OncoScan™ CNV Plus Assay

USER GUIDE

for use with:
OncoScan™ CNV Plus Reagent Kit for Research
OncoScan™ CNV Plus Array
OncoScan™ CNV Plus Assay for Research

Catalog Numbers 902294, 902292, and 902293
Publication Number MAN0027850
Revision B00

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Products manufactured at this site:

OncoScan™ CNV Plus Array

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0027850 B00 (English)

Revision	Date	Description
B00	2 April 2025	• Updated the reagent kit name from OncoScan™ CNV Plus Reagent Kit to OncoScan™ CNV Plus Reagent Kit for Research. • Updated the assay kit name from OncoScan™ CNV Plus Assay Kit to OncoScan™ CNV Plus Assay for Research. • Clarified the instructions in "Prime the fluidics station" on page 102. • Added the recommendation to use a compression pad that is compatible with the thermal cycler throughout the assay. • Corrected Denature time from 15 minutes to 5 minutes in "OncoScan™ CNV Plus Assay thermal cycler protocols for non-Veriti™ thermal cyclers" on page 116, "OncoScan™ CNV Plus Assay thermal cycler protocols for Veriti™ thermal cyclers with firmware less than v2.0.4" on page 123, and "OncoScan™ CNV Plus Assay thermal cycler protocols for Veriti™ thermal cyclers with firmware v2.0.4 or greater" on page 131.
A.0	5 July 2023	Initial release in Thermo Fisher Scientific document control system. Supersedes legacy Affymetrix publication number 703175. Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty. Rewrite and restructure of document for clarity.

The information in this guide is subject to change without notice.

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Introduction to the assay

Obtaining genome wide copy number and loss of heterozygosity profiles from solid tumor samples is a significant challenge due to the difficulty of working with limited amounts of highly modified and degraded DNA derived from heterogeneous formalin-fixed, paraffin-embedded (FFPE) tumor samples. Traditional FFPE sample analysis techniques such as fluorescent *in situ* hybridization (FISH) are limited to locus specific, low-resolution copy number information. Next Generation sequencing approaches require target preparation methods that bias copy number determination. Deep coverage is also required to provide accurate copy number information from heterogeneous FFPE samples which may not be a practical option for most researchers.

The Applied Biosystems™ OncoScan™ CNV Plus Assay utilizes the Molecular Inversion Probe (MIP) assay technology. This technology performs well with highly degraded DNA, such as that derived from FFPE tumor samples, and with low amounts of DNA starting material. The assay can be performed in as little as 48 hours, which makes the assay a powerful solution for cancer research.

The OncoScan™ CNV Plus Assay can be run on existing Applied Biosystems™ microarray instruments. It provides whole-genome coverage with enriched SNP and CN content for over 900 cancer-related genes, plus the ability to detect frequently tested somatic mutations. The assay is capable of accurately identifying CN changes and allelic imbalances, including loss of heterozygosity (LOH), copy-neutral LOH (cnLOH), and chromothripsis across the entire genome. The OncoScan™ CNV Plus Assay is a complete and robust solution for analysis of FFPE solid tumor samples.

Assay workflow

This section provides an overview of the OncoScan™ CNV Plus Assay workflow. The assay typically takes 48 hours over 3 days to complete. It is performed in 2 areas, a Pre-PCR Room and a Post-PCR Room.

Day 1, Pre-PCR Room (PM)

Activities

The DNA sample plate is prepared. The assay begins with overnight annealing of the MIP probes.

- Preparation of non-amplified genomic DNA.
- Annealing of the MIP probes to the DNA samples overnight.

Total time

2—3 hours

Overnight incubation time

16—18 hours

Day 2, Pre-PCR Room (AM)

Activities

The annealed DNA samples are processed through the 1st PCR reaction.

- Addition of Gap Fill Master Mix to the overnight annealed DNA sample.
- Divide each sample reaction into 2 wells on different rows. One well is the AT channel, the other is the GC channel (Channel Split).
- Gap Fill the annealed probe with AT or GC dNTP mixes.
- Exonuclease reaction to remove the unligated (non-gap filled) linear MIP probes.
- Cleavage enzyme reaction to linearize the gap-filled circular MIP probes.
- 1st PCR reaction to amplify the gap filled linearized MIP probes.

Total time

~2.5—3 hours

Day 2, Post-PCR Room (PM)

Activities

The amplified MIP products from the 1st PCR reaction are processed through the 2nd PCR reaction, HaeIII digestion, and array hybridization. The arrays are hybridized overnight.

- 2nd PCR reaction to enrich the MIP products.
- HaeIII digestion of the 2nd PCR product.
- QC Gels are run to determine the size distribution of the 1st PCR reaction, and the HaeIII digested products.
- Preparation of hybridization cocktail with the HaeIII digested product.
- Hybridization of samples onto the arrays.

Total time

~3.5—4.5 hours

Overnight hybridization time

16—18 hours

Day 3, Post-PCR Room (AM)

Activities

The hybridized arrays are processed to generate data for analysis.

- Array wash and stain in the GeneChip™ Fluidics Station 450.
- Array Scan in the GeneChip™ Scanner 3000 7G.

Total time

1.5 hours on the GeneChip™ Fluidics Station 450 and 7 minutes per array in the GeneChip™ Scanner 3000 7G (2 arrays per sample).

Day 3, Post-PCR Room (PM)

The CEL files are available for data analysis and interpretation using the Applied Biosystems™ Chromosome Analysis Suite (ChAS) software.

Optional assay stopping points

- The **DNA Sample Plate** can be frozen if the assay cannot be started.
- The **1st PCR Plate** can be frozen if the assay cannot be continued.
- **HAE Plate** can be frozen at the end of the HaeIII reaction if the assay cannot be continued. (***Do not*** add hybridization cocktail to the **HAE Plate**.)

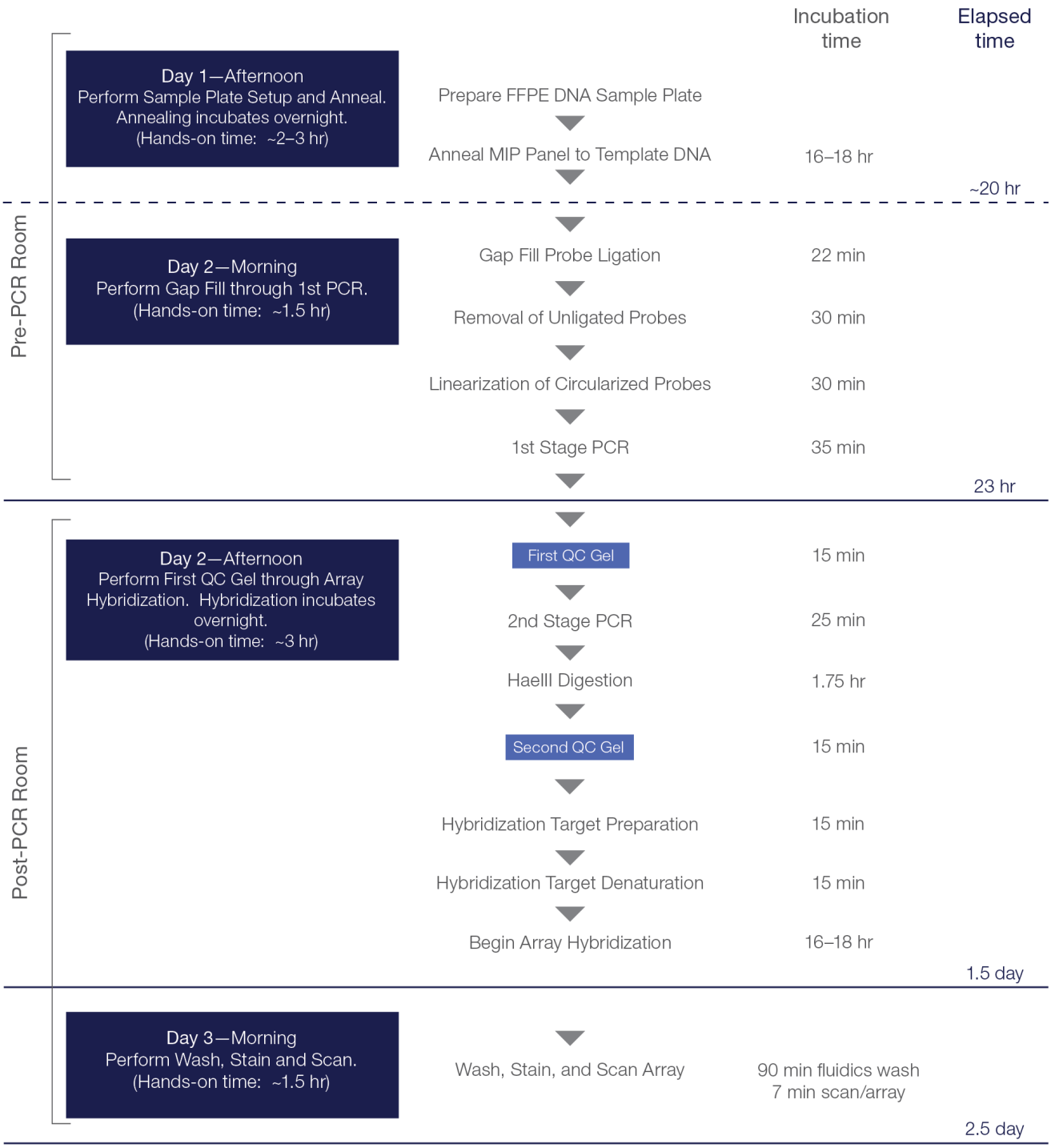


Figure 1 OncoScan™ CNV Plus Assay workflow.

Sample run configuration

This user guide provides workflows to process:

- 7-sample protocol: 5 test samples, 1 positive control, 1 negative control
- 9-sample protocol: 7 test samples, 1 positive control, 1 negative control
- 13-sample protocol: 11 test samples, 1 positive control, 1 negative control
- 25-sample protocol: 23 test samples, 1 positive control, 1 negative control

Note: The negative control is processed through the assay and used for the 2 in-process QC gels. It is not hybridized onto arrays.

Table 1 Detail of the samples and arrays required based on the number of reactions processed per run.

Total number of reactions per run	Number of test samples per run	Positive control sample per run	Negative control per run	Number of runs per assay kit	Total number of samples hybridized per run (excludes negative control)	Number of arrays required per run	Total number of arrays needed for all runs
7	5	1	1	4	6	12	48
9	7	1	1	3	8	16	48
13	11	1	1	2	12	24	48
25	23	1	1	1	24	48	48

2 Guidelines for use

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”.

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

For additional specific warnings, precautions, and procedures, see Appendix H, “Documentation and support”.

Laboratory requirements and recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the OncoScan™ CNV Plus Assay protocol. It is essential to set up the lab to prevent contamination, use equipment that meets specifications, purchase the proper consumables, and use the required reagents. Deviating from this user guide is not recommended as results can be suboptimal.

Proper laboratory setup is necessary to prevent previously amplified PCR product from being a source of contamination. It is strongly recommended to set up 2 separate work areas, a Pre-PCR Room and a Post-PCR Room. The first half of the assay is performed in the Pre-PCR Room (Anneal through 1st PCR) and the second half in the Post-PCR Room (2nd PCR through Array scanning). It is essential to perform assay steps in the appropriate work area and maintain a single direction assay workflow.

Before starting the assay protocol, review the required equipment, consumables, and reagents. See Appendix C, “Equipment, consumables, and reagents required”.

Laboratory setup and contamination prevention

The OncoScan™ CNV Plus Assay requires samples to be amplified using PCR. Proper laboratory setup and precautions are necessary to prevent previously amplified PCR product from being a source of contamination.

- Set up the laboratory areas for a single-direction workflow from Pre-PCR to Post-PCR.
- The Pre-PCR Room should be a low copy DNA template area, and should be free of PCR product (amplicons). No amplified product may be taken into the Pre-PCR Room.
- Do not open the seal of the 1st PCR reaction plate in the Pre-PCR Room.
- The Post-PCR Room has airborne contamination with the PCR amplified MIP-annealed template. It is recommended to not enter the Post-PCR Room while performing the Pre-PCR steps of the assay. After entering the Post-PCR Room, do not re-enter the Pre-PCR Room.
- Keep dedicated equipment (e.g., thermal cyclers, microfuges, pipettes and tips, ice buckets, etc.) in each room or area used for this assay.
- To avoid contamination, do not move equipment between the Pre-PCR Room and the Post-PCR Room.
- Use proper gowning procedures.
- Change gloves frequently throughout the assay and when instructed.
- Store reagents in the appropriate conditions as detailed on the packaging and in this user guide.
- Place separate copies of the assay procedure in the Pre-PCR Room and Post-PCR Room.
- Use nuclease-free pipette tips with aerosol barriers for all pipetting steps.

Equipment and calibration

Laboratory instrumentation plays an important role in the successful execution of this assay. It is critical to use equipment that conforms to the guidelines and specifications detailed in this user guide. To help maintain consistency across assay runs, all equipment must be well maintained and routinely calibrated per manufacturer recommendations.

- Only use calibrated thermal cyclers that meet the specifications outlined in this user guide. It is recommended to service the thermal cyclers at least once per year to help ensure they are operating within the manufacturer's specifications.
- Ensure that the single- and multichannel pipettes are calibrated and accurate.
- The GeneChip™ System 3000 should be serviced at least once a year to help ensure that the components are operating within specifications. The system includes the GeneChip™ Scanner 3000 with the GeneChip™ AutoLoader, the GeneChip™ Fluidics Station 450, and the GeneChip™ Hybridization Oven 645.
- Oven temperature is critical to the performance of the assay. Only use the GeneChip™ Hybridization Oven 645.

Thermal cycler protocols

The thermal cycler protocols used in the OncoScan™ CNV Plus Assay are multistepped and include pausing the protocol, performing a step, then resuming the protocol. Depending on the thermal cycler used, the ability to pause a protocol may differ. This user guide provides different protocol programs based on the thermal cycler. The protocol steps are the same, but additional information can be programed to help pausing and resuming a specific protocol. See Appendix B, "Thermal cycler protocols".

- Program the thermal cyclers before beginning the OncoScan™ CNV Plus Assay.
- It is recommended to use a timer to track the thermal cycler programs and provide notification to pause a program before it moves to the next step.
- It is recommended to use a compression pad that is compatible with the thermal cycler throughout the assay.

To run the OncoScan™ CNV Plus Assay, 2 thermal cyclers are required: 1 in the Pre-PCR Room and 1 in the Post-PCR Room.

Verified thermal cyclers, plates, and seals

The following thermal cyclers, 96-well plates, and adhesive seals have been verified for use with the OncoScan™ CNV Plus Assay. (Table 2 and Table 3.)

Table 2 Thermal cyclers.

Verified thermal cyclers
• Applied Biosystems™ ProFlex™ 96-well PCR System, Cat. No. 4484075 • Applied Biosystems™ Veriti™ 96-Well Thermal Cycler[1], 0.2 mL, Cat. No. 4375786 • Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block), Cat. No. 4314878 • Applied Biosystems™ 2720 Thermal Cycler, Cat. No. 4359659 • Bio-Rad™ T100 Thermal Cycler, Cat. No. 1861096

[1] The Veriti™ *Fast* 96-Well Thermal Cycler, Cat. No. 4375305 and the and the Eppendorf™ Mastercycler™ pro S, Cat. No. 950030020 are not compatible with this assay.

IMPORTANT! Use of non-validated thermal cyclers can result in sub-optimal results. If using non-validated thermal cyclers, consult a Thermo Fisher Scientific technical support representative for guidance.

Table 3 96-well plate and adhesive seals.

Item	Source
96-well half-skirted plate	• (Recommended) Thomas Scientific™ Amplifyt™ 96-Well PCR Plate, Semi-Skirted, Cat. No. 1148A74 • (Alternative) Applied Biosystems™ MicroAmp™ Optical 96-Well Reaction Plate, Cat. No. N8010560 • (Alternative) Bio-Rad™ Multiplate™ 96-Well PCR Plates, high profile, unskirted, clear, Cat. No. MLP9601
MicroAmp™ Clear Adhesive Film	Thermo Fisher Scientific, Cat. No. 4306311

IMPORTANT! The use of 96-well plates that are not compatible with the thermal cyclers can result in poor results. The use of other adhesive seals can result in sample loss and cross-contamination. If using non-validated plates and seals, consult a Thermo Fisher Scientific technical support representative for guidance.

Pipette recommendations

Proficiency with the use of pipettes is essential. To become familiar with the use of multichannel pipettes, it is recommended to practice several times before processing actual samples. Water can be used to get a feel for aspirating and dispensing solutions to multiple wells simultaneously. Take special care to observe complete dispensing of liquid from all pipette tips when using a multichannel pipette.

General pipetting guidance

The OncoScan™ CNV Plus Assay workflow involves a series of ordered stages. The output of one stage directly impacts the performance of the subsequent stage. Many reagents in the OncoScan™ CNV Plus Reagent Kit for Research are viscous. For best results:

- Use pipettes that have been calibrated to ±5%, per the manufacturer's specifications**.**
- Use nuclease-free pipette tips with aerosol barriers for all pipetting steps.
- Pipet slowly to allow enough time for the correct volume of solution to enter the pipette tip.
- Avoid excess solution on the outside of pipette tips.
- To help ensure full volume transfer, check pipette tips after each aspiration and dispense.
- To avoid the formation of bubbles, dispense liquids at the bottom of a well.
- Always use the type and volume of pipette specified in this user guide.

Reagent information, storage conditions, and freeze thaw

This section covers information about reagent handling and freeze-thaw cycles.

