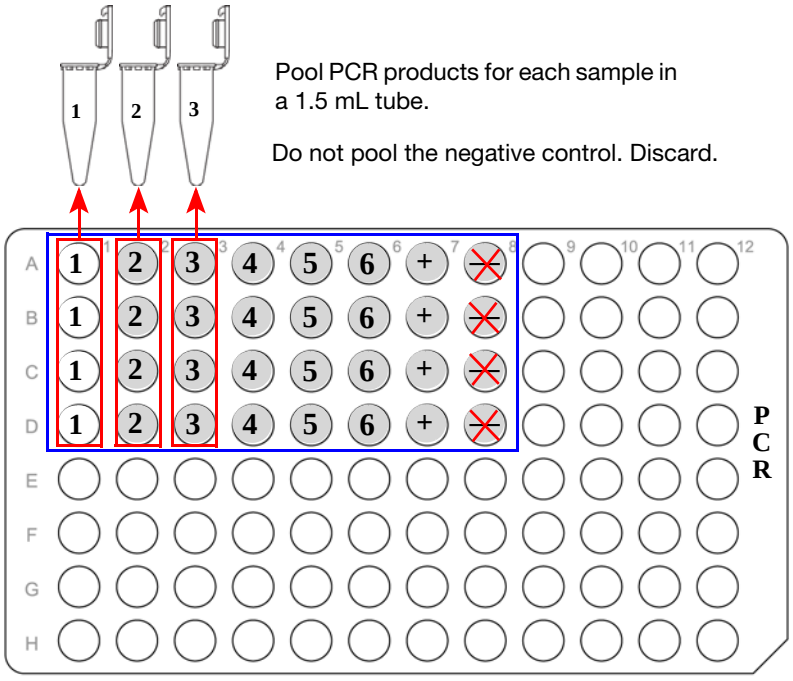


Figure 11 Pool the PCR products

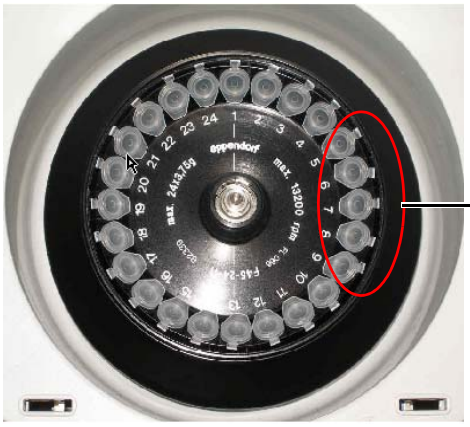
6. Ensure total volume in each well is transferred and pooled.

Volume in each PCR well	= 100 µL
Total volume in 4 PCR wells	= 400 µL
Total volume in each 1.5 mL tube	**= 400 µL/tube –3 µL aliquoted for PCR gel**

Purify PCR products

Change tips between pipetting steps.

1. Thoroughly mix the Purification Beads stock by inverting 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 **720 µL** Purification Beads per sample directly from bottle.
   - **16 and 24 Samples:** Use a multi-channel P1000 pipette to add **720 µL** Purification Beads per sample.
     - 16 samples: Add **15 mL** Purification Beads to reagent reservoir.
     - 24 samples: Add **21 mL** Purification Beads to reagent reservoir.
   - Add **720 µL** 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 room temperature for 10 minutes.
6. Load tubes onto centrifuge with cap hinge facing out and centrifuge 16,000x*g* for 3 minutes.



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

IMPORTANT! Ensure that absolute ethanol has been added to Purification Wash buffer bottle.

1. Use a P1000 pipette to add **1 mL** Purification Wash Buffer to each tube.
   - **16 and 24 Samples:** Pour Purification Wash Buffer into reservoir. With multi-channel pipette add **1 mL** 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 at maximum setting for 2 minutes.

   Note: Bead pellet may not be completely resuspended; this is acceptable.
4. Centrifuge tubes, cap hinges facing out, 16,000x*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, 16,000x*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 room temperature for 10 minutes.

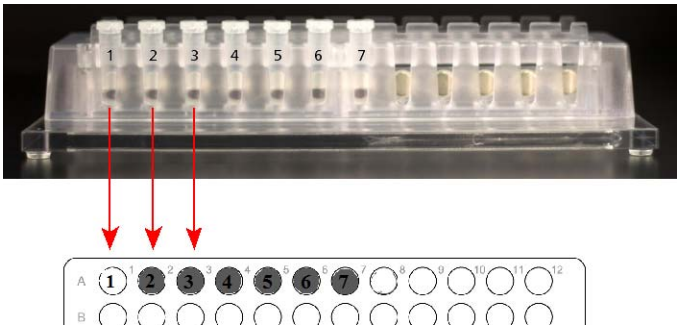
Add Elution Buffer

1. Use a P100 pipette to add **52 µL** 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 at the maximum setting 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 16,000x*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. If not, vortex the tubes to resuspend the pellet, Centrifuge the tubes for 3 minutes at maximum speed (position the tubes with the cap hinges facing out; 16,100 rcf). Place the tubes on the magnetic stand for 10 minutes.

7. Transfer **47 µL** of eluted sample to correct well on new 96-well 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 650x*g* for 1 minute.
10. Ensure plate is tightly sealed.
11. Proceed to "Section 6: Quantitation".

Section 6: 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 12).

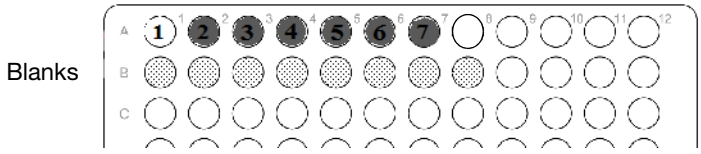


Figure 12 Row for blanks

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 650x*g* for 1 minute. Proceed immediately to quantitation or store at –15°C to –25°C.

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

Quantitate diluted purified PCR product

Note: One absorbance unit at 260 nm = 50 µg/mL (equivalent to 0.05 µg/µL) 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:
   OD_{260} = (sample OD_{260}) – (average water blank OD_{260})
   b. Calculate undiluted concentration for each sample in µg/µL:
   OD_{260} x 0.05 µg/µL x 100
4. Determine acceptable DNA yield.

   Note: For each sample the DNA yield must be ≥2.5 µg/µL. Average DNA yield for 8 or more samples must be ≥3.0 µg/µL. 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.

   - OD_{260}/OD_{280} ratio = 1.7 to 2.1.
   - OD_{320} measurement very close to zero (≤0.1).
5. Immediately go to "Section 7: Fragmentation" or store plate at –25 to –15°C overnight.

   Tip: OPTIONAL STOPPING POINT: The plate maybe stored at –25 to –15°C for up to 10 days.

Section 7: 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 –25 to –15°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 room temperature.

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.

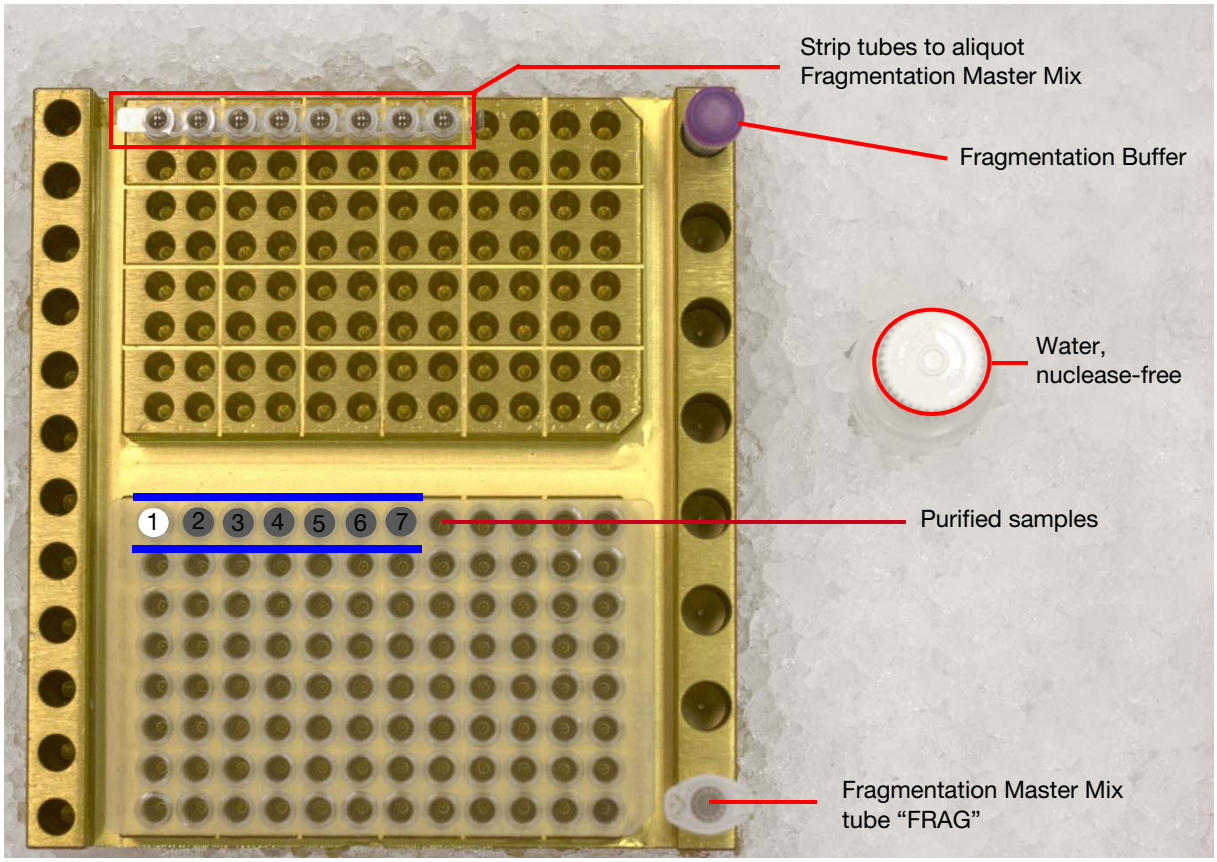


Figure 13 Fragmentation setup (Fragmentation Reagent not pictured, still at –25 to –15°C)

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 tube "FRAG" and keep chilled in cooling block.
5. Cut adhesive film into strips wide enough to seal 8 or 12 strip tubes.
6. Ensure that the plate centrifuge is at 4°C.