Reagent handling and storage

Proper storage and handling of reagents is essential to assay performance. Follow these guidelines to enable best results:

- Do not mix and match reagents from other reagent kits.
- Only use nuclease-free water supplied with the OncoScan™ CNV Plus Reagent Kit for Research, except where otherwise specified in this user guide.
- Store all reagents at the recommended temperatures and conditions. Do not use reagents that have been improperly stored. Storage methods can profoundly impact activity.
- Upon receipt of the reagent kit, store the Nuclease-Free Water at 4°C (Part No. 902253).
- Keep enzymes at –25°C to –15°C until needed. Do not store enzymes at –80°C.
- Do not store enzymes in a frost-free freezer.
- Do not use expired reagents or reagents that have undergone more than 4 freeze-thaw cycles.
- Close all vials and bottle caps after use to prevent evaporation.
- Only store the reagents used for Anneal, Gap Fill, and 1st PCR in the Pre-PCR Room.

When using reagents at the lab bench

Many of the stages in the OncoScan™ CNV Plus Assay workflow must be performed rapidly and on ice to carefully control enzyme activity. It is recommended to set up all the equipment, consumables and reagents before beginning each stage.

- Properly chill equipment such as cooling blocks and reagent coolers before use.
- Keep all plates, tubes, master mixes, and working solutions on a chilled cooling block on ice.
- Unless otherwise indicated, keep all reagents on ice, or on a chilled cooling block on ice.
- Ensure that the enzymes are kept at –25°C to –15°C until needed. When removed from the freezer, immediately place in a benchtop reagent cooler that has been chilled to –25°C to –15°C.
- Enzyme activity is a function of temperature. To enable consistency across sample runs, ensure that the transition from a chilled cooling block to equipment is rapid and well controlled.

Room temperature

Room temperature is defined as 18–25°C. Maintain room temperature throughout the procedure.

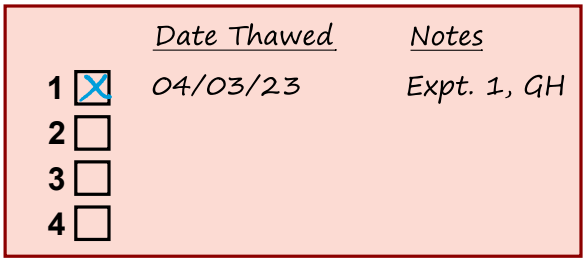
Reagent freeze-thaw cycles and tracking

IMPORTANT! The OncoScan™ CNV Plus Reagent Kit for Research is validated for up to 4 freeze–thaw cycles and for 60 days after opening the reagents. Do not use the kit after the expiration date.

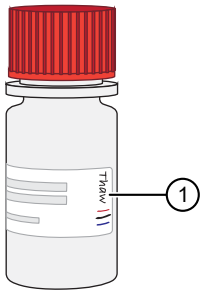
Monitor the freeze–thaw cycles of the reagents by following the recommended guidelines to track usage.

Mark reagent packaging, tubes, and bottles

1. Use a permanent marker to label the module container with the date of each thaw. Other useful information can be added, such as the experiment name and user name.



2. Use a permanent marker to make a tally mark to indicate how many times the reagent has been thawed.



① Thaw tally marks.

3. After use, return all reagent tubes and bottles to the correct module container.
4. Store the module containers at the proper temperature.

Table 4 Storage temperatures for modules in the OncoScan™ CNV Plus Reagent Kit for Research.

Temperature	Module 1 902272	Module 2 902268	Module 3 902269	Module 4 902270	Module 5 902271	Wash Buffers	OncoScan™ CNV Plus Array
15—30°C						✓	
2—8°C					✓		✓
–25°C to –15°C	✓	✓	✓	✓			

About reagents and master mix preparation

This section covers information about reagent kit components and how to handle master mixes.

- About the OncoScan™ CNV Plus Reagent Kit for Research components:
  - Caps on the vials are color-coded according to assay stage.
  - Properly store all enzyme reagents. Improper storage can affect activity.
  - Consult the appropriate Safety Data Sheet for reagent storage and handling requirements.
- About master mix preparation:
  - Keep all tubes, master mixes, and working solutions on the chilled cooling block on ice.
  - Carefully follow each master mix recipe detailed in this user guide.
  - The volume of master mixes prepared is designed to provide accurate pipetting of reagents and consistent assay results. The percent of overage differs for different master mixes, depending on the reagent volumes involved.
  - If you run out of master mix during any of these procedures, a volume error has been made, or the pipettes are not accurate. It is recommended to stop, troubleshoot, and repeat the experiment.
- About adding reagents and master mix to the assay plate:
  - Reagents are *always* added to a chilled PCR plate on the chilled cooling block.
  - Whenever a chilling step is called for, chill the plate on the chilled cooling block for 1 minute, then centrifuge before adding reagents.

Seal, vortex, and centrifuge

This section covers information about how to seal, vortex, and centrifuge plates and tubes. These steps occur repeatedly during the workflow and are critical to the performance of the assay. Unless otherwise noted, follow the instructions in this section.

Handling the plate seal

IMPORTANT! Always ensure that plates are tightly sealed. A tight seal prevents sample loss and cross-well contamination, particularly when plates are being vortexed.

- It is strongly suggested to use MicroAmp™ Clear Adhesive Film to seal plates.
- When applying the seal to a plate, tightly press the seal onto the plate using an adhesive film applicator. Using a plastic lid or a tube storage rack is a potential source of contamination. Ensure that the seal is tight around all plate and well edges.
- *Never reuse a seal.* Discard used seals immediately to avoid contaminating equipment or working surfaces with DNA.
- Whenever a plate is taken out of the thermal cycler, ensure that the seal is tight, centrifuge the plate, then remove and discard the seal.
- Whenever a plate is taken out of the freezer, thaw the plate at room temperature, ensure that the seal is tight, centrifuge, then remove and discard the seal.

Sealing strip tubes

- Seal the strip tubes containing master mix before centrifuging in the benchtop mini centrifuge.
- Adhesive strips can be created by cutting a plate seal into strips wide enough and long enough to seal 7-, 9-, or 12-well strip tubes.

Vortex

When instructed to vortex, follow these guidelines unless otherwise instructed:

- Reagent vials: Vortex at high speed 3 times, 1 second each time. Ensure that there is no precipitate. If a precipitate is observed, repeat vortexing.
- Enzyme vials: Vortex at high speed 1 second, 1 time.
- Master mix tubes: Vortex at high speed 3 times, 1 second each time.
- Vortex plates: Vortex at high speed for 1 second in all corners and in the center. (Figure 2.)

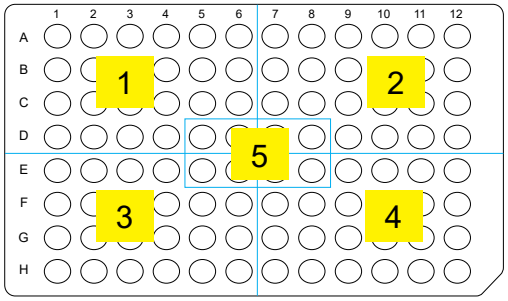


Figure 2 Vortex plates at the corners and center.

Centrifuge

When instructed to centrifuge, follow these guidelines unless otherwise instructed:

- Reagent vials: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Enzyme vials: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Master mix tubes: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Strip tubes: Centrifuge for 3 seconds using a benchtop mini centrifuge.
- Plates:
  - Centrifuge at room temperature or at 4°C in a refrigerated centrifuge if available.
  - Start the centrifuge and allow it to reach 2,400 rpm before starting the centrifuge time.

In-process QC gels

It is recommended to run 2 quality control gels as part of the assay protocol. The purpose of each gel is described as follows:

- The First QC Gel is run after the 1st PCR reaction to identify any samples that did not amplify. For samples in which successful amplification has occurred, 1 single band at approximately 120 bp should be observed.
- The Second QC Gel is run with an aliquot taken during the HaeIII reaction at 37°C. For samples in which a successful 2nd PCR reaction and HaeIII digestion has occurred, two bands at approximately 40 bp and 70 bp should be observed.

Instructions for creating master mixes

This user guide contains reagent master mix tables at each step in the assay to process 7, 9, 13, and 25 samples.

- For processing 7 samples (5 gDNA samples plus 1 positive control and 1 negative control), use information from column-3 in the reagent master mix tables at each step of the assay. This column will provide the reagent volume needed to process 7 anneal reactions, 14 post-channel split reactions, and hybridizing 12 arrays.
- For processing 9 samples (7 gDNA samples plus 1 positive control and 1 negative control), use information from column-4 in the reagent master mix tables at each step of the assay. This column will provide the reagent volume needed to process 9 anneal reactions, 18 post-channel split reactions, and hybridizing 16 arrays.
- For processing 13 samples (11 gDNA samples plus 1 positive control and 1 negative control), use information from column-5 in the reagent master mix tables at each step of the assay. This column will provide the reagent volume needed to process 13 anneal reactions, 26 post-channel split reactions, and hybridizing 24 arrays.
- For processing 25 samples (23 gDNA samples plus 1 positive control and 1 negative control), use information from column-6 in the reagent master mix tables at each step of the assay. This column will provide the reagent volume needed to process 25 anneal reactions, 50 post-channel split reactions, and hybridizing 48 arrays.

Guidelines for all stages of the assay

This section covers general guidelines for each stage of the assay.

- Pre-chill the thawed reagents on ice.
- Pre-chill empty master mix tubes and strip tubes on the chilled cooling block on ice.
- Leave the enzymes at –20°C until ready to use.
- When making a master mix, add reagents in the order detailed in the master mix preparation table. Always add the enzyme last.
- Perform all reagent additions on ice.
- Always carry the sample plate to the centrifuge or the thermal cycler on a chilled cooling block on ice.
- Always prepare the reagents ahead for the next step. Add the reagents for the next step immediately after chilling and spinning down the plate. Vortex, centrifuge and proceed to the next step with 10 minutes of removing the plate from the thermal cycler.
- *Do not* let the reaction plate sit on the chilled cooling block for more than 10 minutes during the Gap Fill thermal cycler protocol.
- *Do not* let the reaction plate sit in the thermal cycler for more than 30 minutes during 1st PCR, 2nd PCR, and the HaeIII thermal cycler protocols.

3 FFPE genomic DNA general requirements

The general requirements for gDNA sources and input amounts, as well as gDNA extraction, purification, and quantification methods are detailed in this chapter. It is recommended to use the protocols provided in this user guide to extract, purify, and quantitate FFPE DNA.

General requirements

- 80 ng of human genomic DNA at 12 ng/µL is required input into the assay.
- This assay has been optimized using a dsDNA-specific quantitation method to determine genomic DNA concentration. Sample concentration determined by UV absorbance or NanoDrop™ Spectrophotometer ***must not be used*** in this assay.
- Use the Applied Biosystems™ OncoScan™ CNV Plus Reagent Kit for Research (Cat. No. 902294). This kit contains the Positive Control (12 ng/µL) which can be used as a positive control during the assay run and to assist in troubleshooting.

Sources of human genomic DNA

The following source of human genomic DNA has been verified for use in the assay:

- Genomic DNA derived from formalin-fixed paraffin-embedded (FFPE) blocks

Non-verified sources of genomic DNA may be used in the assay, but the quality of the results may vary. Contact a Thermo Fisher Scientific technical support representative before using non-verified sample sources, such as:

- Whole blood
- Cell lines
- Fresh frozen
- Bone marrow
- Melanoma

FFPE DNA extraction and purification methods

Numerous commercial kits are available for FFPE DNA extraction and purification. Results may vary based on the extraction method and kit used. Select a kit that matches the sample source type and yields the highest quality genomic DNA possible. It is recommended to use silica-based methods.

The yield of tissue from FFPE is highly dependent on the type of tissue and method used for the initial fixation. For optimal DNA yield, samples submitted for extraction should have a tissue size of 400—700 mm^2.

This user guide includes instructions to extract DNA from FFPE blocks using a modified protocol and the QIAamp™ DNA FFPE Tissue Kit. This modified protocol was developed and tested by Thermo Fisher Scientific and found to generate good quality genomic DNA. It includes steps to improve the tissue digestion process and release more DNA from the tissue sections. See Appendix D, “FFPE DNA extraction protocol for the OncoScan™ CNV Plus Assay”.

Sample quantification

The success of the OncoScan™ CNV Plus Assay is dependent on accurate quantitation of the input genomic DNA. It is mandatory to determine the sample concentration using a dsDNA specific quantification method. The Quant-iT™ PicoGreen™ Assay and the Qubit™ dsDNA Quantification Assay are 2 methods that have been verified for use in the OncoScan™ CNV Plus Assay. Other dsDNA quantitation kits that are commercially available may deliver different results.

IMPORTANT! Sample concentration determined by UV absorbance or NanoDrop™ Spectrophotometer ***must not be used*** in this assay.

Use of controls

The use of a positive and a negative control is required to assess the performance of each assay run.

- Use the Positive Control (12 ng/µL) supplied in the OncoScan™ CNV Plus Assay for Research as a positive control for the entire assay.
- Use the Negative Control supplied in the OncoScan™ CNV Plus Assay for Research as a negative control through the Second QC Gel.

4 Normalize the Test gDNA samples

In this chapter, extracted, purified, and quantified Stock Test gDNA samples are normalized to a single concentration of 12 ng/µL using 1X TE Buffer (Low EDTA).

This procedure should be performed in a clean room such as a Pre-PCR Room. Use caution to not contaminate the gDNA samples with OncoScan™ CNV Plus Assay PCR 1 and PCR 2 amplicons, or other gDNA sources.

Reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 5 Reagents required for genomic DNA preparation.

Quantity	Reagent	Source
1	TE Buffer, 1X Solution pH 8.0, Low EDTA	J75793.AE
5—23	Stock Test gDNA samples	User supplied, stock concentration

Setup the work area in the Pre-PCR Room for Sample Plate preparation

1. Fill the ice bucket with ice, then place a cooling block on the ice.
2. Place the 1X TE Buffer (Low EDTA) on ice.

Prepare the Stock Test gDNA samples

1. If frozen, thaw the Stock Test gDNA samples at room temperature for 15 minutes.
2. Vortex each sample for 4 seconds, then centrifuge for 3 seconds.
3. Incubate the samples at room temperature for 5 minutes.
4. Vortex each sample for 4 seconds, then centrifuge for 3 seconds.
5. Place the Stock Test gDNA samples on ice.

Normalize the Stock Test gDNA samples

1. Label a 96-well plate “DNA Sample”, then place it on the chilled cooling block.

   Note: This plate is called the **DNA Sample Plate**.

2. In separate wells of the **DNA Sample Plate**, dilute the Stock Test gDNA samples to 12 ng/µL using the 1X TE Buffer (Low EDTA).
   a. Prepare enough volume to have a minimum of 6.6 µL of each Normalized Test gDNA sample.
   b. When preparing the dilution, add the volume of 1X TE Buffer (Low EDTA) first, then add the Stock Test gDNA sample. Pipet up and down 3 times to rinse the tip.
3. Complete the **DNA Sample Plate**.
   a. Tightly seal the plate with a new seal, then vortex the plate for 1 second in all corners and center.
   b. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
   c. Place the **DNA Sample Plate** back on the chilled cooling block.
4. Return the Stock Test gDNA samples to –25°C to –15°C storage.
5. Do one of the following:
   - PROCEED:
     If starting the assay: Proceed to “Stage 1: Anneal” on page 30.
   - STOP:
     If stopping and not proceeding with the assay, perform the optional stopping point. (See “Optional stopping point” on page 29.) The **DNA Sample Plate** is stored for future use.

Optional stopping point

IMPORTANT! Only perform the following steps if stopping the assay and storing the **DNA Sample Plate** for future use.

1. Place the **DNA Sample Plate** on a 96-well plate storage rack.
2. Store the plate at –25°C to –15°C in the Pre-PCR Room.

   Note: The plate can be stored for up to 10 days.

5 OncoScan™ CNV Plus Assay workflow

Stage 1: Anneal

About Stage 1

This stage is performed in the Pre-PCR Room. During this stage, the Normalized Test gDNA samples from the **DNA Sample Plate**, and the Positive and Negative controls, are plated into the **Anneal Plate**. The Anneal reaction is setup and the OncoScan Anneal thermal cycler protocol started. The protocol runs for 16—18 hours.

When the Anneal thermal cycler protocol is complete, the assay is continued to Stage 2: Gap Fill through 1st PCR.

Reagents required

Table 6 Reagents required for Stage 1.

Quantity	Reagent and cap color	Module	Part No.
From the OncoScan™ CNV Plus Reagent Kit for Research			
1	Positive Control (12 ng/µL)	OncoScan™ CNV Plus Copy Number Probe Mix 1.0 & Controls (Part No. 902268)	902249
1	Negative Control		902250
1	Buffer A		902246
1	Copy Number Probe Mix 1.0		902248
1	Somatic Mutation Probe Mix 1.0	OncoScan™ CNV Plus Somatic Mutation Probe Mix 1.0 (Part No. 902272)	902247

Table 6 Reagents required for Stage 1. *(continued)*

Quantity	Reagent and cap color	Module	Part No.
Other			
1	**DNA Sample Plate**	Previously prepared	

Set up the work area in the Pre-PCR Room for Stage 1

1. Power on the thermal cycler in the Pre-PCR Room to preheat the lid.
2. Fill the ice bucket with ice, then place 2 cooling blocks on the ice.
3. If using a refrigerated plate centrifuge, set it to 4°C to allow it to reach the proper temperature.
4. Thaw the following at room temperature, then immediately place them on ice.
   - Positive Control (12 ng/µL)
   - Negative Control
   - Buffer A
   - Copy Number Probe Mix 1.0
   - Somatic Mutation Probe Mix 1.0

Prepare the DNA Sample Plate

Note: If the **DNA Sample Plate** has been frozen, thaw at room temperature (≤30 minutes). Once thawed, immediately place it on a chilled cooling block.