Thaw and prepare reagents

1. Thaw the plate of purified, quantitated samples at room temperature (≤30 minutes).
2. Ensure a tight seal and centrifuge plate 650x*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 room temperature (≤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.

1. Add nuclease-free water and Fragmentation Buffer per Table 9.
   a. Vortex tube 3 times for 1 second.
   b. Quick spin 3 seconds.

Table 9 Fragmentation Master Mix

Reagent	Volume
Water, nuclease-free	271.2 µL
Fragmentation Buffer	343.8 µL
Fragmentation Reagent	10.0 µL
Total	**625 µL**

2. Remove the Fragmentation Reagent (2.5 units/µL) from the freezer and immediately place in cooler chilled to –25 to –15°C.
   a. Vortex 1 second.
   b. Quick spin 3 seconds.
   c. Immediately place in –25 to –15°C cooler.
3. Add Fragmentation Reagent per Table 9.

4. Immediately place back in –25 to –15°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.

Note: This is an updated Fragmentation procedure. The previous version is still valid. Refer to Appendix B for the alternative fragmentation Master Mix preparation.

Fragmentation

Add Fragmentation Master Mix to samples

1. Quickly add **78 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. Use a multi-channel P20 pipette to transfer 10 µL Fragmentation Master Mix to each sample (Figure 14). Do not pipet up and down. Do not introduce air bubbles at bottom of tubes.

Tip: It is recommended to use a multi-channel pipette to dispense the Fragmentation Master Mix for any number of samples since Fragmentation is a time and temperature sensitive step.

Sample	Volume/sample
Purified PCR product	45 µL
Fragmentation Master Mix	10 µL
Total	**55 µL**

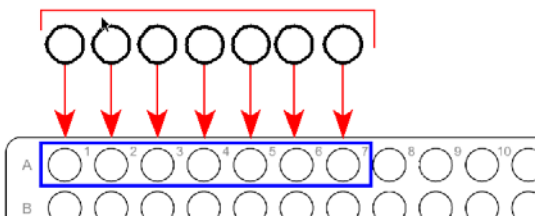


Figure 14 Addition of Fragmentation Master Mix

6. Discard the remaining Fragmentation Master Mix.
7. Seal the plate tightly with new seal.
8. Vortex 1 second at high speed in all corners and center.
9. Bring the sample plate to the centrifuge on cooling block in ice box (2 to 8°C).
10. Centrifuge the plate in pre-chilled centrifuge 650x*g* for 1 minute. Quickly remove the plate from centrifuge and place in cooling block in ice box.
11. Carry the sample plate on the cooling block and immediately load the Fragmentation plate on thermal cycler with preheated lid.

12. Run the **CytoScan Fragment** protocol.

Table 10 CytoScan Fragment thermal cycler protocol

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

13. After removing from thermal cycler, ensure that the plate is sealed tightly and centrifuge 650x*g* for 1 minute and transfer to chilled cooling block on ice.

Check fragmentation reaction

1. Ensure that the plate is tightly sealed; centrifuge 650x*g* for 1 minute. Place on the lower half of the cooling block on ice.
2. Label the correct number of strip-tubes for the number of samples "Dil".
3. Add **28 µL** nuclease-free water to each well of strip tube.
4. Unseal the fragmented DNA plate and discard seal.
5. Add **4 µL** of fragmented samples from plate to corresponding wells of strip tube "Dil" (Figure 15).

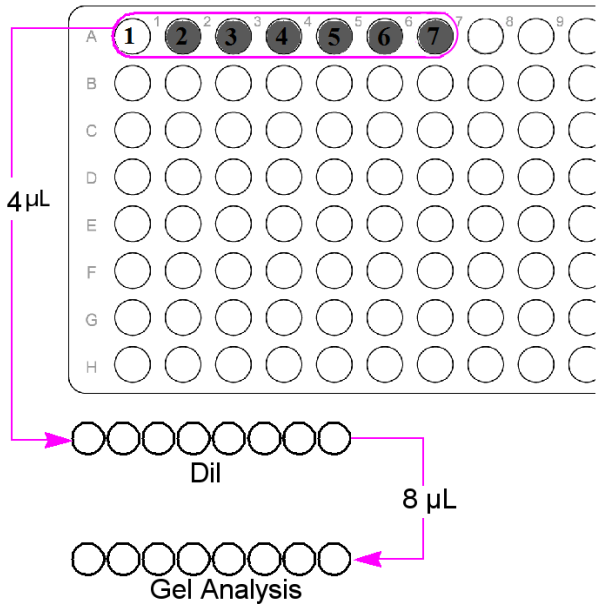


Figure 15 Transfer 2 µL fragmented product to strip tube

6. Seal fragmented DNA plate with new seal and keep on lower half of cooling block on ice.

7. Label the fresh corresponding number of strip-tubes “Gel Analysis”. Add 12 µL of diluted loading buffer to each well.
   a. Add 8 µL diluted fragmented product from Step 5 to 12 µL diluted loading buffer in Step 8.
   b. Pulse vortex.
   c. Centrifuge 650x*g* for 1 minute.
8. Run samples on 4% agarose gel to resolve bands.
9. Inspect gel and follow "Section 11: In-process quality control". Fragment size distribution must be **25 to 125 bp**.
10. Proceed immediately to "Section 8: Labeling".

    Tip: Optional Stopping point: If not proceeding to the next step the fragmented DNA plate maybe held at –25 to –15°C for up to 10 days.

Section 8: Labeling

Preparation

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

Set up work area

1. Place the cooling block on ice.
2. Place a 8- or 12-strip tubes in upper half of cooling block on ice.
3. Label a 1.5 mL microfuge tube "LBL" and place in cooling block.

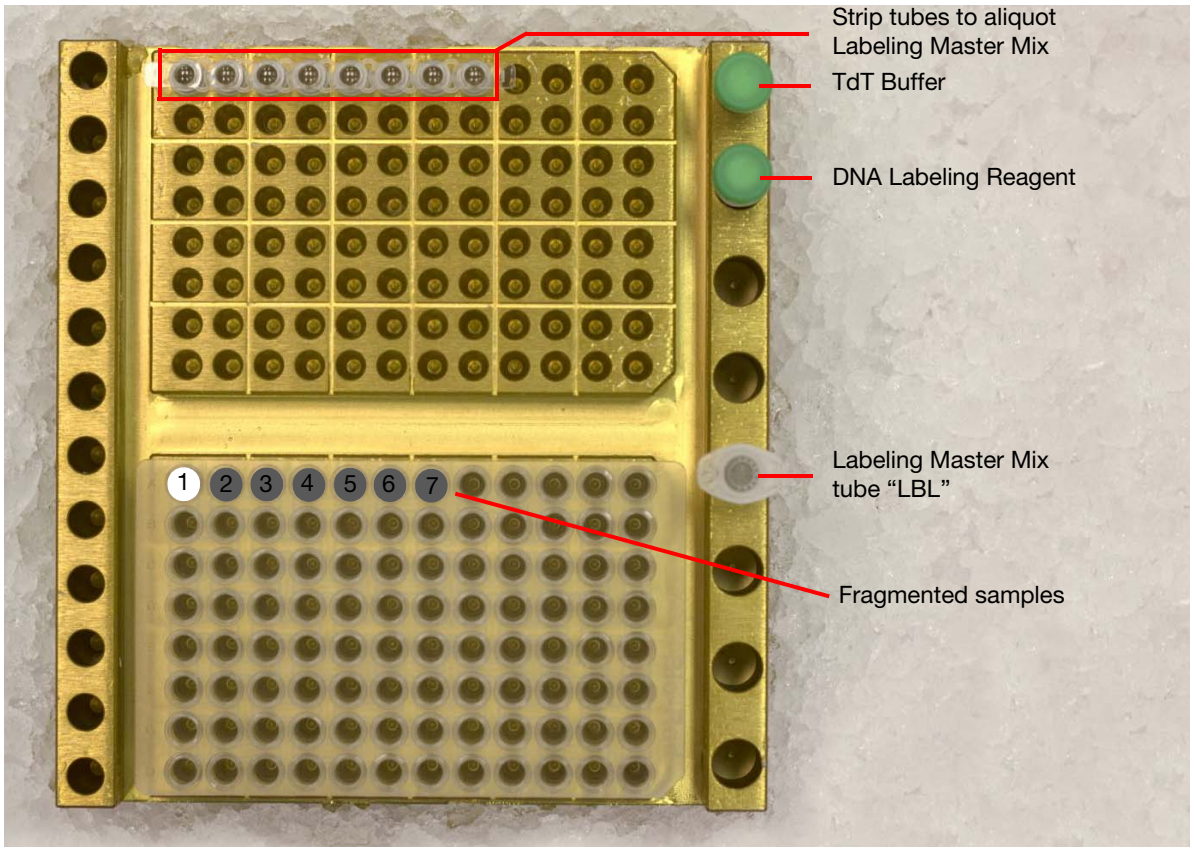


Figure 16 Labeling Setup (TdT Enzyme at –20 to –15°C and hence not shown)