1. Ensure that the **DNA Sample Plate** is tightly sealed, then vortex the plate 1 second in all corners and center.
2. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
3. Place the **DNA Sample Plate** back on the chilled cooling block.

Prepare the Anneal Plate and add controls

Transfer Diluted Test Samples

1. Label a 96-well plate “Anneal”, then place it on a chilled cooling block.

   Note: This plate is called the **Anneal Plate**.

2. Transfer 6.6 µL of each Normalized Test gDNA sample from the **DNA Sample Plate** into the **Anneal Plate**.
   - 7-sample protocol: 5 test samples, transfer into wells A1—A5.
   - 9-sample protocol: 7 test samples, transfer into wells A1—A7.
   - 13-sample protocol: 11 test samples, transfer into wells A1—A11.
   - 25-sample protocol: 23 test samples, transfer into wells A1—A12 and E1—E11.

Add the Positive and Negative Control

1. Prepare the Positive Control (12 ng/µL) and Negative Control that have been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place them on ice.
2. Add 6.6 µL of the Positive Control (12 ng/µL) to the **Anneal Plate**.
   - 7-sample protocol: Add the positive control into well A6.
   - 9-sample protocol: Add the positive control into well A8.
   - 13-sample protocol: Add the positive control into well A12.
   - 25-sample protocol: Add the positive control into well E12.
3. Add 6.6 µL of the Negative Control to the **Anneal Plate**.
   - 7-sample protocol: Add the negative control into well A7.
   - 9-sample protocol: Add the negative control into well A9.
   - 13-sample protocol: Add the negative control into well B1.
   - 25-sample protocol: Add the negative control into well F1.

   Note: The final volume in each well is 6.6 µL.
4. Complete the **Anneal Plate**.
   a. Tightly seal the plate with a new seal, then centrifuge at 2,400 rpm for 30 seconds.
   b. Place the **Anneal Plate** on the chilled cooling block.
5. Return the Positive Control (12 ng/µL) and Negative Control to –25°C to –15°C storage.
6. If there is remaining Normalized Test gDNA samples in the **DNA Sample Plate**, tightly seal the plate with a new seal, place it on a 96-well plate storage rack, then store at –25°C to –15°C.

Prepare and add the Anneal Master Mix

Prepare the Anneal Master Mix

1. Label a 1.5-mL microcentrifuge tube “ANN”, then place it on ice.
2. Label a strip tube “ANN”, then place it on a chilled cooling block. (See Table 7for the correct strip tube.)

3. Prepare the Buffer A, Copy Number Probe Mix 1.0, and Somatic Mutation Probe Mix 1.0 that have been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place them on ice.
4. Prepare the Anneal Master Mix. Use the appropriate column in the following table. (Table 7.)

Table 7 Anneal Master Mix.

Reagent and cap color	1 reaction	7 reactions (~60% overage)	9 reactions (~60% overage)	13 reactions (~60% overage)	25 reactions (~60% overage)
◯ Buffer A	1.53 µL	17.1 µL	22.0 µL	31.8 µL	61.2 µL
● Copy Number Probe Mix 1.0	1.37 µL	15.3 µL	19.7 µL	28.5 µL	54.8 µL
◯ Somatic Mutation Probe Mix 1.0	0.5 µL	5.6 µL	7.2 µL	10.4 µL	20.0 µL
Total volume	**3.40 µL**	**38.0 µL**	**48.9 µL**	**70.7 µL**	**136.0 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		5.0 µL	5.0 µL	5.0 µL	10.0 µL

5. Add the Buffer A, Copy Number Probe Mix 1.0, and Somatic Mutation Probe Mix 1.0 to the 1.5-mL microcentrifuge tube labeled “ANN”.
   a. Pipet the Copy Number Probe Mix 1.0 and Somatic Mutation Probe Mix 1.0 up and down 3 times to rinse the tips.
   b. Between each addition, change tips.
   c. Between each addition, vortex the master mix 3 times for 1 second each, then centrifuge 3 seconds.
   d. Place on ice.
6. Complete the Anneal Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge 3 seconds.
   b. Place on ice.
7. Aliquot the appropriate volume of Anneal Master Mix into the chilled strip tube, “ANN”:
   - 7-sample protocol: Add 5.0 µL into each well of a 7-well strip tube.
   - 9-sample protocol: Add 5.0 µL into each well of a 9-well strip tube.
   - 13-sample protocol: Add 5.0 µL into each well of a 12-well strip tube.
   - 25-sample protocol: Add 10.0 µL into each well of a 12-well strip tube.
8. Ensure that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.

9. Place the strip tube on a chilled cooling block.

10. Place the Anneal Master Mix tube, “ANN”, on ice. Do not discard.

Add the Anneal Master Mix to the Anneal Plate

1. Remove the seal from the **Anneal Plate**, then discard the seal.

2. Use a multichannel P20 pipette to add 3.4 µL of the Anneal Master Mix to each sample. (Figure 3.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 3.4 µL of the Anneal Master Mix from the “ANN” microcentrifuge tube to the Negative Control well. Pipet up and down 3 times to rinse the tip. (Figure 3.)

Note: The final volume in each well is 10.0 µL.

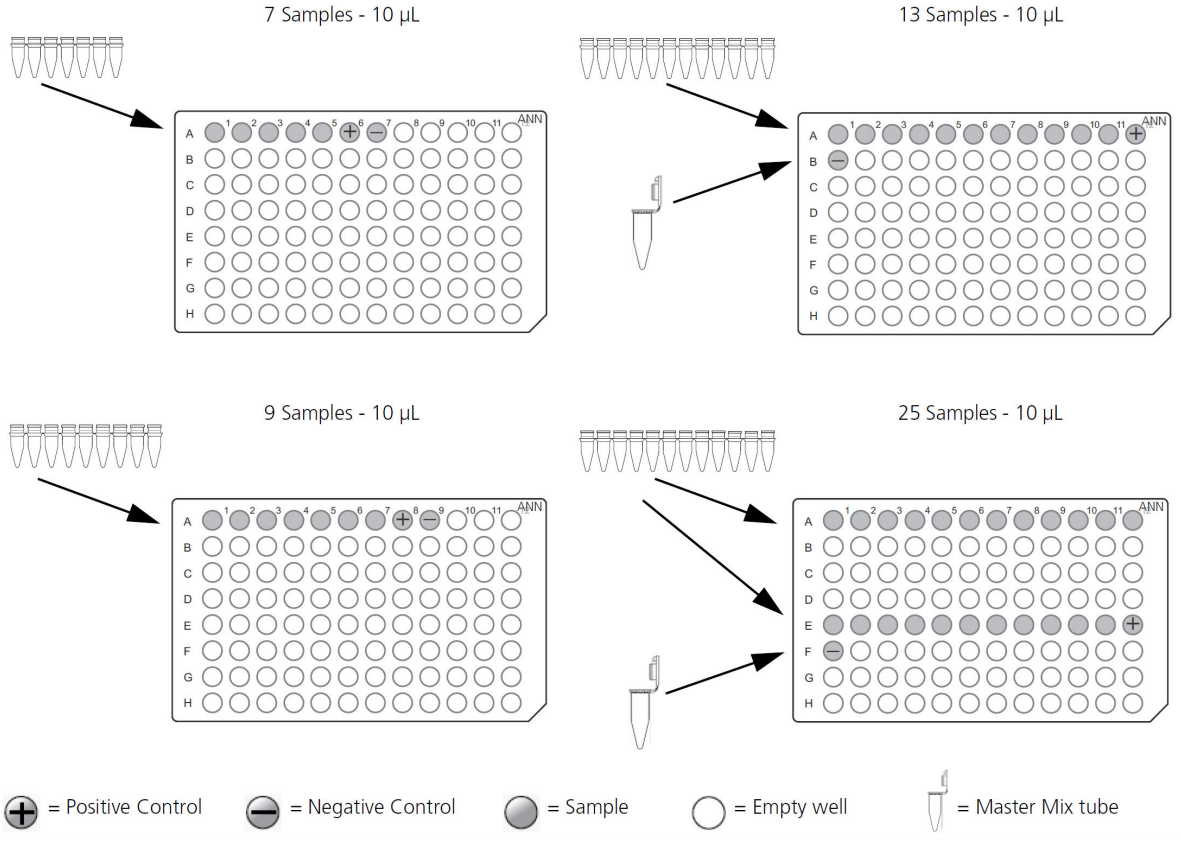


Figure 3 Add Anneal Master Mix to the Anneal Plate.

3. Tightly seal the **Anneal Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

5. Place the **Anneal Plate** back on the chilled cooling block.

Run the OncoScan Anneal thermal cycler protocol

1. Load the **Anneal Plate** into the thermal cycler.

2. Start the OncoScan Anneal thermal cycler protocol. (Figure 4.)
   a. After 6 minutes into the OncoScan Anneal protocol, pause the program at 58°C (1 minute into 58°C).

   Note: Ensure that the thermal cycler protocol is paused.

   b. Remove the **Anneal Plate** from the thermal cycler and place it on a chilled cooling block for 1 minute.

   c. After 1 minute, ensure that the plate is tightly sealed.

   d. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.

   e. Place the **Anneal Plate** back on the chilled cooling block.

   f. Load the **Anneal Plate** into the thermal cycler, then resume the OncoScan Anneal thermal cycler protocol.

   Note: Ensure that the thermal cycler protocol has resumed and is running.

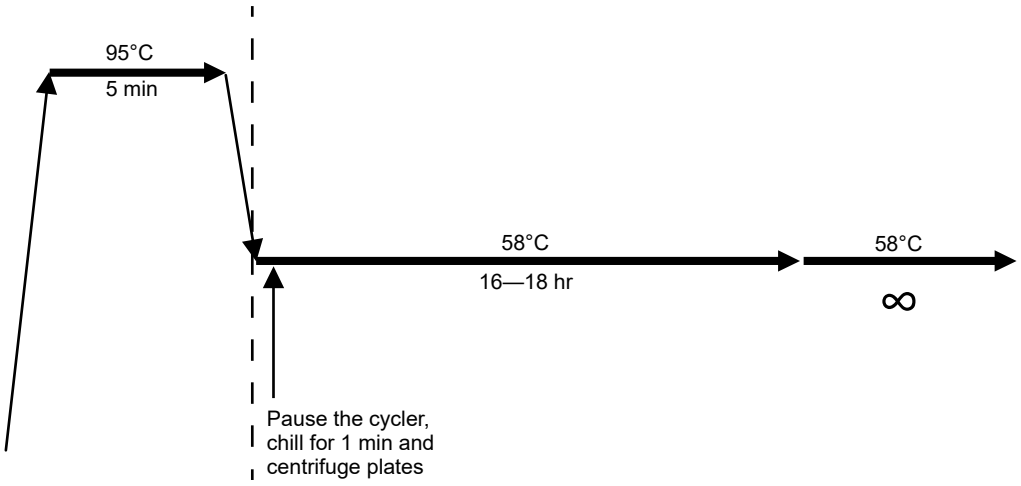


Figure 4 OncoScan Anneal thermal cycler protocol.

3. Allow the protocol to run overnight, 16—18 hours.

   IMPORTANT! Do not incubate samples more than 18 hours. Prepare the AT Master Mix, GC Master Mix, and Gap Fill Master Mix before the 18-hour incubation completes, then immediately begin the Gap Fill step.

4. Discard the Anneal Master Mix microcentrifuge tube and strip tube. Discard the ice in the ice bucket, then place the cooling blocks in the refrigerator.

5. Return the Buffer A, Copy Number Probe Mix 1.0, and Somatic Mutation Probe Mix 1.0 to the –25°C to –15°C storage.

Stage 2: Gap Fill through 1st PCR

About Stage 2

This stage is performed in the Pre-PCR Room. During this stage, the **Anneal Plate** is removed from the thermal cycler and the Gap Fill Master Mix is added to the samples.

Each sample is then split into 2 separate rows of the **1st PCR Plate**. This is called the Channel Split. Each sample will now have 2 separate reactions performed through the end of the assay.

The **1st PCR Plate** is placed in a thermal cycler and the OncoScan Gap Fill thermal cycler protocol is started. During this protocol, 3 master mixes are added to the **1st PCR Plate**. Prior to each addition, the plate is removed from the thermal cycler, placed on a chilled cooling block for 1 minute, then centrifuged.

The master mixes that are added during the OncoScan Gap Fill thermal cycler protocol are:

1. AT dNTP and GC dNTP Master Mixes
2. Exo Master Mix
3. Cleavage Master Mix

When the Gap Fill thermal cycler protocol is complete, the 1st PCR Master Mix is added to the **1st PCR Plate**, and the OncoScan 1st PCR thermal cycler protocol is started. Once the protocol is complete, the **1st PCR Plate** is transferred to the Post-PCR Room.

The assay can be stopped or continued to Stage 3: 2nd PCR and First QC Gel.

Reagents required

Table 8 Reagents required for Stage 2.

Quanty	Reagent and cap color	Module	Part No.
From the OncoScan™ CNV Plus Reagent Kit for Research			
1	Buffer A	OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269)	902246
1	Gap Fill Enzyme Mix		902252
1	SAP, Recombinant (1 U/µL)		902251
1	dNTP Mix (A/T)		902254
1	dNTP Mix (G/C)		902255
1	Nuclease-Free Water		902253
1	Exo Mix		902256
1	Cleavage Buffer		902257
1	Cleavage Enzyme (2 U/µL)		902258
1	PCR Mix		902259
1	Taq Polymerase (5 U/µL)		902260

Table 8 Reagents required for Stage 2. *(continued)*

Quanty	Reagent and cap color	Module	Part No.
Other			
1	**Anneal Plate**	From Stage 1	

Set up the work area in the Pre-PCR Room for Stage 2

1. Fill the ice bucket with ice, then place 4 cooling blocks on the ice.
2. If using a refrigerated plate centrifuge, set it to 4°C to allow it to reach the proper temperature.
3. Thaw the following reagents at room temperature, then immediately place them on ice.
   - Nuclease-Free Water
   - dNTP Mix (A/T)
   - dNTP Mix (G/C)
   - Buffer A
   - Cleavage Buffer
   - PCR Mix

Note: The reagents are mixed and centrifuged in later steps.

Prepare the AT Master Mix and the GC Master Mix

IMPORTANT! During this stage, each sample is split into 2 separate reactions. This is called the Channel Split. One reaction will have AT dNTPs added. The other reaction will have GC dNTPs added. Contamination between the 2 channels will result in poor data. To prevent channel contamination, follow these precautions:

- Prepare the AT Master Mix and GC Master Mix separately.
- Change gloves between making the AT Master Mix and the GC Master Mix. Change gloves regularly throughout the assay.
- Label all wells, tubes, and strips for the AT Master Mix in a different colored marker than the GC Master Mix. This helps identify the different dNTP reactions and avoid a mix-up.
- Keep the prepared AT Master Mix and the GC Master Mix separated.

1. Label a 1.5-mL microcentrifuge tube “AT” with a blue marker.
2. Label a 1.5-mL microcentrifuge tube “GC” with a red marker.
3. Place the labeled microcentrifuge tubes on ice.
4. Label a strip tube “AT” with blue marker. (See Table 9 for the correct strip tube.)
5. Label a strip tube “GC” with a red marker. (See Table 10 for the correct strip tube.)
6. Place the labeled strip tubes on separate chilled cooling blocks.

Prepare the AT Master Mix

1. Prepare the dNTP Mix (A/T) that has been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
2. Prepare the AT Master Mix. Use the appropriate column in the following table. (Table 9.)

   IMPORTANT! Due to the small volume of the reagents used in the AT Master Mix preparation, prepare as shown.

Table 9 AT Master Mix.

Reagent and cap color	1 reaction	7 reactions	9 reactions	13 reactions	25 reactions
Nuclease-Free Water	3.93 µL	118.0 µL	118.0 µL	118.0 µL	118.0 µL
dNTP Mix (A/T)	0.07 µL	2.2 µL	2.2 µL	2.2 µL	2.2 µL
Total volume	**4.0 µL**	**120.2 µL**	**120.2 µL**	**120.2 µL**	**120.2 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		9.6 µL	9.6 µL	9.6 µL	9.6 µL

3. Add the Nuclease-Free Water and the dNTP Mix (A/T) to the 1.5-mL microcentrifuge tube labeled “AT”.
   a. Pipet the dNTP Mix (A/T) up and down 3 times to rinse the tips.
   b. Place on ice.
4. Complete the AT Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
5. Aliquot the appropriate volume of AT Master Mix into the chilled strip tube, “AT”:
   - 7-sample protocol: Add 9.6 µL into each well of a 7-well strip tube.
   - 9-sample protocol: Add 9.6 µL into each well of a 9-well strip tube.
   - 13-sample protocol: Add 9.6 µL into each well of a 12-well strip tube.
   - 25-sample protocol: Add 9.6 µL into each well of a 12-well strip tube.
6. Cover the “AT” strip tube with strip of adhesive film.
7. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, centrifuge for 3 seconds.

8. Place the strip tube on a chilled cooling block until the AT Master Mix addition step.

9. Place the AT Master Mix tube, “AT”, on ice. Do not discard.

IMPORTANT! Discard the gloves used to prepare the AT Master Mix. Don fresh gloves to prepare the GC Master Mix.