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

Thaw and prepare reagents

1. Thaw at room temperature (≤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 min.

1. Add to the 1.5 mL tube on ice per Table 11:
   - TdT Buffer
   - DNA Labeling Reagent

Table 11 Labeling Master Mix (≥8 samples, 20% overage)

Reagent	1 Sample	8 Samples	16 Samples	24 Samples
TdT Buffer	14 µL	134.4 µL	268.8 µL	403.2 µL
DNA Labeling Reagent	2 µL	19.2 µL	38.4 µL	57.6 µL
TdT Enzyme	3.5 µL	33.6 µL	67.2 µL	100.8 µL
Total	**19.5 µL**	**187.2 µL**	**374.4 µL**	**561.6 µL**

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 –25 to –15°C.
5. Mix TdT Enzyme.
   a. Vortex 1 second.
   b. Quick spin 3 seconds, place in cooling block.
6. Immediately place in –25 to –15°C cooler.
7. Add the TdT Enzyme to the Labeling Master Mix. Place in –25 to –15°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.
4. Remove and discard the plate seal.
5. Use a P20 multi-channel pipette to add **19.5 µL** Labeling Master Mix to each sample.

Sample	Volume/sample
Fragmented DNA (less 2.0 µL for gel analysis)	51 µL
Labeling Master Mix	19.5 µL
Total	**70.5 µL**

6. Use a new seal and tightly seal the plate.

7. Vortex 1 second each in all corners and center.
8. Centrifuge 650x*g* for 1 minute.
9. Place the labeling plate in thermal cycler and run **CytoScan Label** protocol.

Table 12 CytoScan Label thermal cycler protocol

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

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

    Note: If not proceeding to the next step, the plate maybe held at –25 to –15°C for up to 10 days.

Section 9: 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, please refer to the *Applied Biosystems™ GeneChip™ Command Console™ 4.0 User Guide* (Pub. 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 GeneChip™ Hybridization Oven 645

Note: Confirm that the GeneChip 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 hr before loading arrays.

Prepare the arrays and create a Batch Registration file

Note: Remove array from storage 1 hour before hybridization to equilibrate to room temperature. Do not remove arrays from pouch.

To prepare the arrays:

1. Unwrap the arrays and place on the bench top (clean, lint-free surface), 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 18).

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, please 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 17).

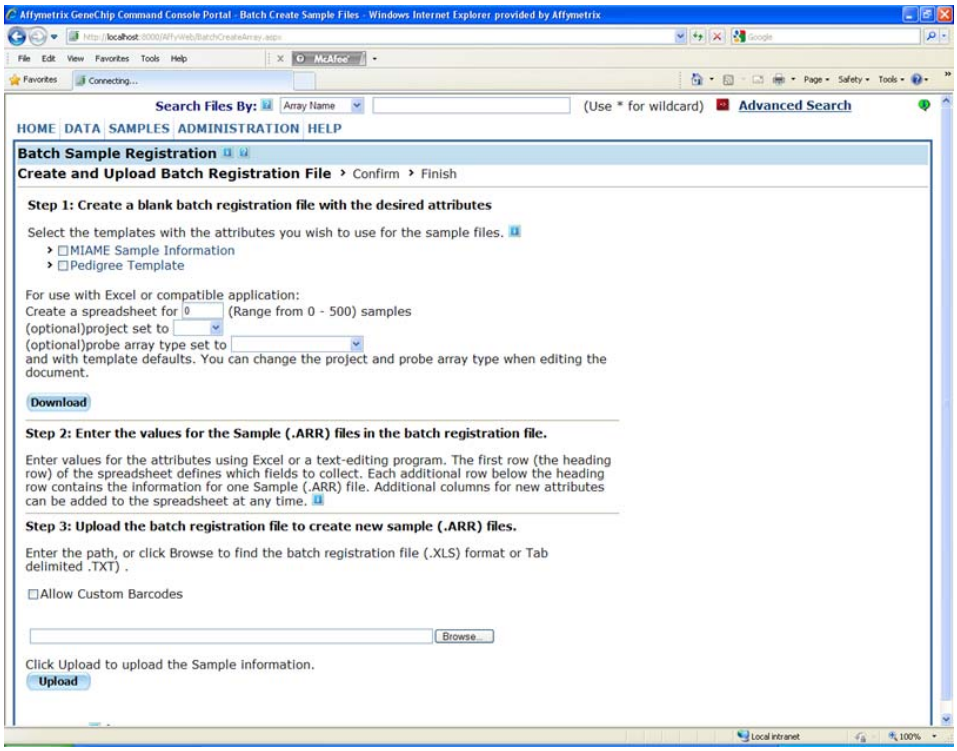


Figure 17 Batch Registration window

3. Within Step 1:
   a. Enter the number of samples for which a spreadsheet needs to be created under **Create a Spreadsheet for**.
   b. Select **Default** from the **Project Set to** drop-down list.
   c. Select the appropriate array type from the **Probe Array type** set to drop-down list:
      - If using the CytoScan HD array, select **CytoScanHD_Array**
      - If using the CytoScan 750K array, select **CytoScan750K_Array**
   d. Click **Download**.
   A Microsoft® Excel® spreadsheet opens.
4. Within Step 2:
   a. Name the experiment file using the following convention: "SampleName_PlateCoordinate_ExperimentDescriptionString_ArrayType_OperatorInitials_yyyymmdd".
   b. The sample file name and the 'Array name' would be identical.
   c. Scan the corresponding barcodes for each Sample name.
   d. 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 18).

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.

Figure 18 Arrays prepared for sample loading

Prepare the reagents and consumables

Set up the work area

To set up the work area:

1. Place a double cooling block on ice (Figure 19 on page 45).
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 650x*g* 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 19.

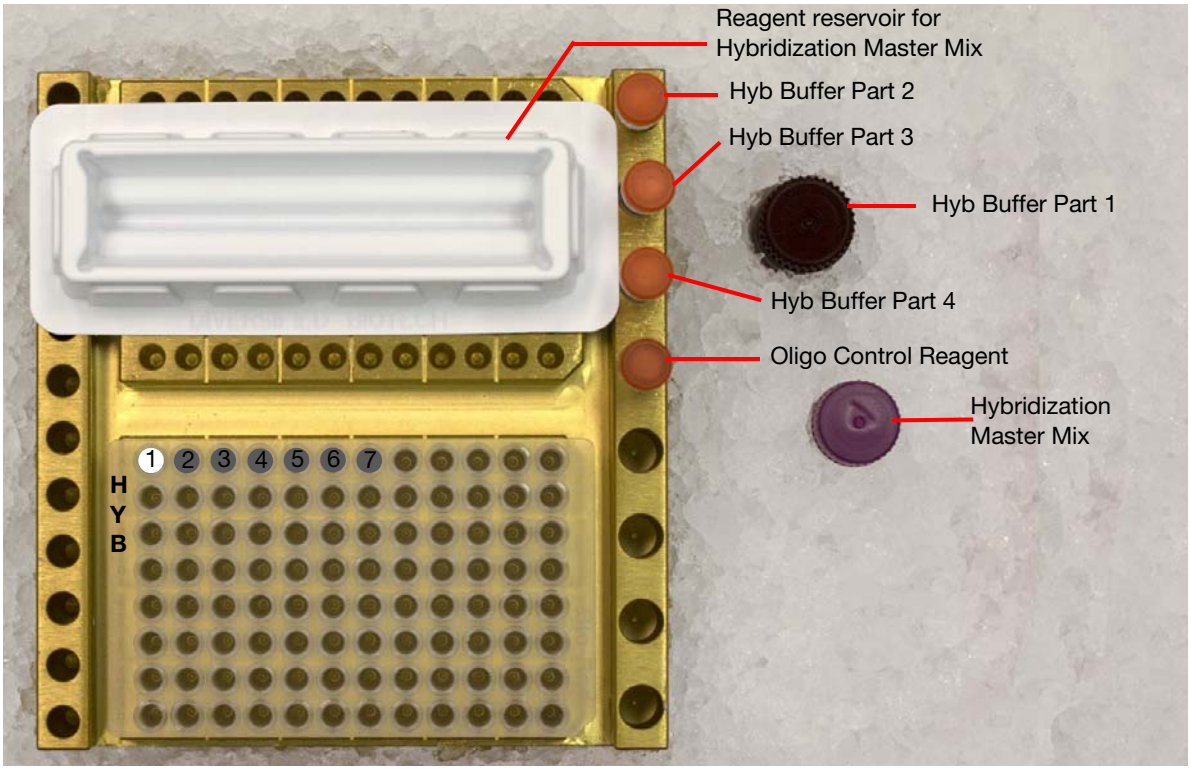


Figure 19 Hybridization setup

Prepare the Hybridization Master Mix

1. To the 15 mL Hyb Master Mix centrifuge tube on ice, add the appropriate volume of each reagent in the order shown in Table 13.