Prepare the GC Master Mix

1. Prepare the dNTP Mix (G/C) that has been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.

2. Prepare the GC Master Mix. Use the appropriate column in the following table. (Table 10.)

   IMPORTANT! Due to the small volume of the reagents used in the GC Master Mix preparation, prepare as shown.

Table 10 GC Master Mix.

Reagent and cap color	1 reaction	7 reactions	9 reactions	13 reactions	25 reactions
◯ Nuclease-Free Water	3.93 µL	118.0 µL	118.0 µL	118.0 µL	118.0 µL
● dNTP Mix (G/C)	0.07 µL	2.2 µL	2.2 µL	2.2 µL	2.2 µL
Total volume	**4.0 µL**	**120.2 µL**	**120.2 µL**	**120.2 µL**	**120.2 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		9.6 µL	9.6 µL	9.6 µL	9.6 µL

3. Add the Nuclease-Free Water and the dNTP Mix (G/C) to the 1.5-mL microcentrifuge tube labeled “GC”.
   a. Pipet the dNTP Mix (G/C) up and down 3 times to rinse the tips.
   b. Place on ice.

4. Complete the GC Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.

5. Aliquot the appropriate volume of GC Master Mix into the chilled strip tube, “GC”:
   - 7-sample protocol: Add 9.6 µL into each well of a 7-well strip tube.
   - 9-sample protocol: Add 9.6 µL into each well of a 9-well strip tube.
   - 13-sample protocol: Add 9.6 µL into each well of a 12-well strip tube.
   - 25-sample protocol: Add 9.6 µL into each well of a 12-well strip tube.

6. Cover the “GC” strip tube with strip of adhesive film.

7. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, centrifuge for 3 seconds.

8. Place the strip tube on a chilled cooling block until the GC Master Mix addition step.

9. Place the GC Master Mix tube, “GC”, on ice. Do not discard.

IMPORTANT! Discard the gloves used to prepare the GC Master Mix. Don fresh gloves to prepare the Gap Fill Master Mix.

Prepare the Gap Fill Master Mix

1. Label a 1.5-mL microcentrifuge tube “G”, then place it on ice.

2. Label a strip tube “G”, then place it on a chilled cooling block. (See Table 11 for the correct strip tube.)

3. Remove the SAP, Recombinant (1 U/µL) and the Gap Fill Enzyme Mix from the freezer, then immediately place them in a –25°C to –15°C cooler.

4. Prepare the SAP, Recombinant (1 U/µL), and the Gap Fill Enzyme Mix.
   a. Centrifuge for 1 second. Vortex for 1 second, then centrifuge for 3 seconds.
   b. Return the SAP, Recombinant (1 U/µL), and the Gap Fill Enzyme Mix to the cooler.

5. Prepare the Buffer A that has been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.

6. Prepare the Gap Fill Master Mix. Use the appropriate column in the following table. (Table 11.)

Table 11 Gap Fill Master Mix.

Reagent and cap color	1 reaction	7 reactions (~20% overage)	9 reactions (~20% overage)	13 reactions (~20% overage)	25 reactions (~20% overage)
◯ Nuclease-Free Water	10.58 µL	89.0 µL	114.0 µL	165.0 µL	317.0 µL
◯ Buffer A	1.18 µL	9.9 µL	12.7 µL	18.4 µL	35.4 µL
● SAP, Recombinant (1 U/µL)	0.84 µL	7.1 µL	9.1 µL	13.1 µL	25.2 µL
● Gap Fill Enzyme Mix	1.40 µL	11.8 µL	15.1 µL	21.8 µL	42.0 µL
Total Volume	**14.0 µL**	**117.8 µL**	**150.9 µL**	**218.3 µL**	**419.6 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		16.0 µL	16.0 µL	16.0 µL	33.0 µL

7. Add the Nuclease-Free Water, Buffer A, SAP, Recombinant (1 U/µL), and the Gap Fill Enzyme Mix to the 1.5-mL microcentrifuge tube labeled “G”.
   a. Pipet the Buffer A, SAP, Recombinant (1 U/µL), and Gap Fill Enzyme Mix up and down 3 times to rinse the tips.
   b. Between each addition, change tips.
   c. Between each addition, vortex the master mix 3 times for 1 second each, then centrifuge 3 seconds.
   d. Place on ice.
8. Return the SAP, Recombinant (1 U/µL) and Gap Fill Enzyme Mix to the cooler.
9. Complete the Gap Fill Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
10. Aliquot the appropriate volume of Gap Fill Master Mix into the chilled strip tube, “G”:
    - 7-sample protocol: Add 16.0 µL into each well of a 7-well strip tube.
    - 9-sample protocol: Add 16.0 µL into each well of a 9-well strip tube.
    - 13-sample protocol: Add 16.0 µL into each well of a 12-well strip tube.
    - 25-sample protocol: Add 33.0 µL into each well of a 12-well strip tube.
11. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.
12. Place the strip tube on a chilled cooling block until the Gap Fill Master Mix addition step.
13. Place the Gap Fill Master Mix tube, “G”, on ice. Do not discard.

Complete the Anneal Plate

When the OncoScan Anneal thermal cycler protocol is complete, do the following:

1. Remove the **Anneal Plate** from the thermal cycler and place it on a chilled cooling block for 1 minute.
2. After 1 minute, ensure that the plate is tightly sealed.
3. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.
4. Place the **Anneal Plate** back on the chilled cooling block.

Prepare the thermal cycler for the OncoScan Gap Fill protocol

1. Stop the OncoScan Anneal protocol on the thermal cycler.
2. Start the OncoScan Gap Fill protocol on the thermal cycler. When the thermal cycler block temperature reaches 58°C, pause the protocol at 58°C. (Figure 5.)

Note: Ensure that the protocol is paused.

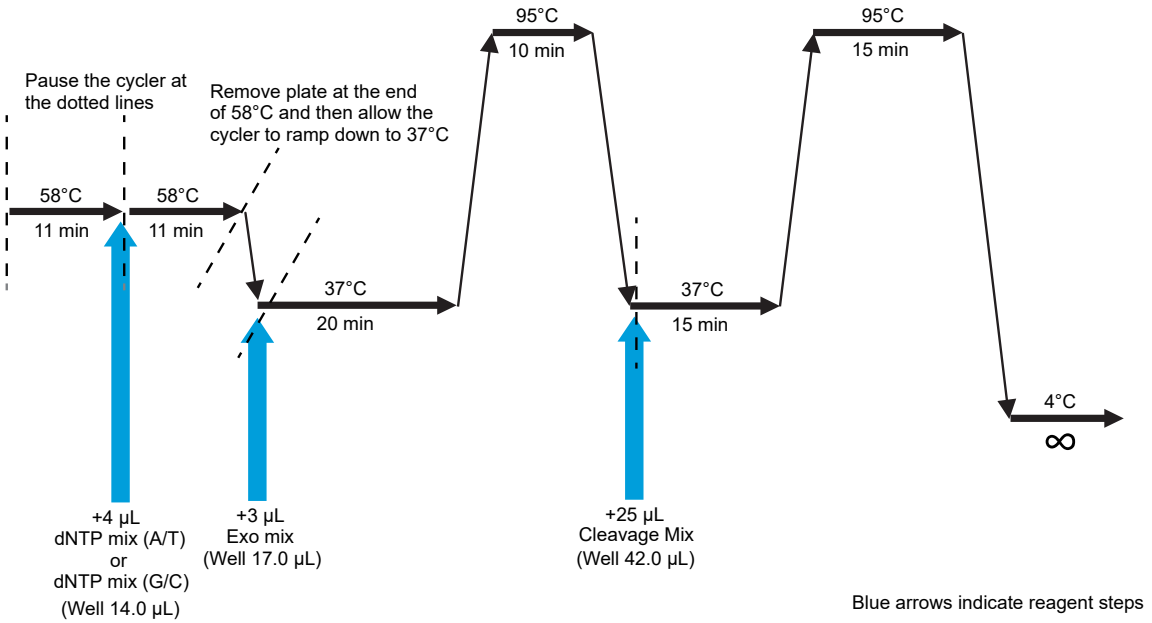


Figure 5 OncoScan Gap Fill thermal cycler protocol.

IMPORTANT! The thermal cycler protocols used in the OncoScan™ CNV Plus Assay are multi-stepped and include pausing the protocol, performing a step, then resuming the protocol. Depending on the thermal cycler used, the ability to pause a protocol may differ. This user guide provides different protocol programs based on the thermal cycler. The protocol steps are the same, but additional information can be programed to aid pausing and resuming a specific protocol. Ensure the pause and resume characteristics of the instrument are understood. See Appendix B, “Thermal cycler protocols”.

Add the Gap Fill Master Mix to the Anneal Plate

1. Remove the seal from the **Anneal Plate**, then discard the seal.
2. Use a multichannel P20 pipette to add 14.0 µL of Gap Fill Master Mix to each sample. (Figure 6.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- and 25-sample reaction, use a single channel pipette to add 14.0 µL of the Gap Fill Master Mix from the “G” microcentrifuge tube to the Negative Control well. Pipet up and down 3 times to rinse the tip. (Figure 6.)

Note: The final volume in each well is 24.0 µL.

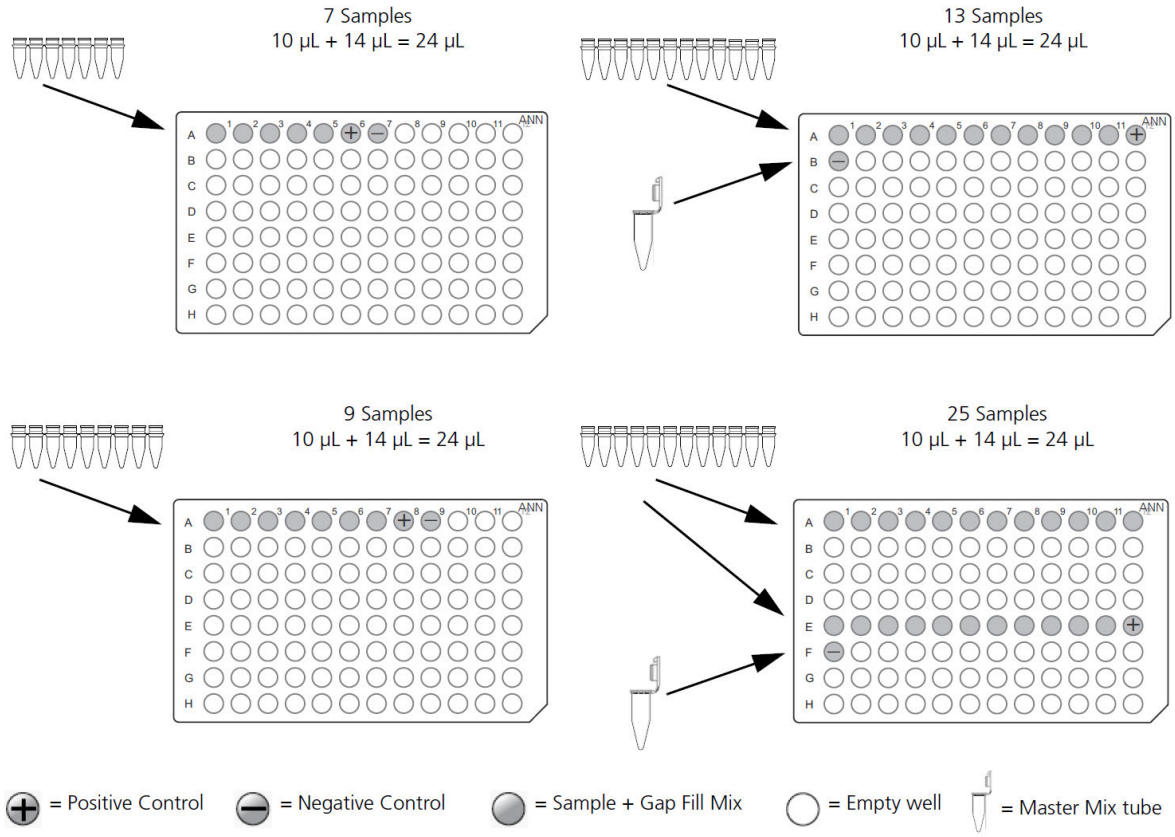


Figure 6 Adding Gap Fill Master Mix to the Anneal Plate.

3. Tightly seal the **Anneal Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

5. Place the **Anneal Plate** back on the chilled cooling block.

6. Discard the Gap Fill Master Mix microcentrifuge tube and strip tube.

Perform the channel split

IMPORTANT! In this section, each sample is split into 2 separate reactions. This is called the Channel Split. Each sample will now have 2 separate reactions performed through the end of the assay.

1. Label a 96-well plate “1st PCR”, then place it on a chilled cooling block.

Note: This plate is called the **1st PCR Plate**.

2. To the **1st PCR Plate**, label the AT wells with a blue marker and the GC wells with a red marker as follows:
   - 7-sample protocol: Label wells A1—A7 as AT (Blue) and wells C1—C7 as GC (Red).
   - 9-sample protocol: Label wells A1—A9 as AT (Blue) and wells C1—C9 as GC (Red).
   - 13-sample protocol: Label wells A1—A12, B1 as AT (Blue) and wells C1—C12, D1 as GC (Red).
   - 25-sample protocol: Label wells A1—A12, E1—E12, F1 as AT (Blue) and wells C1—C12, G1—G12, H1 as GC (Red).
3. Confirm that the **Anneal Plate** and the **1st PCR Plate** are in the same orientation with well A1 at the top left.
4. Remove the seal from the **Anneal Plate**, then discard the seal.
5. Use a multichannel P20 pipette to transfer 10.0 µL of Anneal product from the **Anneal Plate** to the **1st PCR Plate**. (Figure 7.)

   Note: For the 13- or 25-sample protocol, use a single channel pipette to add 10.0 µL of the Negative Control Anneal product from the **Anneal Plate** to the **1st PCR Plate**.

   - 7-sample protocol:
     - 10 µL from A1—A7 (**Anneal Plate**) to A1—A7 **1st PCR Plate**-AT Channel.
     - 10 µL from A1—A7 (**Anneal Plate**) to C1—C7 **1st PCR Plate**-GC Channel.
   - 9-sample protocol:
     - 10 µL from A1—A9 (**Anneal Plate**) to A1—A9 **1st PCR Plate**-AT Channel.
     - 10 µL from A1—A9 (**Anneal Plate**) to C1—C9 **1st PCR Plate**-GC Channel.
   - 13-sample protocol:
     - 10 µL from A1—A12, B1 (**Anneal Plate**) to A1—A12, B1 **1st PCR Plate**-AT Channel.
     - 10 µL from A1—A12, B1 (**Anneal Plate**) to C1—C12, D1 **1st PCR Plate**-GC Channel.
   - 25-sample protocol:
     - 10 µL from A1—A12 (**Anneal Plate**) to A1—A12 **1st PCR Plate**-AT Channel.
     - 10 µL from A1—A12 (**Anneal Plate**) to C1—C12 **1st PCR Plate**-GC Channel.
     - 10 µL from E1—E12, F1 (**Anneal Plate**) to E1—E12, F1 **1st PCR Plate**-AT Channel.
     - 10 µL from E1—E12, F1 (**Anneal Plate**) to G1—G12, H1 **1st PCR Plate**-GC Channel.

   Note: The total volume of each well in the **1st PCR Plate** is 10 µL.

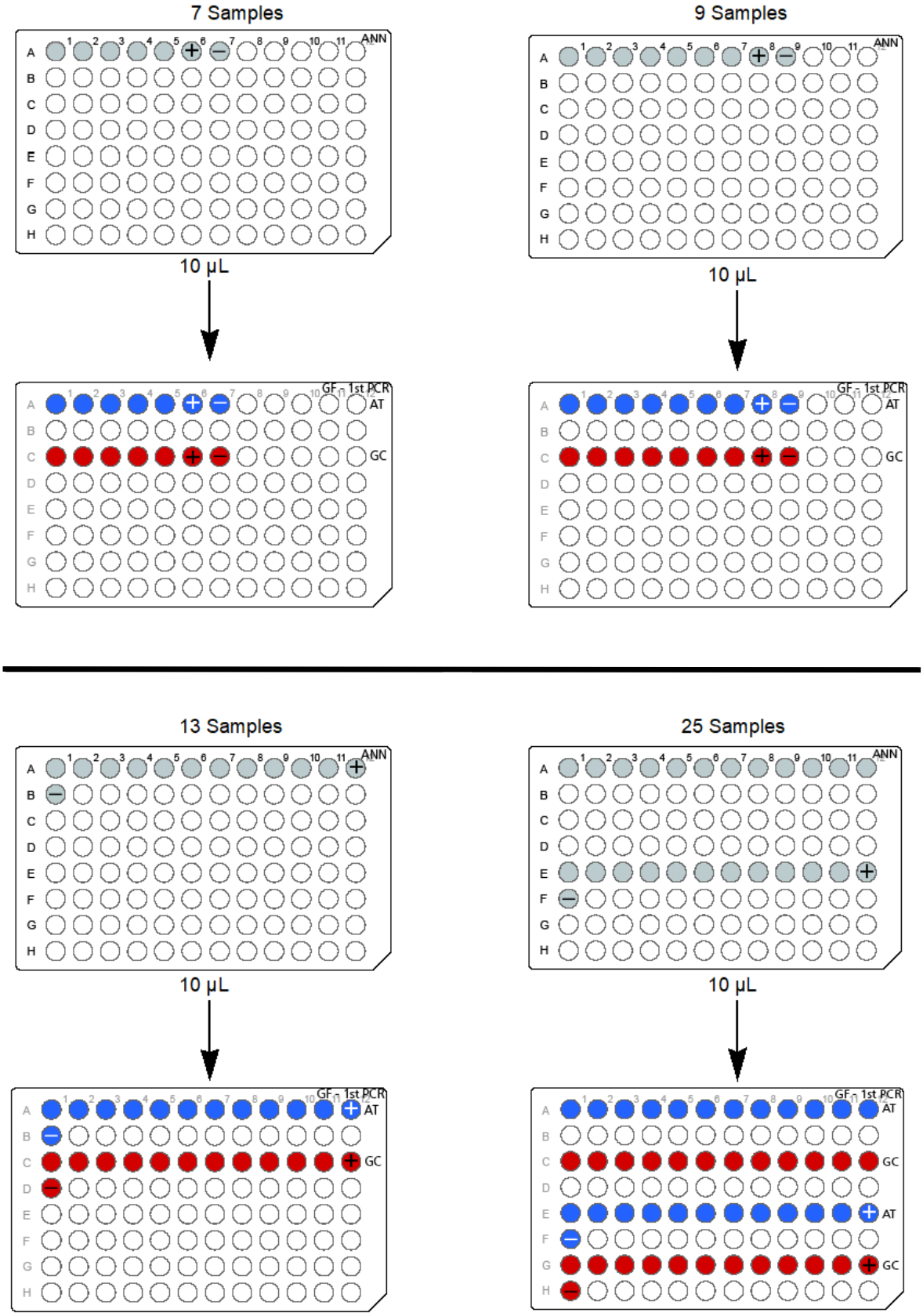


Figure 7 Perform the Channel Split Split the Anneal Product into the 1st PCR Plate.

6. Tightly seal the **1st PCR Plate** with a new seal.
7. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.
8. Place the **1st PCR Plate** back on the chilled cooling block.
9. Discard the **Anneal Plate**.

Resume the OncoScan Gap Fill thermal cycler protocol

1. Load the **1st PCR Plate** into the thermal cycler.
2. Resume the OncoScan Gap Fill thermal cycler protocol. (Figure 8.)

   Note: Ensure that the thermal cycler protocol has resumed and is running.

3. Start a timer for 10 minutes.

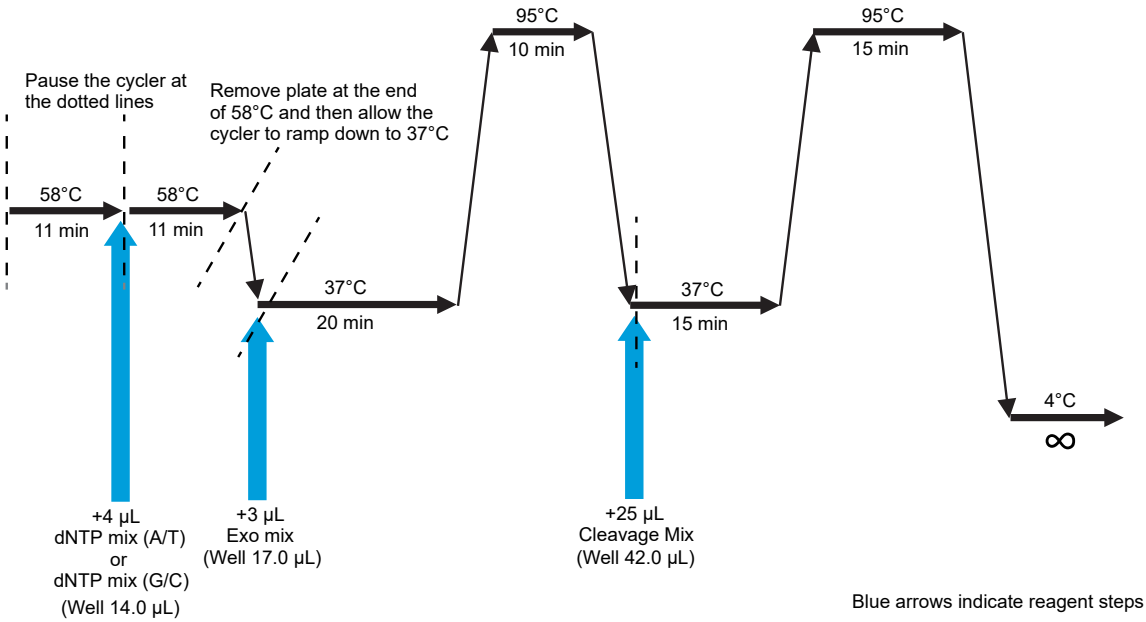


Figure 8 OncoScan Gap Fill thermal cycler protocol.

4. Return the the Nuclease-Free Water, dNTP Mix (A/T), dNTP Mix (G/C), Buffer A, SAP, Recombinant (1 U/µL), and Gap Fill Enzyme Mix to –25°C to –15°C storage.

Add the dNTP AT Master Mix and dNTP GC Master Mix

1. After 11 minutes, pause the OncoScan Gap Fill thermal cycler protocol at 58°C. (Figure 9.)

Note: Ensure that the thermal cycler protocol is paused.

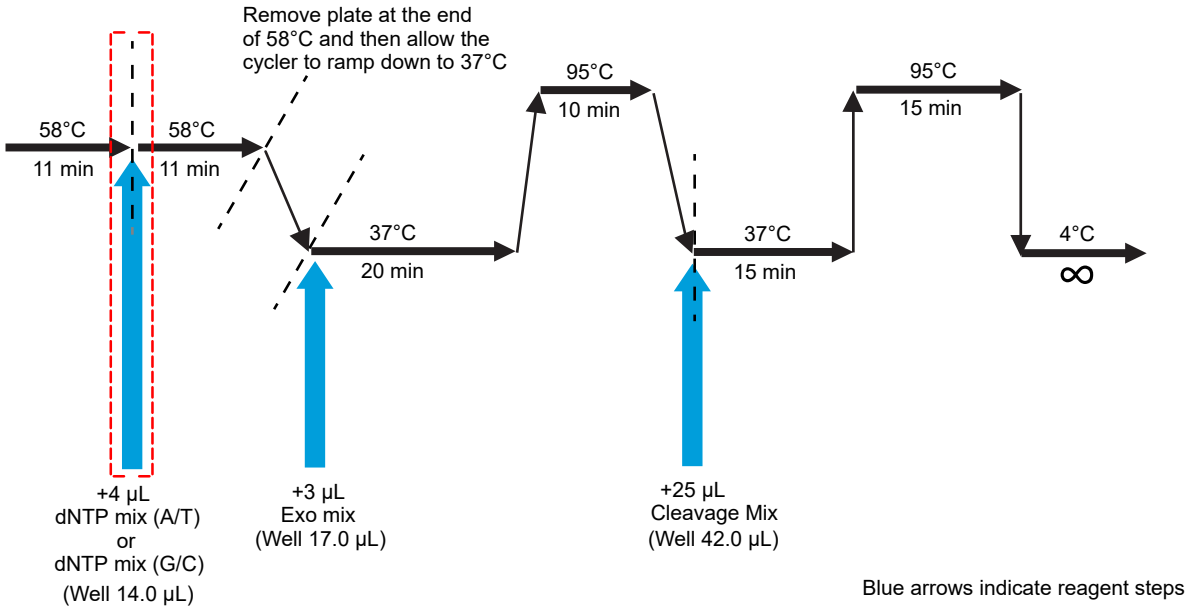


Figure 9 AT Master Mix and GC Master Mix addition.

2. Remove the **1st PCR Plate** from the thermal cycler and place it on a chilled cooling block for 1 minute.
3. After 1 minute, ensure that the plate is tightly sealed.
4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.
5. Place the **1st PCR Plate** back on the chilled cooling block.
6. Confirm the plate is in the correct orientation with well A1 at the top left.
7. Remove the seal from the **1st PCR Plate**, then discard the seal.

Add the AT Master Mix to the 1st PCR Plate

Use a multichannel P20 pipette to transfer 4.0 µL of AT Master Mix to the **1st PCR Plate** AT channel (Figure 10).

- 7-sample protocol:
  - 4.0 µL from the “AT” strip tube to wells A1—A7 of the **1st PCR Plate**-AT Channel.
  - Pipet up and down 3 times to rinse tips.

- 9-sample protocol:
  - 4.0 µL from the “AT” strip tube to wells A1—A9 of the **1st PCR Plate**-AT Channel.
  - Pipet up and down 3 times to rinse tips.
- 13-sample protocol:
  - 4.0 µL from the “AT” strip tube to wells A1—A12 of the **1st PCR Plate**-AT Channel.
  - Use the AT Master Mix tube “AT” to transfer 4.0uL to well B1.
  - Pipet up and down 3 times to rinse tips.
- 25-sample protocol:
  - 4.0 µL from the “AT” strip tube to wells A1—A12 of the **1st PCR Plate**-AT Channel.
  - 4.0 µL from the “AT” strip tube to wells E1—E12 of the **1st PCR Plate**-AT Channel.
  - Use the AT Master Mix tube “AT” to transfer 4.0 µL to well F1.
  - Pipet up and down 3 times to rinse tips.

Add the GC Master Mix to the 1st PCR Plate

1. Use a multichannel P20 pipette to transfer 4.0 µL of GC Master Mix to the **1st PCR Plate** GC channel. (Figure 10.)
   - 7-sample protocol:
     - 4 µL from the “GC” strip tube to wells C1—C7 of the **1st PCR Plate**-GC Channel.
     - Pipet up and down 3 times to rinse tips.
   - 9-sample protocol:
     - 4 µL from the “GC” strip tube to wells C1—C9 of the **1st PCR Plate**-GC Channel.
     - Pipet up and down 3 times to rinse tips.
   - 13-sample protocol:
     - 4 µL from the “GC” strip tube to wells C1—C12 of the **1st PCR Plate**-GC Channel.
     - Use the GC Master Mix tube “GC” to transfer 4.0 µL to well D1.
     - Pipet up and down 3 times to rinse tips.
   - 25-sample protocol:
     - 4 µL from the “GC” strip tube to wells C1—C12 of the **1st PCR Plate**-GC Channel.
     - 4 µL from the “GC” strip tube to wells G1—G12 of the **1st PCR Plate**-GC Channel.
     - Use the GC Master Mix tube “GC” to transfer 4.0 µL to well H1.
     - Pipet up and down 3 times to rinse tips.

Note: The final volume in each well is 14 µL.

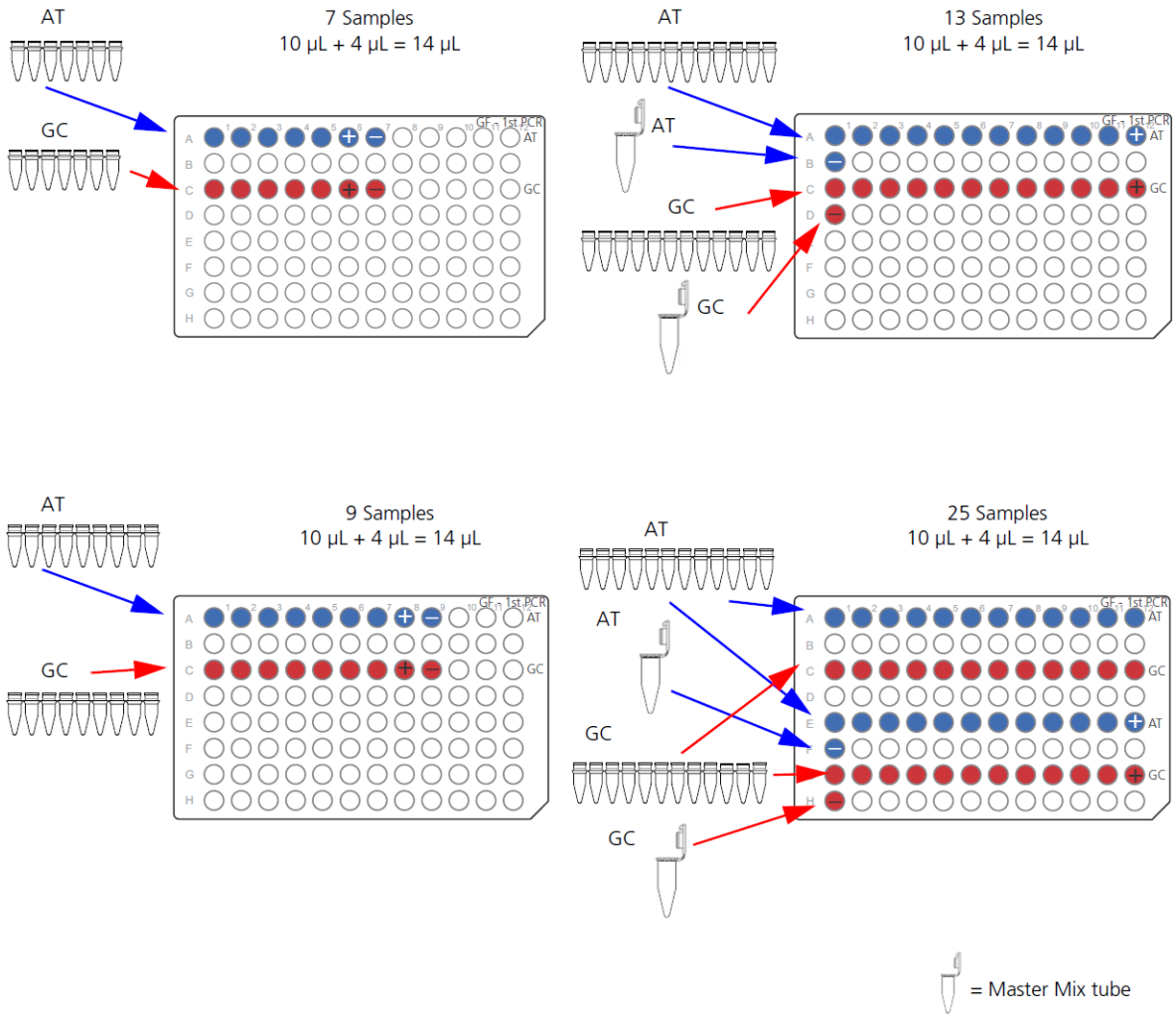


Figure 10 Adding the AT Master Mix and the GC Master Mix to the 1st PCR Plate.

2. Tightly seal the **1st PCR Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

3. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

4. Place the **1st PCR Plate** back on the chilled cooling block.

Resume the OncoScan Gap Fill thermal cycler protocol

1. Load the **1st PCR Plate** into the thermal cycler.

2. Resume the OncoScan Gap Fill thermal cycler protocol. (Figure 9.)

3. Start a timer for 10 minutes.

Note: Ensure that the thermal cycler protocol has resumed and is running.

4. Discard the AT and GC Master Mix microcentrifuge tubes and strip tubes.

5. Begin preparing the Exo Mix.

IMPORTANT! When the timer ends, return to the thermal cycler to pause the protocol. (Figure 11.) It is critical to pause and remove the plate from the thermal cycler at the end of the 58°C incubation. ***Do not*** allow the plate to ramp down to 37°C. This will result in non-specific Gap Fill and compromised data.

Prepare and add the Exo Mix

Note: Starting from this step until the end of the assay, the number of reactions double due to the AT and GC channel split. All the master mixes, from the addition of Exo Mix and beyond, will be prepared to account for twice the number of samples. The reactions must be carried out in the same plate layout until the end of the assay.

- 7-sample protocol will be processed as 14 reactions.
- 9-sample protocol will be processed as 18 reactions.
- 13-sample protocol will be processed as 26 reactions.
- 25-sample protocol will be processed as 50 reactions.

Prepare the Exo Mix

1. Label a strip tube “EXO”, then place it on a chilled cooling block.

2. Remove the Exo Mix from the freezer, then immediately place it in a –25°C to –15°C cooler.

3. Prepare the Exo Mix.
   a. Centrifuge for 1 second. Vortex for 1 second, then centrifuge for 3 seconds.

   b. Return the Exo Mix to the cooler.

4. Aliquot the appropriate volume of Exo Mix into the chilled strip tube, “EXO”:
   - 7-sample protocol: Add 7.5 µL into each well of a 7-well strip tube.
   - 9-sample protocol: Add 7.5 µL into each well of a 9-well strip tube.
   - 13-sample protocol: Add 7.5 µL into each well of a 12-well strip tube.
   - 25-sample protocol: Add 16.0 µL into each well of a 12-well strip tube.

5. Return the Exo Mix to the cooler.

6. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.

7. Place the strip tube on a chilled cooling block until the Exo Mix addition step.

Add the Exo Mix to the 1st PCR Plate

1. After 10 minutes, return to the thermal cycler.
2. When the protocol reaches the end of the 58°C incubation, pause the protocol at 58°C. (Figure 11.)

IMPORTANT! It is critical to remove the plate before the thermal cycler protocol begins ramping down to 37°C.