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

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

Table 13 Hybridization Master Mix (≥8 Samples, 20% overage)

Reagent	1 Sample	8 Samples	16 Samples	24 Samples
Hyb Buffer Part 1	165 µL	1584 µL	3168 µL	4752 µL
Hyb Buffer Part 2	15 µL	144 µL	288.0 µL	432.0 µL
Hyb Buffer Part 3	7 µL	67.2 µL	134.4 µL	201.6 µL
Hyb Buffer Part 4	1 µL	9.6 µL	19.2 µL	28.8 µL
Oligo Control Reagent	2 µL	19.2 µL	38.4 µL	57.6 µL
Total	**190 µL**	**1824 µL**	**3648 µL**	**5472 µL**

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:

1. Remove and discard the plate seal.
2. 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 190 µL of Hybridization mix to the samples.

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

3. 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.

4. 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 650x*g* for 1 minute.
5. Place the plate onto the pre-heated thermal cycler and run the **CytoScan Hyb** protocol. If not proceeding to the next step, the plate maybe held at –25 to –15°C for up to 10 days.

Table 14 CytoScan Hyb thermal cycler protocol

Temperature	Time
95°C	10 minutes
49°C	∞

Load the samples onto arrays

To load the samples onto arrays:

1. When the thermal cycler reaches 49°C, leave the samples at 49°C for at least one minute, 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.

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

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

Figure 20 Loading samples onto arrays

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

3. Using a P200 pipette, remove 200 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 20).

   Press firmly to ensure a tight seal to prevent evaporation and leakage.
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.

      Note: Do not allow loaded arrays to sit at room temperature for more than approximately 1 minute. Ensure that the oven is balanced as the trays are loaded, and ensure that the trays are rotating at 60 rpm at all times.
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.

8. When you finish loading the 200 µL of the hybridization mix to the arrays, remove the hybridization plate from the thermal cycler.
9. Seal the plate and spin down at 2000 rpm for 1 min.
10. Store the leftover at –15°C to –25°C. If you need to re-hybridize the samples please refer to Appendix B "Rehybridization step" on page 62.

Section 10: 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. Place 3 empty tubes in the sample holders.
3. Prime the fluidics station.
   a. From Applied Biosystems Command Console application, start the Launcher.
   b. From the Launcher, open 'AGCC Fluidics Control' application.
   c. From the AGCC Fluidics Control panel, select **PRIME_450** script for the specific fluidics stations and the modules.
   d. Intake buffer reservoir A: use Wash A.
   e. Intake buffer reservoir B: use Wash B.
4. 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 and Array Holding Buffer 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. Choose CytoScan HD_Array.Mapping or CytoScan750K_Array.Mapping under **Probe Array Type**.
4. Select a protocol from the AGCC Fluidics from the AGCC Fluidics Control Panel:
   - Select **CytoScan_HD_Array_450** when washing CytoScan HD arrays.
   - Select **CytoScan_750K_Array_450** when washing CytoScan 750K arrays.
5. 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 document (Pub. No. 08-0093 for the Fluidics Station 450).

   Eject the wash block to avoid sensor time out.
6. Remove any previously loaded empty vials.
7. 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.
      - After 16-18 hrs hybridization time, remove the PCR plate containing the remaining hybridization target from the –20°C freezer and thaw at RT for 15 minutes. Centrifuge at 2,000 rpm for 1 minute at RT.
8. After 16 to 18 hrs of hybridization, remove no more than 4 arrays at a time from the oven. Remove the Tough-Spots® from the arrays.
9. Use a P200 to remove all hybridized target and place it back to the corresponding well of the plate containing the left over hybridization mix.

   IMPORTANT! When you recover the hybridized target be sure to return the sample to the corresponding well in the hybridization plate. Avoid mixing samples.

   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.
10. 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.
11. 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.

12. 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.
13. 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.
14. 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.
15. If the array has no bubble, it is ready for scanning. Proceed to "Scanning arrays" on page 50.

    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 hr.
16. Pull up on the cartridge lever to engage wash block. Remove the microcentrifuge vials containing stain and replace with three empty vials as prompted.
17. When washing and staining are complete, shut down the fluidics station following the procedure on page 51.

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* (Pub. 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 21).

   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.

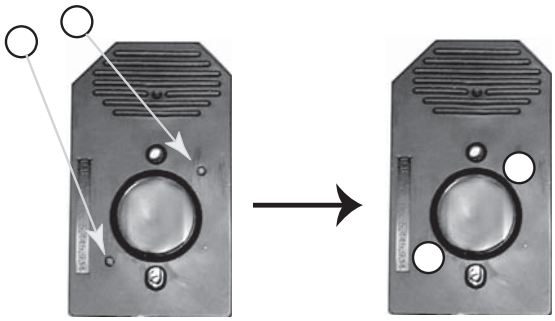


Figure 21 Applying Tough-Spots® to arrays

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  .

   The GeneChip Scanner message appears.

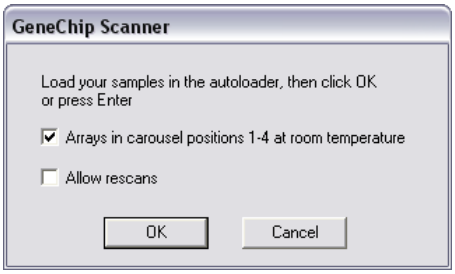
Figure 22 GeneChip Scanner message

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 following message is displayed, click **OK**.



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

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 F, "Fluidics station care and maintenance" for weekly maintenance of the fluidics stations.

Section 11: In-process quality control

The protocol is optimized to be run on an E-Gel.

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.

Diluting the TrackIt™ Cyan/Orange Loading Buffer

1. Add 50 µL of Track it Cyan/Orange Loading Buffer to 49.95 mL nuclease-free water (total volume = 50 mL).
2. Mix well and store at room temperature.

PCR product

1. Prepare the loading buffer dilution.
2. From the first row, add **3 µL** PCR product to **17 µL** diluted loading buffer (total = 20 µL)
3. Vortex.
4. Centrifuge 650x*g* for 1 minute.
5. Load 20 µL from each well onto a 2% agarose gel.
6. Prepare the PCR marker dilutions.
7. Dilute the marker (1:3 dilutions, 5 µL of Marker in 10 µL of nuclease-free water) and load all 15 µL in to each of the marker wells (as needed).
8. Follow manufacturer's instructions to run gel that meets requirements (Figure 23 and Figure 24).

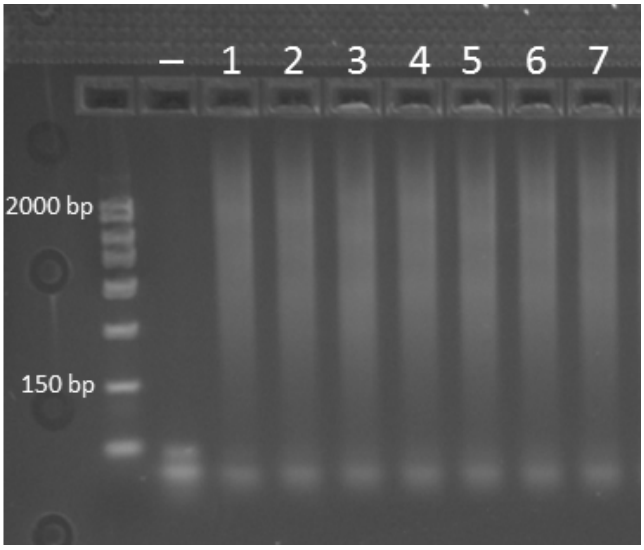


Figure 23 Example PCR gel image on 2% agarose gel with negative control.

Requirements – PCR Product Gel Quality Control Interpretation

- Target distribution of smear covers **150 to 2,000 bp**

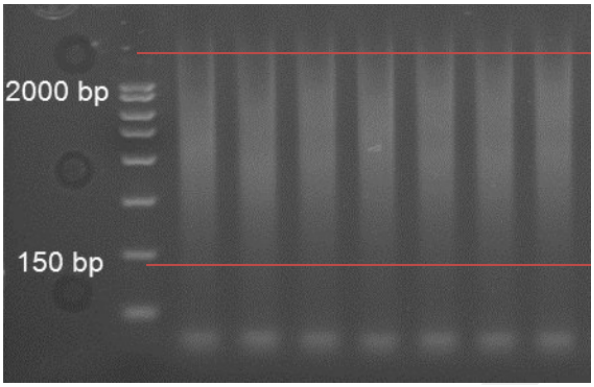


Figure 24 Example PCR gel image on 2% agarose gel.

Fragmented product

1. Prepare loading buffer dilution.
2. Add:
   a. 4 µL fragmented product
      to
   b. 28 µL nuclease-free water (1:8 dilution)
3. Add:
   a. 8 µL diluted fragmented product
      to
   b. 12 µL diluted loading buffer
4. Pulse vortex.
5. Centrifuge 650xg for 1 minute.
6. Load onto gel.
7. Prepare DNA marker dilutions (1:15 dilution, 2 µL in 28 µL nuclease-free water) and load all 15 µL into each of the marker wells (as needed).
8. Follow manufacturer's instructions and run on 4% agarose gel to meet requirements (Figure 25 and Figure 26).