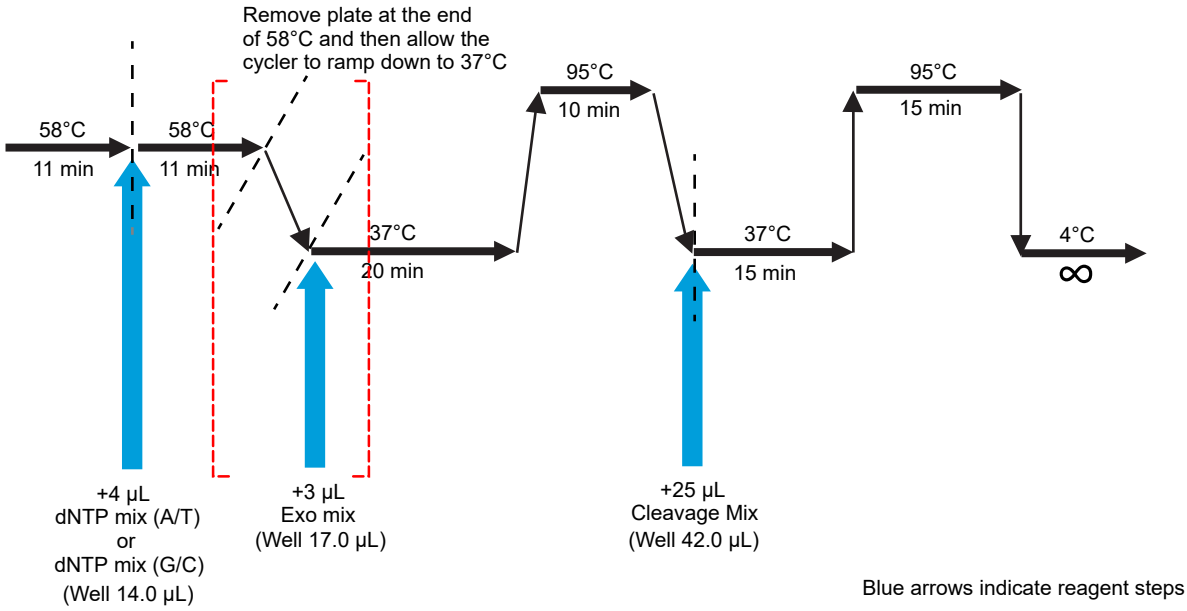


Figure 11 Exo Mix addition.

3. Remove the **1st PCR Plate** from the thermal cycler and place it on a chilled cooling block for 1 minute.
4. Resume the OncoScan Gap Fill protocol on the thermal cycler. Allow it to ramp down to the start of the 37°C step. Pause the protocol at the start of the 37°C step. (Figure 11.)

Note: Ensure that the thermal cycler protocol is paused.

5. After 1 minute, ensure that the plate is tightly sealed.
6. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.
7. Place the **1st PCR Plate** back on the chilled cooling block.
8. Confirm the plate is in the correct orientation with well A1 at the top left.
9. Remove the seal from the **1st PCR Plate**, then discard the seal.
10. Use a multichannel P20 pipette to add 3.0 µL of the Exo Mix to each sample. (Figure 12.)
    a. Pipet up and down 3 times to rinse the tips.

b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 3.0 µL of the Exo Mix directly from the Exo Mix tube to the Negative Control wells. Pipet up and down 3 times to rinse the tip. (Figure 12.)

Note: The final volume in each well is 17.0 µL.

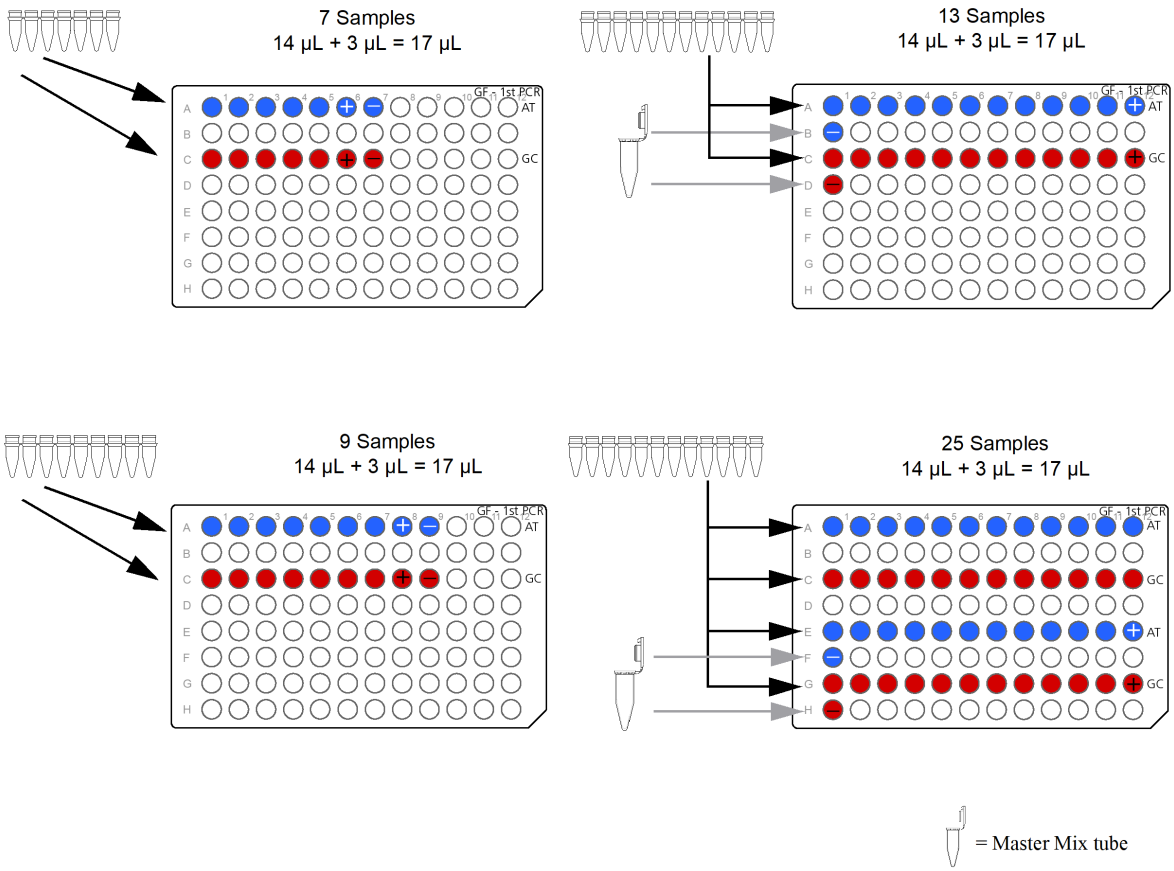


Figure 12 Adding the Exo Mix to the 1st PCR Plate.

11. Tightly seal the **1st PCR Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

12. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

13. Place the **1st PCR Plate** back on the chilled cooling block.

Resume the OncoScan Gap Fill thermal cycler protocol

1. Load the **1st PCR Plate** into the thermal cycler.
2. Resume the OncoScan Gap Fill thermal cycler protocol. (Figure 11.)

   Note: Ensure that the thermal cycler protocol has resumed and is running.

3. Start a timer for 20 minutes.
4. Discard the Exo Mix strip tube.
5. Return the Exo Mix to the –25°C to –15°C storage.
6. Begin preparing the Cleavage Master Mix.

Note: At 20 minutes, prepare the Cleavage Master Mix. At 30 minutes return to the thermal cycler to pause the protocol. (Figure 13.) The protocol is paused after the block ramps down to 37°C, then the **1st PCR Plate** is removed.

Prepare and add the Cleavage Master Mix

Prepare the Cleavage Master Mix

1. Label a 1.5-mL microcentrifuge tube “CM”, then place it on ice.
2. Label a strip tube “CM”, then place it on a chilled cooling block. (See Table 12 for the correct strip tube).

   Note: At 20 minutes (the last 10 minutes of the 95°C step on the thermal cycler) prepare the Cleavage Master Mix.

3. Remove the Cleavage Enzyme (2 U/µL) from the freezer, then immediately place it in a –25°C to –15°C cooler.
4. Prepare the Cleavage Enzyme (2 U/µL).
   a. Centrifuge for 1 second. Vortex for 1 second, then centrifuge for 3 seconds.
   b. Return the Cleavage Enzyme (2 U/µL) to the cooler.
5. Prepare the Cleavage Buffer that has been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.

6. Prepare the Cleavage Master Mix. Use the appropriate column in the following table. (Table 12.)

Table 12 Cleavage Master Mix.

Reagent and cap color	1 reaction	14 reactions (~25% overage)	18 reactions (~25% overage)	26 reactions (~25% overage)	50 reactions (~25% overage)
Cleavage Buffer	25.0 µL	438.0 µL	563.0 µL	813.0 µL	1,563.0 µL
Cleavage Enzyme (2 U/µL)	0.2 µL	3.5 µL	4.5 µL	6.5 µL	12.5 µL
Total volume	**25.2 µL**	**441.5 µL**	**567.5 µL**	**819.5 µL**	**1,575.5 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		60.0 µL	60.0 µL	60.0 µL	120.0 µL

7. Add the Cleavage Buffer and the Cleavage Enzyme (2 U/µL) to the 1.5-mL microcentrifuge tube labeled “CM”.
   a. Pipet the Cleavage Enzyme (2 U/µL) up and down 3 times to rinse the tips.
   b. Between each addition, change tips.
   c. Place on ice.
8. Return the Cleavage Enzyme (2 U/µL) to the cooler.
9. Complete the Cleavage Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
10. Aliquot the appropriate volume of Cleavage Master Mix into the chilled strip tube, “CM”:
    - 7-sample protocol: Add 60.0 µL into each well of a 7-well strip tube.
    - 9-sample protocol: Add 60.0 µL into each well of a 9-well strip tube.
    - 13-sample protocol: Add 60.0 µL into each well of a 12-well strip tube.
    - 25-sample protocol: Add 120.0 µL into each well of a 12-well strip tube.
11. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.
12. Place the strip tube on a chilled cooling block until the Cleavage Master Mix addition step.
13. Place the Cleavage Master Mix tube, “CM”, on ice. Do not discard.

Add the Cleavage Master Mix to the 1st PCR Plate

1. Return to the thermal cycler.
2. When the protocol reaches the end of the 95°C incubation and ramps down to the start of the 37°C step, pause the protocol. (Figure 13.)

Note: Ensure that the thermal cycler protocol is paused.

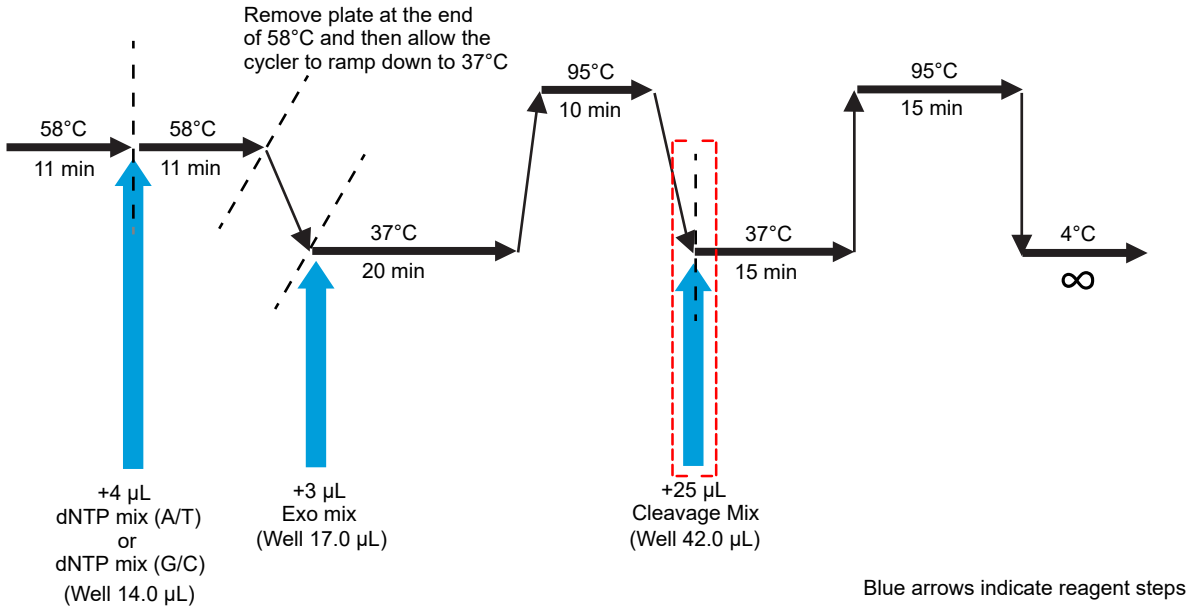


Figure 13 Cleavage Master Mix addition.

3. Remove the **1st PCR Plate** from the thermal cycler and place it on a chilled cooling block for 1 minute.
4. After 1 minute, ensure that the plate is tightly sealed.
5. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.
6. Place the **1st PCR Plate** back on the chilled cooling block.
7. Confirm the plate is in the correct orientation with well A1 at the top left.
8. Remove the seal from the **1st PCR Plate**, then discard the seal.
9. Use a multichannel P200 pipette to add 25.0 µL of the Cleavage Master Mix to each sample. (Figure 14.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 25.0 µL of the Cleavage Master Mix from the "CM" microcentrifuge tube to the Negative Control wells. Pipet up and down 3 times to rinse the tip. (Figure 14.)

Note: The final volume in each well is 42.0 µL.

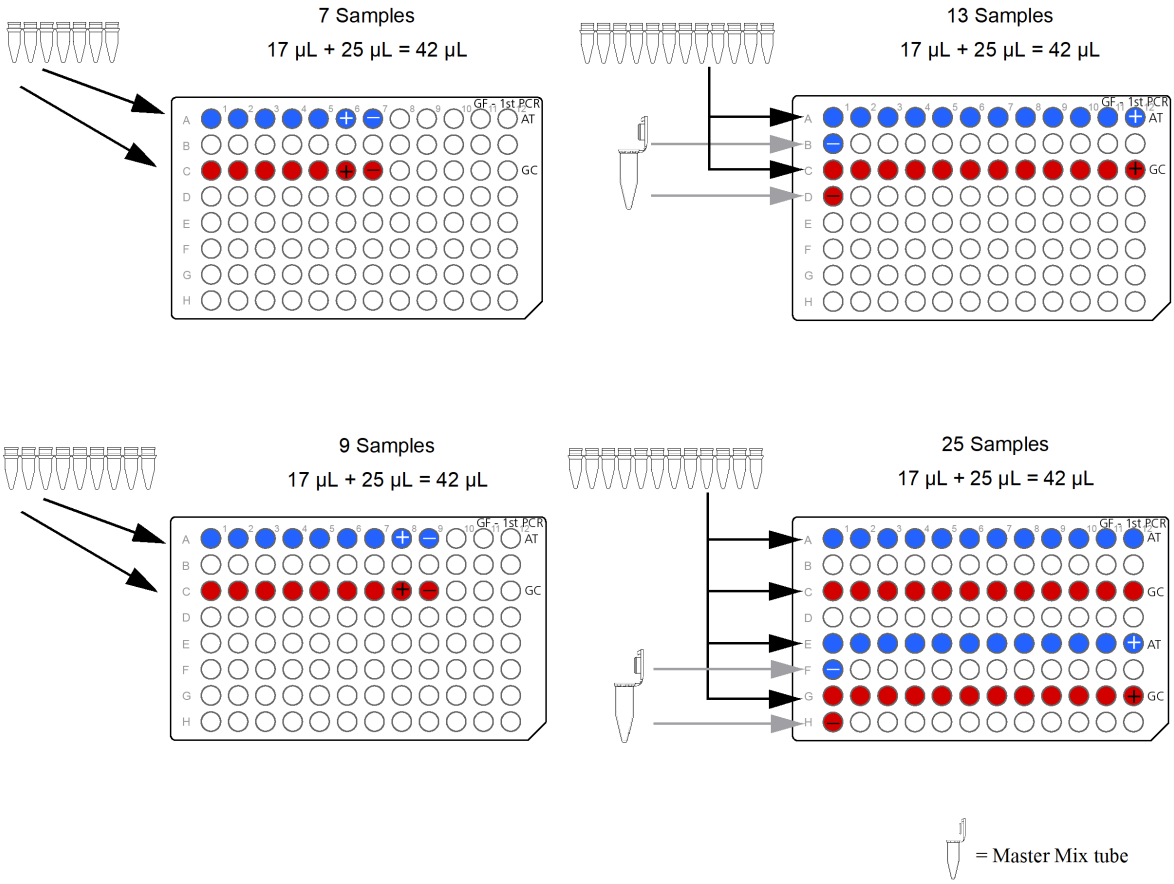


Figure 14 Adding Cleavage Master Mix to the 1st PCR Plate.

10. Tightly seal the **1st PCR Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

11. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

12. Place the **1st PCR Plate** back on the chilled cooling block.

Resume the OncoScan Gap Fill thermal cycler protocol

1. Load the **1st PCR Plate** into the thermal cycler.

2. Resume the OncoScan Gap Fill thermal cycler protocol. (Figure 13.)

Note: Ensure that the thermal cycler protocol has resumed and is running.

3. Start a timer for 25 minutes.
4. Discard the Cleavage Master Mix microcentrifuge tube and strip tube.
5. Return the Cleavage Buffer and Cleavage Enzyme (2 U/µL) to the –25°C to –15°C storage.
6. Begin preparing 1st PCR Master Mix.

Prepare and add the 1st PCR Master Mix

Prepare the 1st PCR Master Mix

1. Label a 1.5-mL microcentrifuge tube "PCR 1", then place it on ice. If performing the 25-sample protocol (50 reactions), use a 2.0-mL microcentrifuge tube.
2. Label a strip tube "PCR 1", then place it on a chilled cooling block. (See Table 13 for the correct strip tube.)

   Note: At 25 minutes (the last 5 minutes of the 95°C step on the thermal cycler) prepare the 1st PCR Master Mix.

3. Remove the Taq Polymerase (5 U/µL) from the freezer, then immediately place it in a –25°C to –15°C cooler.
4. Prepare the Taq Polymerase (5 U/µL).
   a. Centrifuge for 1 second. Vortex for 1 second, then centrifuge for 3 seconds.
   b. Return the Taq Polymerase (5 U/µL) to the cooler.
5. Prepare the PCR Mix that has been on ice.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
6. Prepare the 1st PCR Master Mix. Use the appropriate column in the following table. (Table 13.)

Table 13 1st PCR Master Mix.

Reagent and cap color	1 reaction	14 reactions (~25% overage)	18 reactions (~25% overage)	26 reactions (~25% overage)	50 reactions (~25% overage)
◯ PCR Mix	24.4 µL	427.0 µL	549.0 µL	793.0 µL	1,525.0 µL
◯ Taq Polymerase (5 U/µL)	0.56 µL	9.8 µL	12.6 µL	18.2 µL	35.0 µL
Total volume	**24.9 µL**	**436.8 µL**	**561.6 µL**	**811.2 µL**	**1,560.0 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		60.0 µL	60.0 µL	60.0 µL	120.0 µL

7. Add the PCR Mix and the Taq Polymerase (5 U/µL) to the 1.5- (or 2.0-) mL microcentrifuge tube labeled “PCR 1”.
   a. Pipet the Taq Polymerase (5 U/µL) up and down 3 times to rinse the tips.
   b. Between each addition, change tips.
   c. Place on ice.
8. Return the Taq Polymerase (5 U/µL) to the cooler.
9. Complete the 1st PCR Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
10. Aliquot the appropriate volume of 1st PCR Master Mix into the chilled strip tube, “PCR 1”:
    - 7-sample protocol: Add 60.0 µL into each well of a 7-well strip tube.
    - 9-sample protocol: Add 60.0 µL into each well of a 9-well strip tube.
    - 13-sample protocol: Add 60.0 µL into each well of a 12-well strip tube.
    - 25-sample protocol: Add 120.0 µL into each well of a 12-well strip tube.
11. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.
12. Place the strip tube on a chilled cooling block until the 1st PCR Master Mix addition step.
13. Place the 1st PCR Master Mix tube, “PCR 1”, on ice. Do not discard.

Complete the OncoScan Gap Fill thermal cycler protocol

When the OncoScan Gap Fill protocol is complete and ramps down to 4°C, do the following:

1. Remove the **1st PCR Plate** from the thermal cycler, then place it on a chilled cooling block for 1 minute.
2. After 1 minute, ensure that the plate is tightly sealed.
3. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 30 seconds.
4. Place the **1st PCR Plate** back on the chilled cooling block.

Prepare the thermal cycler for the OncoScan 1st PCR protocol

1. Stop the OncoScan Gap Fill protocol on the thermal cycler.
2. Start the OncoScan 1st PCR thermal cycler protocol. When the thermal cycler block temperature reaches 60°C, pause the protocol. (Figure 15.)

Note: Ensure that the thermal cycler protocol is paused.

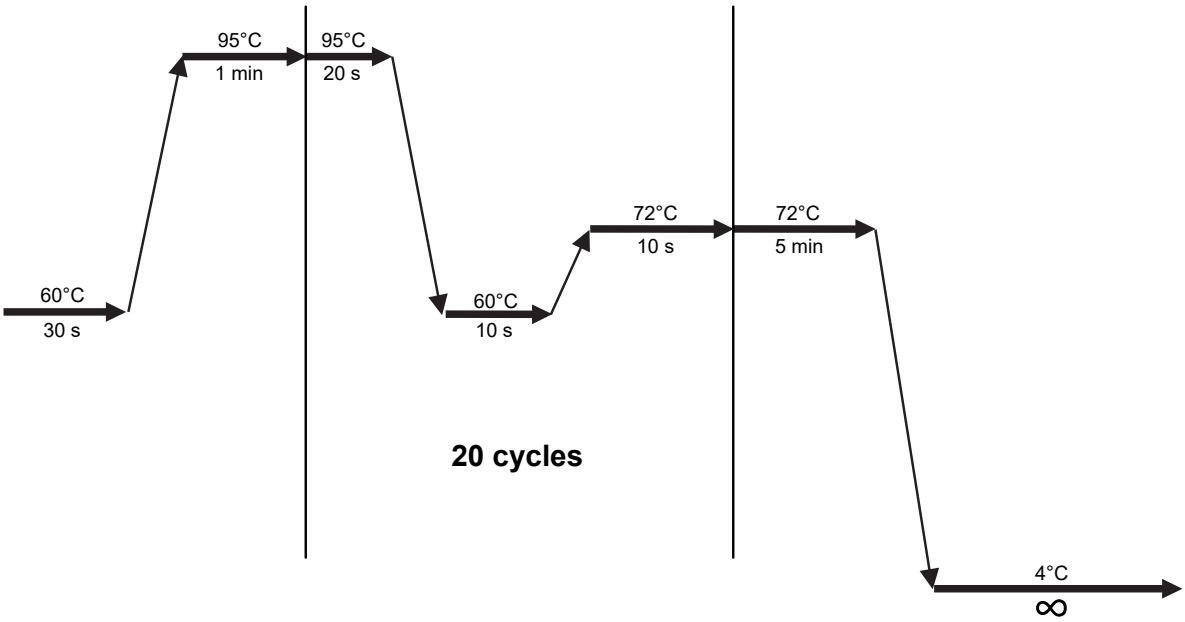


Figure 15 OncoScan 1st PCR thermal cycler protocol.

Add the 1st PCR Master Mix to the 1st PCR Plate

1. Confirm that the plate is in the correct orientation with well A1 at the top left.
2. Remove the seal from the **1st PCR Plate**, then discard the seal.
3. Use a multichannel P200 pipette to add 25.0 µL of the 1st PCR Master Mix to each sample. (Figure 16.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 25.0 µL of the 1st PCR Master Mix from the "PCR 1" microcentrifuge tube to the Negative Control wells. Pipet up and down 3 times to rinse the tip. (Figure 16.)

Note: The final volume in each well is 67.0 µL.

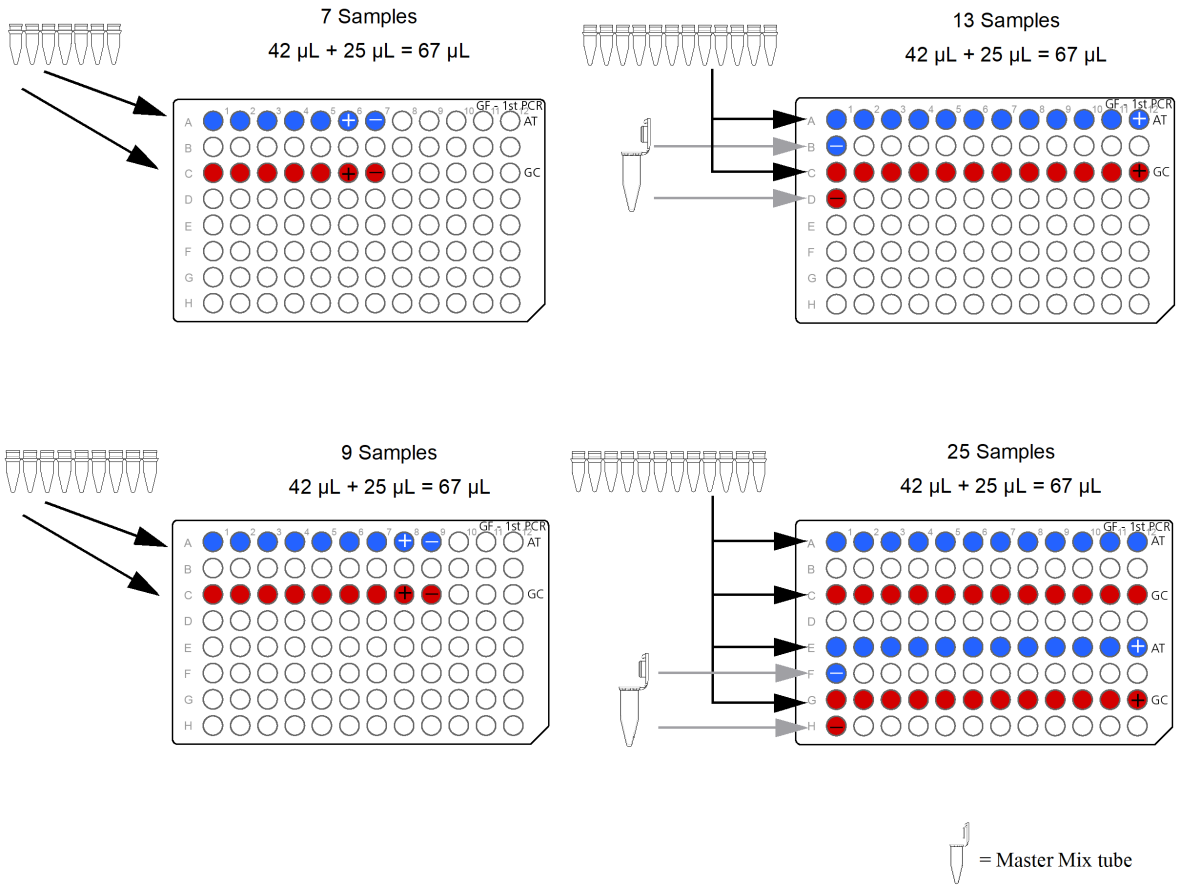


Figure 16 Adding the 1st PCR Master Mix to the 1st PCR Plate.

4. Tightly seal the **1st PCR Plate** with a new seal, then vortex the plate for 1 second in all corners and center.
5. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
6. Place the **1st PCR Plate** back on the chilled cooling block.

Resume the OncoScan 1st PCR thermal cycer protocol

1. Load the **1st PCR Plate** into the thermal cycler.
2. Resume the OncoScan 1st PCR thermal cycler protocol. (Figure 15.)

   Note: Ensure that the thermal cycler protocol has resumed and is running.

3. Discard the 1st PCR Master Mix microcentrifuge tube and strip tube.
4. Return the the PCR Mix and Taq Polymerase (5 U/µL) to the –25°C to –15°C storage.
5. Empty the ice bucket, then place the cooling blocks in the refrigerator.

Complete the OncoScan 1st PCR thermal cycler protocol

1. When the OncoScan 1st PCR thermal cycler protocol is complete and ramps down to 4°C, do the following:
   a. Remove the **1st PCR Plate** from the thermal cycler, then place it on a small tray of ice.
   b. Stop the OncoScan 1st PCR protocol, then power off the thermal cycler.
   c. Transfer the **1st PCR Plate** to the Post-PCR Room on ice.

IMPORTANT! To prevent contamination from PCR products, the **1st PCR Plate** must remain sealed until it has been transferred to the Post-PCR Room. ***Do not*** open the seal, centrifuge, or store the **1st PCR Plate** in the Pre-PCR Room.

---

2. Do one of the following:
   - PROCEED:
     If continuing the assay: Proceed to “Stage 3: 2nd PCR and First QC Gel” on page 64.
   - STOP:
     If stopping and not proceeding with the assay, perform the “Optional stopping point” on page 62. When resuming the assay, perform the step, “Resume the assay” on page 63.

Optional stopping point

IMPORTANT! Only perform the following steps if stopping the assay and storing the **1st PCR Plate** for future use.

---

1. Place the **1st PCR Plate** on a 96-well plate storage rack.
2. Store the plate at –25°C to –15°C in the Post-PCR Room.

Note: The plate can be stored for up to 10 days.

Resume the assay

When resuming the assay, perform the following steps in the Post-PCR Room:

1. Fill the ice bucket with ice, then place 3 cooling blocks on the ice.
2. Thaw the **1st PCR Plate** at room temperature (≤30 minutes). Once thawed, immediately place it on a chilled cooling block.
3. Ensure that the plate is tightly sealed, then vortex the plate for 1 second in all corners and center.
4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
5. Place the **1st PCR Plate** back on the chilled cooling block.

IMPORTANT! PROCEED: Immediately proceed to “Stage 3: 2nd PCR and First QC Gel” on page 64.

Stage 3: 2nd PCR and First QC Gel

About Stage 3

This stage is performed in the Post-PCR Room. During this stage, the 2nd PCR reaction is setup using product from the **1st PCR Plate**. The OncoScan 2nd PCR thermal cycler protocol is started.

The First QC Gel is run to check that the 1st PCR reaction occurred, and the correct size DNA product was generated.

When the 2nd PCR thermal cycler protocol is complete, and the First QC Gel has been assessed, the assay is continued to Stage 4: HaeIII Digest and Second QC Gel.

Reagents required

Table 14 Reagents required for Stage 3.

Quantity	Reagent and cap color	Source	
From the OncoScan™ CNV Plus Reagent Kit for Research		**Module**	**Part No.**
1	◯ PCR Mix	OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270)	902259
1	◯ Taq Polymerase (5 U/µL)		902260
QC Gel Materials			**Cat. No.**
1	Nuclease-Free Water (from different source)		10977015
1	Invitrogen™ E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4%		G820804
1	Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer		10482028
1	Applied Biosystems™ 25-bp DNA Ladder		931343
Other			
1	**1st PCR Plate**	From Stage 2	

Set up the work area in the Post-PCR Room for Stage 3

1. If using a refrigerated plate centrifuge, set it to 4°C to allow it to reach the proper temperature.
2. Thaw the PCR Mix at room temperature, then immediately place it on ice.

Prepare the 1st PCR Plate for the 2nd PCR

1. Fill the ice bucket with ice, then place 3 cooling blocks on the ice.
2. Place the **1st PCR Plate** on a chilled cooling block.
3. Ensure that the plate is tightly sealed, then vortex the plate for 1 second in all corners and center.
4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
5. Place the **1st PCR Plate** back on the chilled cooling block.

Prepare thermal cycler for 2nd PCR protocol

1. Power on the thermal cycler.
2. Start the OncoScan 2nd PCR protocol on the thermal cycler. When the thermal cycler block temperature reaches 60°C, pause the protocol. (Figure 17.)

Note: Ensure that the thermal cycler protocol is paused.

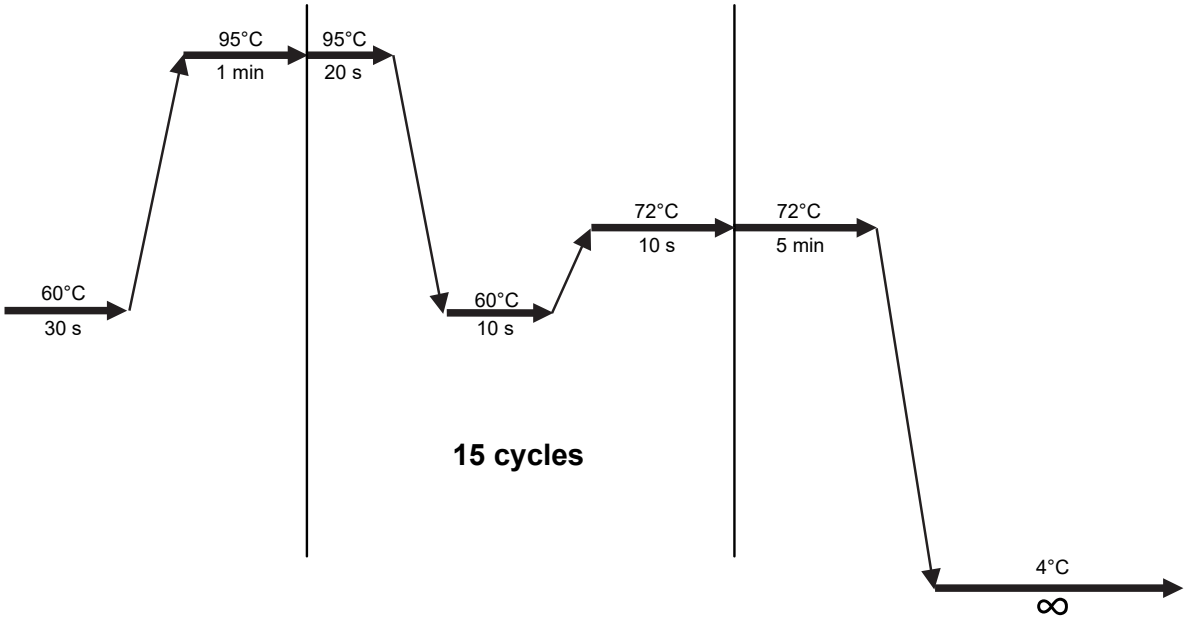


Figure 17 OncoScan 2nd PCR thermal cycler protocol.

Prepare the 2nd PCR Plate

1. Label a 96-well plate “2nd PCR”, then place it on a chilled cooling block.

Note: This plate is called the **2nd PCR Plate**.

2. Confirm that the **1st PCR Plate** and the **2nd PCR Plate** are in the same orientation with well A1 at the top left.

3. To the **2nd PCR Plate**, label the AT wells with a blue marker and the GC wells with a red marker as follows:
   - 7-sample protocol: Label wells A1—A7 as AT (Blue) and wells C1—C7 as GC (Red).
   - 9-sample protocol: Label wells A1—A9 as AT (Blue) and wells C1—C9 as GC (Red).
   - 13-sample protocol: Label wells A1—A12, B1 as AT (Blue) and wells C1—C12, D1 as GC (Red).
   - 25-sample protocol: Label wells A1—A12, E1—E12, F1 as AT (Blue) and wells C1—C12, G1—G12, H1 as GC (Red).
4. Cover the **2nd PCR Plate** with an unopened seal to prevent contamination.

Prepare and add the 2nd PCR Master Mix

Prepare the 2nd PCR Master Mix

1. Label a 1.5-mL microcentrifuge tube “PCR 2”, then place it on ice. If performing the 25-sample protocol (50 reactions), use a 2.0-mL microcentrifuge tube.
2. Label a strip tube “PCR 2”, then place it on a chilled cooling block. (See Table 15 for the correct strip tube).
3. Remove the Taq Polymerase (5 U/µL) from the freezer, then immediately place it in a –25°C to –15°C cooler.
4. Prepare the Taq Polymerase (5 U/µL).
   a. Centrifuge for 1 second. Vortex for 1 second, then centrifuge for 3 seconds.
   b. Return the Taq Polymerase (5 U/µL) to the cooler.
5. Prepare the PCR Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
6. Prepare the 2nd PCR Master Mix. Use the appropriate column in the following table. (Table 15.)