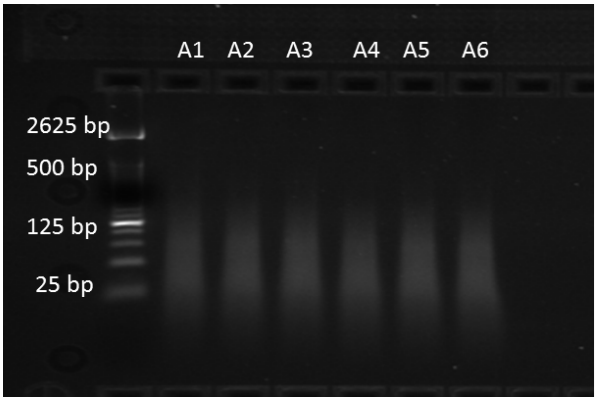


Figure 25 Example fragmentation gel image on 4% agarose gel

Requirements for Fragmentation Product Gel Quality Control Interpretation

- Target distribution of smear covers **25 to 125 bp**

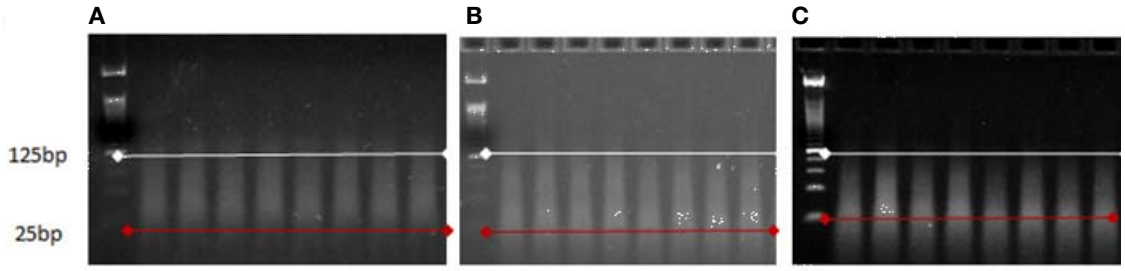


Figure 26 Examples of good fragmentation gels: passing performance besides variability in smear patterns

The three gels in Figure 27 have the majority of high intensity bands above 125 bp (left); at 125 bp (middle); or below 25 bp (right).

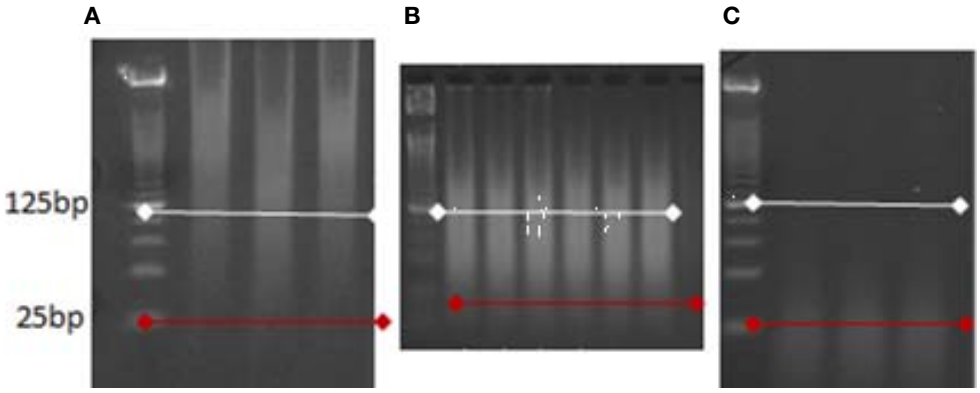


Figure 27 Examples of failing fragmentation gels

Table 15

If majority of target distribution is...	Then sample is...
At 125 bp or extends to 2652 bp	Under fragmented (Figure 27, A and B)
Below 25 bp	Over fragmented (Figure 27, C)

Section 12: Array quality control threshold

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

- MAPD ≤0.25
- SNPQC ≥15
- Waviness-SD≤0.12

For samples not passing these QC threshold, please refer to the troubleshooting section in this user guide (Appendix E). Other metrics can be used as guidance for sample performance. For more information on how these or other metrics are calculated please refer to the *Chromosome Analysis Suite (ChAS) User Guide* (Pub. No. 702943).

Workflow

The 3.5-day workflow for one operator and 8 to 24 samples is given in Figure 28. The 2.5-day workflow for one operator and 8 to 24 samples is given in Figure 29.

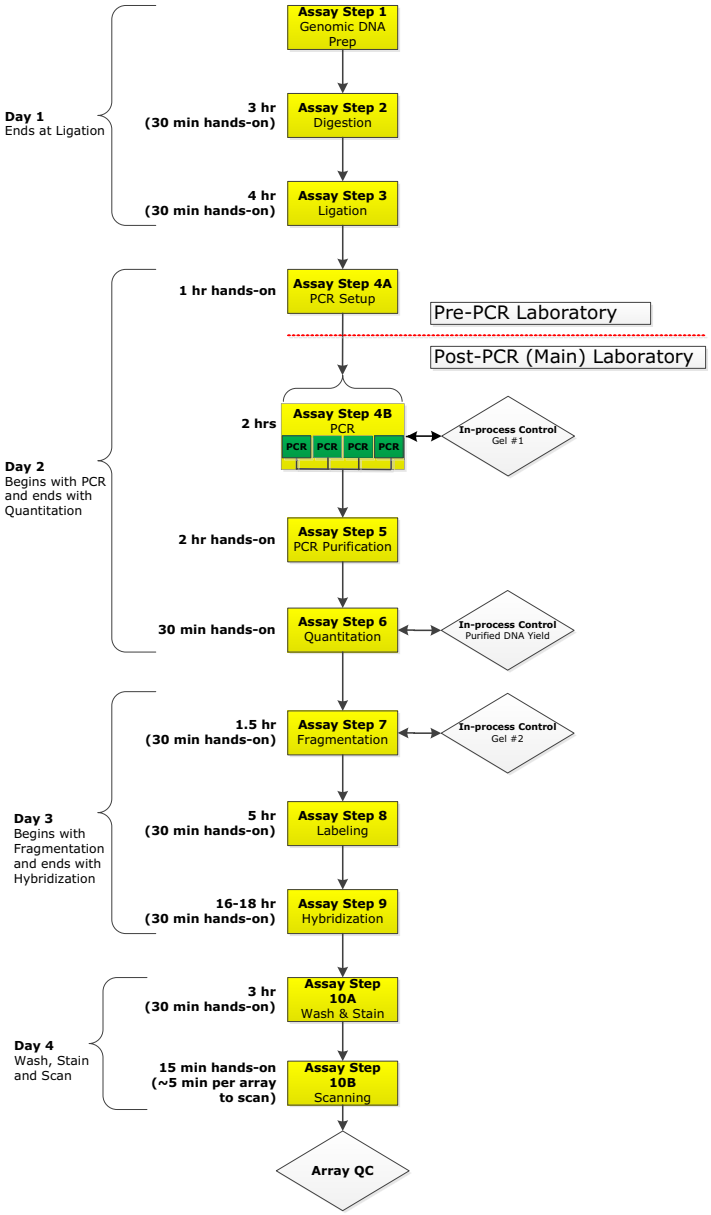


Figure 28 3.5-day workflow

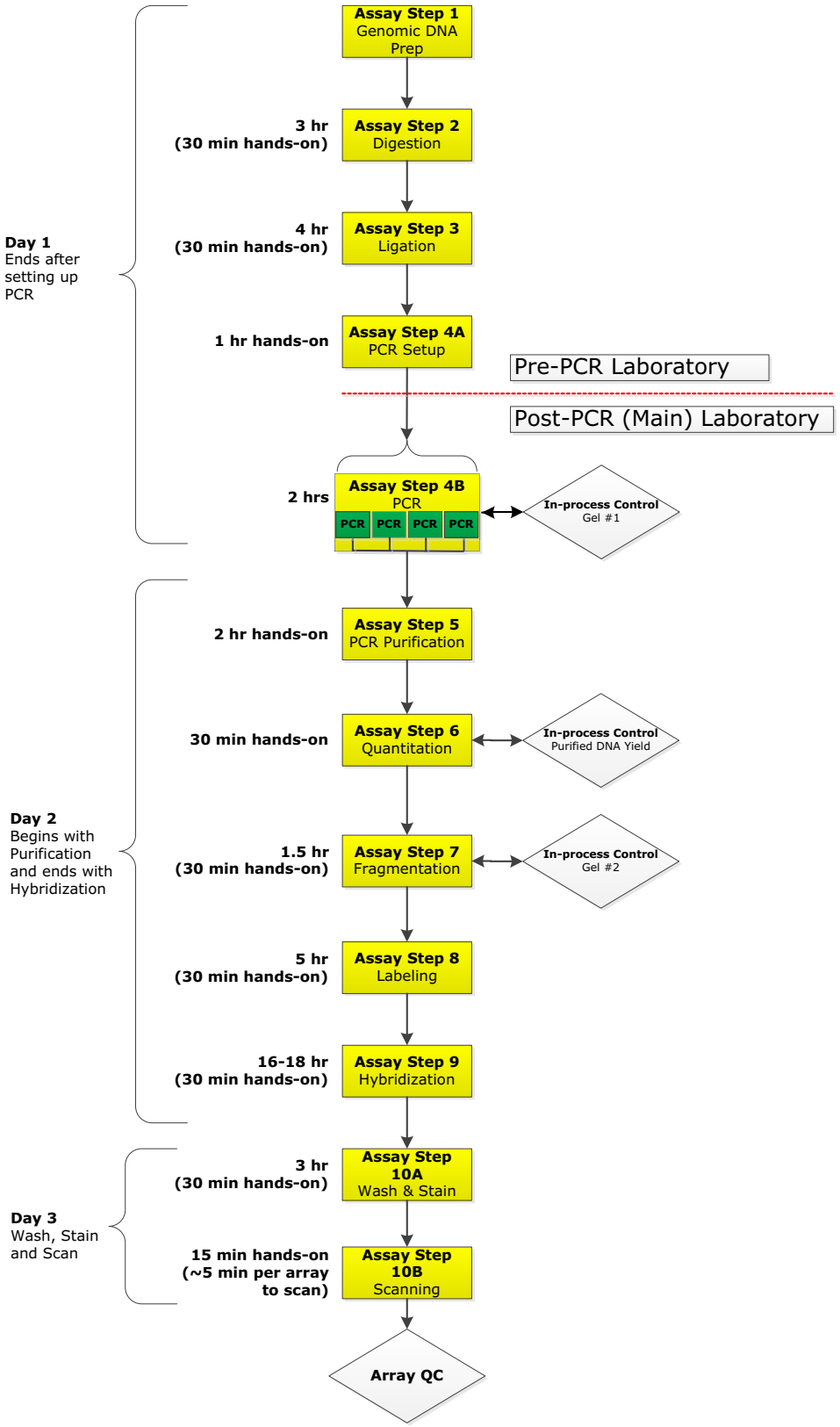


Figure 29 2.5-day workflow

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 room temperature
  b. Ensure tight seal
  c. Centrifuge 650x*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 30), repeat once for high volume reagents: PCR (100 µL), hybridization-ready samples (260 µL)
- **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

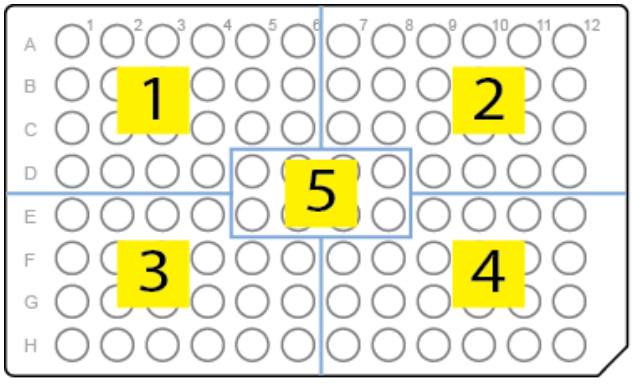


Figure 30 Plate vortexing

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 µL
- 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 10 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.

B Alternative assay steps

Alternative preparation of Fragmentation Master Mix

Thaw and prepare the reagents

IMPORTANT! Leave the Fragmentation Reagent at –20°C until ready to use.

1. Remove the plate of purified, quantitated samples from the –20°C freezer and thaw at room temperature. Once thawed completely, make sure the plate is sealed tightly, then vortex and spin down the plate. Place the plate on lower half of the cooling block on ice and chill for 10 minutes prior to use.
2. Thaw the Fragmentation Buffer (10X) at room temperature. Immediately place on cooling block on ice when thawed.
3. Prepare the Fragmentation Buffer as follows:
   a. Vortex 3 times, 1 second each time.
   b. Pulse spin for 3 seconds.
   c. Place in the cooling block.

Prepare the Fragmentation Master Mix

IMPORTANT! All additions in this procedure must be performed on ice.

We strongly recommend preparing the Fragmentation Master Mix following Table 16 only. The Fragmentation Master Mix is sufficient for 1 to 24 samples.

IMPORTANT! Do not make less than the recommended volume of Master Mix.

Table 16 Fragmentation Master Mix

Reagent	Fragmentation Master Mix
Chilled nuclease-free water	123.8 µL
10X Fragmentation Buffer	158.4 µL
Fragmentation Reagent	5.8 µL
Total	**288.0 µL**

4. Remove the Fragmentation Reagent (2.5 units/µL) from the freezer and immediately place it in the cooler, chilled to –20°C.
   a. Vortex the Fragmentation Reagent at high speed one time for 1 second.
   b. Immediately pulse spin for 3 seconds to bring down any reagent that may be clinging to the top of the tube.
   c. Immediately place in the –20°C cooler.

5. Add the appropriate volume of Fragmentation Reagent from Table 16. Immediately place it back in the –20°C cooler.
6. Vortex the master mix at high speed 3 times, 1 second each time.
7. Pulse spin for 3 seconds and immediately place in the cooling block.
8. Proceed immediately to the next set of steps, "Add Fragmentation Master Mix to the samples" on page 62.

Add Fragmentation Master Mix to the samples

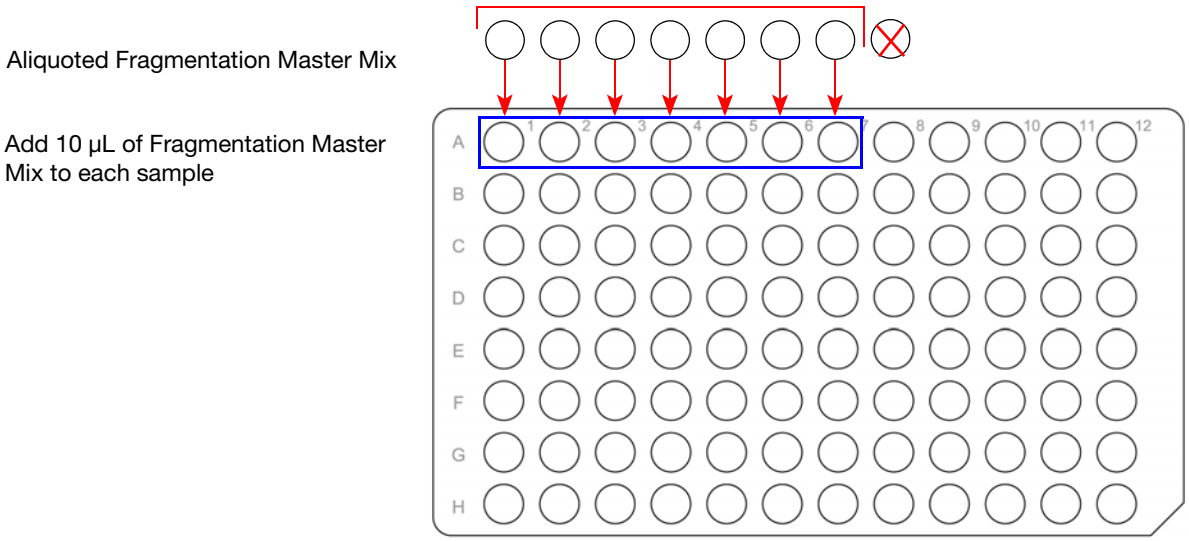


Figure 31 Adding Fragmentation Master Mix to samples

Rehybridization step

One troubleshooting step that can be performed when an array fails to generate a .CEL file or has a QC metrics issue that could be related to an array with manufacturing issues is the rehybridization of the target sample on a new array.

If you have any array issue, please contact your Field Applications Scientist before proceeding to rehybridizing a sample.

Rehybridization procedure

1. After loading 200 µL of hybridization target onto each array, save the left over hybridization target (~60 µL) in the original PCR plate by removing the plate from the thermal cycler (refer to "Section 9: Target hybridization via AGCC" on page 42).
2. Seal the plate and spin down at 2000 rpm for 1 min at room temperature.
3. Store the plate at –20°C for 16-18 hrs during hybridization time.
4. On the next day, after 16-18 hrs hybridization time, remove the PCR plate containing the remaining hybridization target from the –20°C freezer and thaw at RT for 15 minutes. Centrifuge at 2,000 rpm for 1 minute at RT.

5. Remove the hybridized arrays from the oven (4 arrays are removed at a time). Use a P200 to remove all hybridized target and place back into the corresponding well of the plate containing left over hybridization mix from day 1. The hybridized arrays can be immediately loaded on fluidics station to undergo the wash/stain protocol and scanning.
6. The recovered hybridization target shall be sealed in the PCR plate and spun down at 2000 rpm for 1 min.

   Note: Target can be stored at –25 to –15°C for up to 10 days. Precipitation may be visible in wells; however, no impact to hybridization has been observed
7. For re-hybridization the sealed PCR plate shall be removed from the –20°C freezer, incubated at room temperature for 15 min and vortex at maximum speed for 3 seconds. Spin down at 2000 rpm for 1 min at room temperature.
8. Place the plate into a thermal cycler and run the **CytoScan Hyb** protocol.

Table 17 CytoScan Hyb thermal cycler protocol

Temperature	Time
95°C	10 minutes
49°C	∞

9. Continue with loading denatured hyb cocktail onto arrays as described in "Section 9: Target hybridization via AGCC" on page 42.

C Thermal cycler protocols

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

Pre- and Post-PCR protocols

Table 18 Pre-PCR clean room

Number of thermal cyclers required	Protocol name
1	CytoScan Digest
	CytoScan Ligate

Table 19 Post-PCR room

Number of thermal cyclers required	Protocol name
1	CytoScan PCR Assay
	CytoScan Assay Fragment
	CytoScan Assay Label
	CytoScan Assay Hyb

Thermal cycler protocols

CytoScan Digest

Table 20 CytoScan Digest thermal cycler protocol

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

CytoScan Ligate

Table 21 CytoScan Ligate thermal cycler protocol

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

CytoScan 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 22 CytoScan PCR thermal cycler protocol

Temperature	Time	Cycles
94°C	3 minutes	1X
94°C 60°C 68°C	30 seconds 45 seconds 15 seconds	} 30X
68°C	7 minutes	1X
4°C	Hold (for up to 24 hrs)	

CytoScan Fragment

Table 23 CytoScan Fragment thermal cycler protocol

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

CytoScan Label

Table 24 CytoScan Label thermal cycler protocol

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

CytoScan Hyb

Table 25 CytoScan Hyb thermal cycler protocol

Temperature	Time
95°C	10 minutes
49°C	∞

D Required equipment, consumables, and reagents

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

From Thermo Fisher Scientific™

Table 26 Thermo Fisher Scientific equipment and software required

✓	Item	Qty	Cat. No.
Equipment			
❑	GeneChip™ System 3000 – GeneChip™ Scanner 3000 with AutoLoader – GeneChip™ Fluidics Station 450 – Workstation	1	00-0210
❑	GeneChip™ Hybridization Oven 645		00-0331
❑	Tubing, Silicone peristaltic for Fluidics Station 450		400110
Software			
❑	Applied Biosystems™ GeneChip Command Console Software		Version 3.2.2 or higher
❑	Chromosome Analysis Suite (ChAS 2.1 or higher)		901394

Table 27 Applied Biosystems array and reagent bundles available

Bundle name	Items	Cat. No.
CytoScan™ HD Kit Plus 24	CytoScan HD Arrays+ CytoScan Reagent Kit + Applied Biosystems Amplification Kit, sufficient for 24 reactions	905824
CytoScan HD Kit Plus 96	CytoScan HD Arrays + CytoScan reagent kit + Applied Biosystems Amplification Kit, sufficient for 96 reactions	905896
CytoScan™ 750K Kit Plus 24	CytoScan 750K Arrays+ CytoScan Reagent Kit + Applied Biosystems Amplification Kit, sufficient for 24 reactions	905924
CytoScan 750K Plus 96	CytoScan 750K Arrays+ CytoScan Reagent Kit + Applied Biosystems Amplification Kit, sufficient for 96 reactions	905996
CytoScan™ HD Kit	CytoScan HD Arrays + CytoScan Reagent Kit sufficient for 24 reactions	901835
CytoScan™ 750K Kit	CytoScan 750K Arrays + CytoScan Reagent Kit sufficient for 24 reactions	901859

CytoScan™ Reagent Kit

Table 28 CytoScan™ Assay Kit–24 Reaction Kit components (Cat. No. 901808)

Cap color	Component	Part No.	Storage
CytoScan™ Module 1: Pre-Lab Restriction and Ligation		904004	
●	Nsp I	901718	–25°C to –15°C
●	Nsp I Buffer	901719	
●	100X BSA	901720	
●	Adaptor,Nsp I	902702	
●	DNA Ligase buffer	901722	
●	DNA Ligase	901723	
○	REF DNA 103 (50 ng/µL)	900421	
○	PCR Primer	902674	
CytoScan™ Module 2: Pre-Lab TE Buffer and Water		904001	
●	Low EDTA TE Buffer	902979	2°C to 8°C
●	Water, Nuclease-Free	902976	
CytoScan™ Module 3: Post-Lab Fragmentation, Labeling and Hybridization		904002	
●	Fragmentation Reagent	902428	–25°C to –15°C
●	Fragmentation Buffer	903001	
●	TdT Enzyme	902675	
●	TdT Buffer	902676	
●	DNA Labeling Reagent	902677	
●	Oligo Control Reagent	902678	
●	Hyb. Buffer Part 1	901725	
●	Hyb. Buffer Part 2	901726	
●	Hyb. Buffer Part 3	901727	
●	Hyb. Buffer Part 4	901728	
CytoScan™ Module 4: Post-Lab Stain, Holding Buffer, Beads and Water		904005	
●	Stain Buffer 1	901751	2°C to 8°C
●	Stain Buffer 2	901752	
●	Array Holding Buffer	901733	
○	Purification Beads	901807	
○	Water, Nuclease-free	901781	
CytoScan™ Module 5: Post-Lab Elution Buffer and Purification Wash Buffer		903000	15°C to 30°C
○	Elution Buffer	901738	
○	Purification Wash Buffer	901372	
○	Wash A	901680	15°C to 30°C
○	Wash B	901681	

From other suppliers

Reagents required but not provided.

Table 29 Reagents from other suppliers, required

Item
Bleach, Sodium Hypochlorite prepared from a concentrate solution without additives at final working concentration of 0.615% (v/v)
PCR gel DNA ladder: 50 to 2000 bp
Fragmentation gel DNA ladder: 25 to 2600 bp
Ethanol, absolute
Gel-loading buffer

Pre-PCR Clean Room—equipment required but not provided

Table 30 Pre-PCR Clean Room equipment, required

Item
If assay is to be performed in one room: • 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, –25 to –15°C; deep freeze; manual defrost; 17 cu ft
Microfuge (for tubes and strip tubes)
Pipettors: • 12-channel, 2 to 20 µL • 12-channel, 20 to 200 µL • single-channel, 100 to 1000 µL • single-channel, 2 to 20 µL • single-channel, 20 to 200 µL
Rectangular ice tray large, 9L (16 x 13 in, 41 x 33 cm)
Storage racks, tube, 96-well
Thermal cycler: capable of holding 200 µL volume and 96-well plate; heat block capable of holding temperature of 4–99.9°C; temperature accuracy of ±0.25°C (at 35–99.9°C); average heating and cooling rate of 2.6°C per second; thermal uniformity of ±0.5°C.
Vortexer, 60 Hz, 75 W, 600 to 3200 RPM

Post-PCR Room—equipment required but not provided

Table 31 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 µL
Electrophoresis supplies
Freezer, –25 to –15°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 16,200x*g*
Microfuge (for tubes and strip tubes)
Microtube foam insert
Pipettors: • 12-channel, 100 to 1200 µL • 12-channel, 2 to 20 µL • 12-channel, 20 to 200 µL • single-channel, 100 to 1000 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 13 in; 41 x 33 cm)
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 4–99.9°C; temperature accuracy of ±0.25°C (at 35–99.9°C); average heating and cooling rate of 2.6°C per second; thermal uniformity of ±0.5°C.
Vortexer, 60 Hz, 75W, 600-3200 RPM

Consumables required but not provided

Table 32 Consumables from other suppliers, required

Item
Adhesive films, clear, PCR-certified, 96-well plates
Adhesive label dot (Tough-Spots®), 1/2-inch and 3/8-inch
Agarose gel or E-Gel, 2%
Agarose gel or E-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 1000 µL
Plate, OD for UV, 96-well (required only if using microplate spectrophotometer)
Plates, unskirted PCR with a maximum volume of 330 µL
Reagent reservoir, 25 mL
Tube strips, nuclease-free, sterile, 8-well, 0.2 mL polypropylene

Symbols

Table 33

Symbol/Label	Statement/Meaning
	Part/Catalog Number
	Lot Number
	Expiration Date YYYY-MM-DD
	Temperature Limitation

Troubleshooting the CytoScan™ Assay

Table 34

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.	• 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.
	Non-optimal PCR conditions.	Use only calibrated thermal cyclers. Double-check PCR protocols to ensure that they have been entered correctly.
		Check the PCR reagents. Use only those reagents recommended by Thermo Fisher Scientific.
		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.
		• 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.

Table 34 (Continued)

PCR Gel QC step	Likely cause	Solution
Faint or no PCR product visible on the gel. Samples are affected but positive control is OK.	Insufficient or degraded genomic DNA.	• 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 page 8.
	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 Chapter 1.
Wrong size distribution of PCR product.	Mispipetting of PCR primer volume in the master mix.	• Verify pipette calibration and function. • Repeat PCR from the remaining digestion/ligation material if available; otherwise restart from the beginning.
	Mispipetting of Taq polymerase in the master mix.	
PCR product evident in the negative control.	Reagents or equipment contaminated with ligated product or amplified product.	• 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.

Table 35

Purification Yield QC step	Likely cause	Solution
Low eluate volume (<47 µL)	Insufficient volume due to pipetting error or pipet out of calibration.	Check pipette calibration. Make sure 52 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 or individual yield is <2.5 µg/µL)	Loss of sample prior to purification.	If the yield is not adequate, repeat the assay.
	Possible problems with input genomic DNA.	• 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 1.
	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.
	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.

Table 35 (Continued)

Purification Yield QC step	Likely cause	Solution
High yields (>5.0 µg/µL)	Too little Elution Buffer added to the Purification Beads.	Verify pipette calibration and function. Make sure 52 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.
OD 260/280 ratio is not between 1.8 and 2.0	PCR product may not have been adequately washed.	Ensure that proper volume of absolute ethanol is added to the Purification Wash Buffer and follow the procedure provided in Chapter 2 on page 28.
	An error may have been made while taking the O.D readings.	Retake the O.D following the instructions provided in Chapter 2 on page 32.
OD 320 measurement is > 0.1	Purification beads may have been carried over into purified samples.	Spin down the sample for 5 minutes. Place on the MagnaRack and pipette out the eluate. Retake the OD measurement.
	Scratches or dust particles on the OD plate.	Ensure that the bottom surface of the OD plate is clean and scratch-free.
	Air bubbles are present in the diluted DNA within the OD plate.	Vortex the OD plate, spin it down again following the guidelines provided in Chapter 2 on page 32, and retake the OD.

Table 36

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.	• 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.
	Purified samples or assembled reactions were allowed to warm to room temperature during reaction assembly or prior to incubation.	Ensure that the plate centrifuge is completely chilled to 4°C before spinning the assembled fragmentation plate.
		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.
		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.
		Ensure that the master mix tube and strip tubes are pre-chilled before reaction setup.
Under fragmentation: PCR product is still visible in 150-2000 bp size region on a 4% agarose gel.	Improper storage or handling of the Fragmentation Reagent.	• The Fragmentation Reagent should be stored at –25°C to –15°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 –25°C to –15°C inside a cooler to preserve its activity. • Do not over vortex the Fragmentation Reagent.
	• 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.	• 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.

Table 36 (Continued)

Fragmentation QC step (Gel)	Likely cause	Solution
(continued) Under fragmentation: PCR product is still visible in 150-2000 bp size region on a 4% agarose gel	Samples were frozen during fragmentation reaction assembly or centrifugation.	• Make sure that cold blocks are not chilled to –25°C to –15°C as sample freezing can occur. • Before centrifugation, ensure that the interior of the chilled plate centrifuge is not lower than 2-8°C.
	Thermal cycler was not programmed correctly or is out of calibration.	• 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.

Table 37

.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.	• 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 those staining reagents provided by Thermo Fisher Scientific.
	Bright hybridization artifact(s) obscure gridding oligo locations on the array.	Try to manually align the grid. See the *Applied Biosystems™ GeneChip™ Command Console™ User Guide* (Pub. No. 702569) for instructions. If manual grid alignment fails to produce a .CEL file, repeat the experiment.

Table 38

Data QC failures	Likely cause	Solution
Low or failing SNPQC	• Maternal cell contamination (MCC), triploidy or whole genome mosaic samples. • Cross-contamination between samples within a plate. • Contaminated reagents, equipment, or input DNA.	• Be sure you are using the appropriate Reference file. The reference file is selected in the analysis workflow. • Analyze the allelic difference track on ChAS, if it shows altered patterns compatible with MCC, 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. – 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.
		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.
		Perform all steps after removal of arrays from the oven to the time the washing begins with minimal delays.
	Over or under fragmentation of the PCR product	See above.
	Hybridization oven out of calibration or oven model is not compatible with this assay.	• 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.	• Recalibrate pipettes to ensure accurate delivery of reagent volumes. • Consider operator retraining or review by an Thermo Fisher Scientific Field Applications Scientist if the problem persists. • Review Best practices under Chapter 1 and Appendix A.
	Over fragmentation	See above.
	Degraded starting material.	• 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.

Table 38 (Continued)

Data QC failures	Likely cause	Solution
High MAPD with low SNPQC	Error during washing the array.	• 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 guide.

Fluidics station care and maintenance

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

Thermo Fisher Scientific recommends 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. Thermo Fisher Scientific recommends running this protocol weekly, regardless of the frequency of use.

The current version of the protocol can be found on the Thermo Fisher Scientific website at www.thermofisher.com

The bleach cycle

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

Table 39 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 32 on page 81.
2. Prepare 500 mL of 0.525% sodium hypochlorite solution using deionized water.

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.

Figure 32 Disengaged washblocks showing cartridge levers in the down position. Remove any cartridges.

3. As shown in Figure 33 on page 82:
   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.

      The Bleach protocol requires approximately one liter of DI water.

   b. Insert the waste line into the waste bottle.
   c. Immerse all three wash and water lines into the bleach solution.

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

4. Open the instrument control software (AGCC).
5. Choose the current bleach protocol for each module.

Immerse the tubes into the 0.52% sodium hypochlorite solution.

The waste line remains in the waste bottle.

Figure 33 Bleach cycle

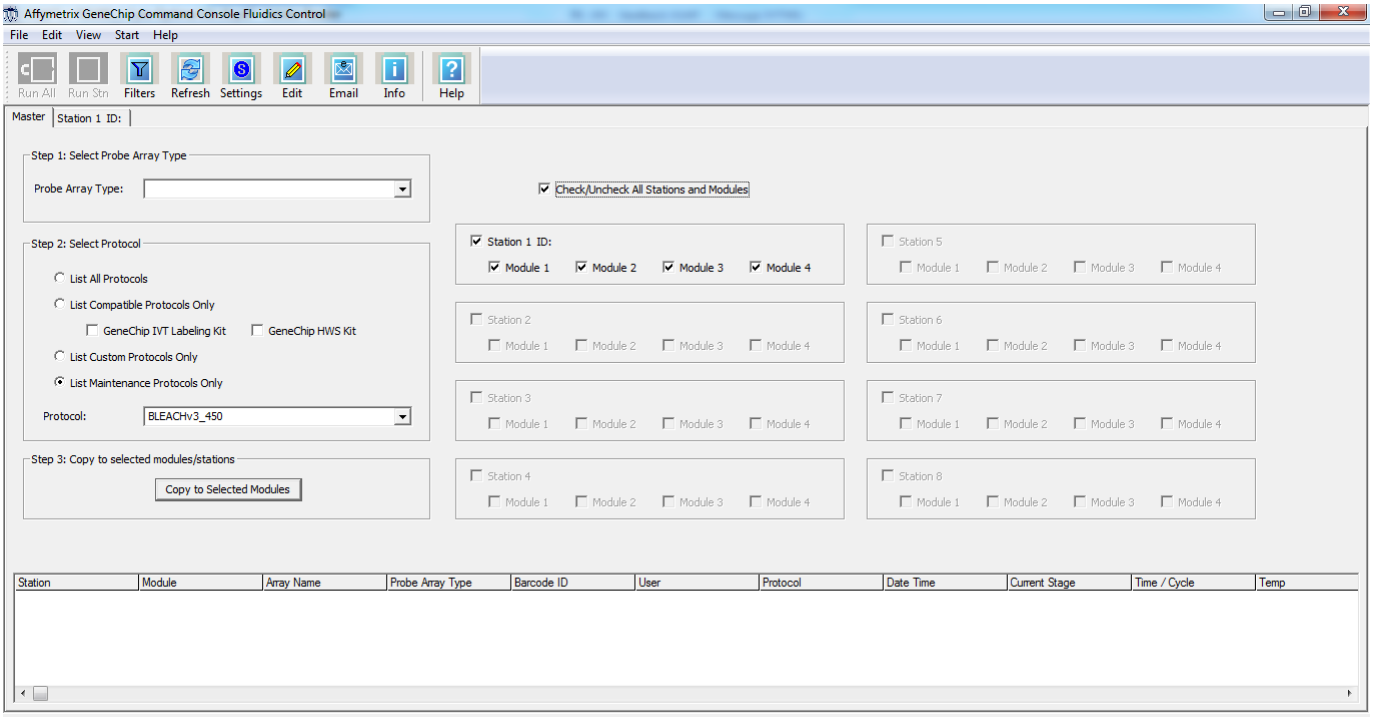


Figure 34 Fluidics Station Protocol window: Select All Modules

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

   Note: The fluidics station will not start until the needle lever is pressed down (Figure 35 on page 84). The temperature will ramp up to 50°C.

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 35).

Figure 35 Press down on the needle levers to start the 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 36).

   At this step, there is no need to be concerned about the bleach remaining in the lines.

Figure 36 Immerse the three wash and water lines in the DI water bottle.

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 40.
7. Please run Shutdown_450 protocol twice after the bleach protocol is completed.

Table 40 Storage suggestions for the Fluidics Station 450

If:	Then do this:
Planning to use the system immediately	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). • Perform a prime protocol without loading your probe arrays. **Failure to run a prime protocol will result in irreparable damage to the loaded hybridized probe arrays.**
Not planning to use the system immediately	Since the system is already well purged with water, there is no need to run an additional shutdown protocol. Remove the old DI water bottle and replace it with a fresh bottle.
Not planning to use the system for an extended period of time (longer than one week)	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. Also, remove the pump tubing from the peristaltic pump rollers.

Safety

⚠ **WARNING! GENERAL SAFETY**. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

⚠ **WARNING!** GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/ provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/ CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Table 41 Documents related to the CytoScan™ Assay

Document	Publication number	Description
CytoScan™ Assay User Guide	703038	Provides detailed instruction for running the CytoScan Assay.
CytoScan™ Assay Site Preparation Guide	703040	Provides guidance on reagents, instruments, and supplies required to run the CytoScan Assay.
Applied Biosystems ™ GeneChip™ Command Console™ Software User Guide	702569	This user guide provides instructions on using Applied Biosystems GeneChip Command Console Software (AGCC) used to control GeneChip instrument systems. Command Console Software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip Arrays.
Chromosome Analysis Suite (ChAS) User Guide (Pub. No.	702943	Provides information on using Chromosome Analysis Suite (ChAS) software for analysis tailored for cytogenetics. ChAS 3.1 includes a database and automatic diploid-centering algorithm.
Applied Biosystems™ GeneChip™ Fluidics Station 450 User Guide for AGCC	08-0295	This user guide provides instructions for using the GeneChip Fluidics Station 450 to wash and stain GeneChip cartridge arrays.
GeneChip™ Scanner 3000 Quick Reference	08-0075	An abbreviated set of instructions for the experienced for using the GeneChip Scanner 3000 (GCS 3000) to scan next-generation higher-density arrays.
GeneChip™ Hybridization Oven 645 User Guide	08-0255	This user guide provides instructions for using the GeneChip Hybridization Oven 645 for hybridization of up to 64 GeneChip cartridge arrays at one time.

Customer and technical support

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  - 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

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