Table 15 2nd PCR Master Mix.

Reagent and cap color	1 reaction	14 reactions (~25% overage)	18 reactions (~25% overage)	26 reactions (~25% overage)	50 reactions (~25% overage)
◯ PCR Mix	24.4 µL	427.0 µL	549.0 µL	793.0 µL	1,525.0 µL
◯ Taq Polymerase (5 U/µL)	0.56 µL	9.8 µL	12.6 µL	18.2 µL	35.0 µL
Total volume	**24.9 µL**	**436.8 µL**	**561.6 µL**	**811.2 µL**	**1,560.0 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		60 µL	60 µL	60 µL	120 µL

7. Add the PCR Mix and the Taq Polymerase (5 U/µL) to the 1.5- (or 2.0-) mL microcentrifuge tube labeled “PCR 2”.
   a. Pipet the Taq Polymerase (5 U/µL) up and down 3 times to rinse the tips.
   b. Between each addition, change tips.
   c. Place on ice.
8. Return the Taq Polymerase (5 U/µL) to the cooler.
9. Complete the 2nd PCR Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
10. Aliquot the appropriate volume of 2nd PCR Master Mix into the chilled strip tube, “PCR 2”:
    - 7-sample protocol: Add 60.0 µL into each well of a 7-well strip tube.
    - 9-sample protocol: Add 60.0 µL into each well of a 9-well strip tube.
    - 13-sample protocol: Add 60.0 µL into each well of a 12-well strip tube.
    - 25-sample protocol: Add 120.0 µL into each well of a 12-well strip tube.
11. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.
12. Place the strip tube on a chilled cooling block until the 2nd PCR Master Mix addition step.
13. Place the 2nd PCR Master Mix tube, “PCR 2”, on ice. Do not discard.

Add the 2nd PCR Master Mix to the 2nd PCR Plate

Use a multichannel P200 pipette to add 25.0 µL of the 2nd PCR Master Mix to each empty sample well of the **2nd PCR Plate**. (Figure 18.)

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 25.0 µL of the 2nd PCR Master Mix from the “PCR 2” microcentrifuge tube to the Negative Control wells. (Figure 18.)

Note: The current volume in each well is 25.0 µL.

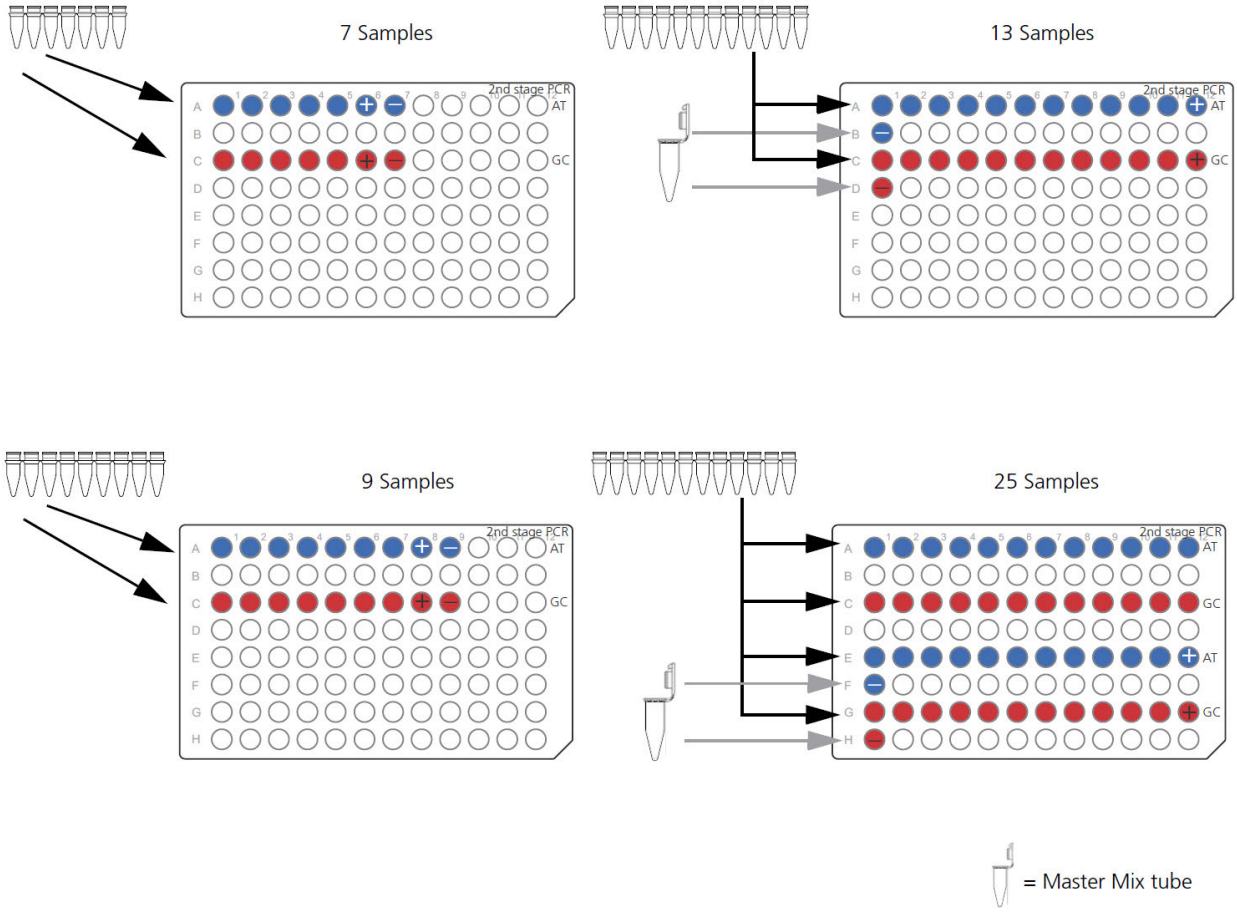


Figure 18 Add the 2nd PCR Master Mix to the empty 2nd PCR Plate.

Add the 1st PCR product to the 2nd PCR Plate

1. Confirm that the **1st PCR Plate** and the **2nd PCR Plate** are in the same orientation with well A1 at the top left.
2. Remove the seal from the **1st PCR Plate**, then discard the seal.
3. Remove the unopened seal from the **2nd PCR Plate** and set it aside.
4. Use a multichannel P20 pipette to transfer 2.0 µL of the 1st PCR product from the **1st PCR Plate** to the corresponding well of the **2nd PCR Plate**. (Figure 19.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 2.0 µL of the Negative Control 1st PCR product from the **1st PCR Plate** to the corresponding wells of the **2nd PCR Plate**. Pipet up and down 3 times to rinse the tip. (Figure 19.)

Note: The final volume in each well is 27.0 µL.

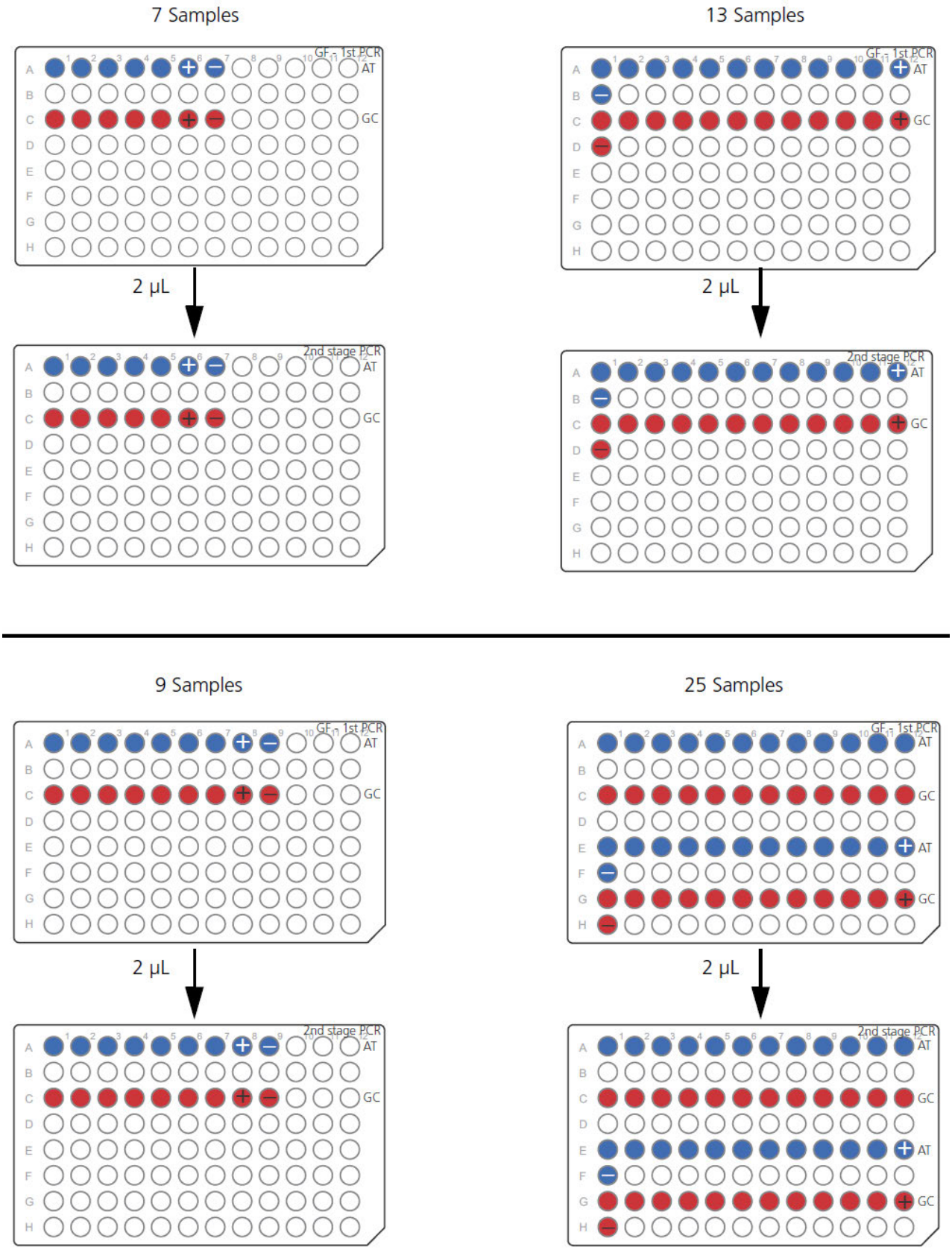


Figure 19 Add the 1st PCR Product to the 2nd PCR Plate.

5. Cover the **1st PCR Plate** with an unopened seal. Leave the plate on the chilled cooling block until ready for the First Gel QC.

6. Tightly seal the **2nd PCR Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

7. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

8. Place the **2nd PCR Plate** back on the chilled cooling block.

Resume the OncoScan 2nd PCR thermal cycler protocol

1. Load the **2nd PCR Plate** into the thermal cycler.

2. Resume the OncoScan 2nd PCR thermal cycler protocol. (Figure 17.)

   Note: Ensure that the thermal cycler protocol has resumed and is running.

3. While the OncoScan 2nd PCR thermal cycler protocol is running, perform the following:
   a. Discard the 2nd PCR Master Mix microcentrifuge tube and strip tube.
   b. Return the PCR Mix and Taq Polymerase (5 U/µL) to the –25°C to –15°C storage.
   c. Proceed to “Setup the area for the First QC Gel” on page 71.

Setup the area for the First QC Gel

Setup the work area in the Post-PCR Room

1. Assemble the E-Gel™ Power Snap Plus Electrophoresis System.

2. Place the following items on the benchtop:
   - Nuclease-Free Water (from different source)
   - E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4%
   - TrackIt™ Cyan/Orange Loading Buffer
   - 25-bp DNA Ladder

   Note: The OncoScan™ CNV Plus Assay protocol is optimized to be run on E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4% using the Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System.

Prepare the Loading Buffer and 25-bp DNA Ladder

Dilute the TrackIt™ Cyan/Orange Loading Buffer

Note: This recipe makes 10.0 mL of 1:100 diluted Loading Buffer. It is enough volume to be used for numerous gels and assays. Make the 1:100 diluted Loading Buffer using the Nuclease-Free Water (from different source). The Nuclease-Free Water supplied in the OncoScan™ CNV Plus Reagent Kit for Research does not have enough volume to make 10.0 mL

Note: If a previously made 1:100 diluted Loading Buffer is not available, prepare new bulk stock.

1. Label a 15-mL conical centrifuge tube “1:100 diluted Loading Buffer”.
2. Add 100.0 µL of TrackIt™ Cyan/Orange Loading Buffer to 9.9 mL of Nuclease-Free Water (from different source).
3. Vortex thoroughly, then place the tube on the benchtop. It can be stored at room temperature for long term use.

Dilute the 25-bp DNA Ladder

Note: The diluted 25-bp DNA Ladder should be made fresh.

Prepare 162.0 µL of a fresh 1:6 dilution of 25-bp DNA Ladder.

1. Label a 1.5-mL microcentrifuge tube “1:6 25 bp DNA Ladder”.
2. Add 27.0 µL of 25-bp DNA Ladder to 135.0 µL of Nuclease-Free Water (from different source).
3. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
4. Place the tube on the bench top. It will be used during the First QC Gel and the Second QC Gel.

Prepare the Gel 1 Plate

1. Label a 96-well plate “GEL 1”, then place it on a chilled cooling block.

   Note: This plate is called the **Gel 1 Plate**.

2. To the **Gel 1 Plate**, label the AT wells with a blue marker and the GC wells with a red marker as follows:
   - 7-sample protocol: Label wells A1—A7 as AT (Blue) and wells C1—C7 as GC (Red).
   - 9-sample protocol: Label wells A1—A9 as AT (Blue) and wells C1—C9 as GC (Red).
   - 13-sample protocol: Label wells A1—A12, B1 as AT (Blue) and wells C1—C12, D1 as GC (Red).
   - 25-sample protocol: Label wells A1—A12, E1—E12, F1 as AT (Blue) and wells C1—C12, G1—G12, H1 as GC (Red).
3. Add 16.0 µL of 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer to all the labeled wells of the **Gel 1 Plate**.

4. Remove the unopened seal from the **1st PCR Plate**.
5. Confirm the **1st PCR Plate** and the **Gel 1 Plate** are in the same orientation with well A1 at the top left.
6. Use a multichannel P20 pipette to transfer 4.0 µL of the 1st PCR product in the **1st PCR Plate** to the corresponding well of the **Gel 1 Plate**.
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 4.0 µL of the Negative Control 1st PCR product from the **1st PCR Plate** to the corresponding wells of the **Gel 1 Plate**. Pipet up and down 3 times to rinse the tip.

Note: The final volume in each well is 20.0 µL.

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7. Tightly seal the **1st PCR Plate** with a new seal, then place it on a 96-well plate storage rack. Store the plate at –25°C to –15°C in the Post-PCR Room until the end of the assay. It can then be discarded.
8. Tightly seal the **Gel 1 Plate** with a new seal, then vortex the plate for 1 second in all corners and center.
9. Transfer to the plate centrifuge, then centrifuge at 2,400 rpm for 1 minute.
10. Place the **Gel 1 Plate** on a 96-well storage rack on the benchtop.

Run the First QC Gel, capture, and inspect the image

Run the First QC Gel

1. Power on the E-Gel™ Power Snap Plus Electrophoresis Device.
2. Gently remove the combs and place the gel into the E-Gel™ Adaptor.
3. Starting from the right edge, insert the gel cassette and adaptor into the E-Gel™ Power Snap Plus Electrophoresis Device. Press down on the left side of the cassette to secure it into the device.
4. Remove the seal from the **Gel 1 Plate**, then discard the seal.
5. Load 20.0 µL of each sample from the **Gel 1 Plate** into the gel wells.
6. Load 15.0 µL of the 1:6 diluted 25-bp DNA Ladder into the marker wells.
7. Load 15.0 µL of the 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer into any remaining empty wells.
8. Select the number of wells by selecting **Set up run** ▸ **Category** ▸ **48 wells**.
9. Select **Type** ▸ **E-Gel SYBR 4%**.

10. Select **Duration** and adjust the time using the **+/–** buttons.

    Note: It is suggested to start with 15 minutes. Adjust time as needed to adequately discern a band at 120 bp. DNA separation can be viewed in real time by using the E-Gel™ Power Snap Plus Camera.

11. Select **Start run** to begin running the gel.

12. Discard the **Gel 1 Plate**.

Capture the image

The gel run stops and beeps after the programmed time has elapsed.

1. On the E-Gel™ Power Snap Plus Electrophoresis Device, select **Done** to end the protocol.

2. Attach the E-Gel™ Power Snap Plus Camera.

3. Select **View Gel** to access the view gel screen and visualize the bands on the gel. Adjust the exposure setting if necessary.

4. Select **Capture** to access the capture screen and save an image to the camera. Adjust the capture settings if necessary.

Inspect the image

1. Select **Gallery** to view the image.

2. Inspect the image and ensure that:
   a. The 1st PCR product sample wells have a single band at approximately 120 bp. This indicates that the steps leading up to and including the 1st PCR reaction were successful. (Figure 20.)

b. The Negative Control does not have a smear. It will have a band for the primer dimer. This indicates that no sample or cross contamination has occured. (Figure 20.)

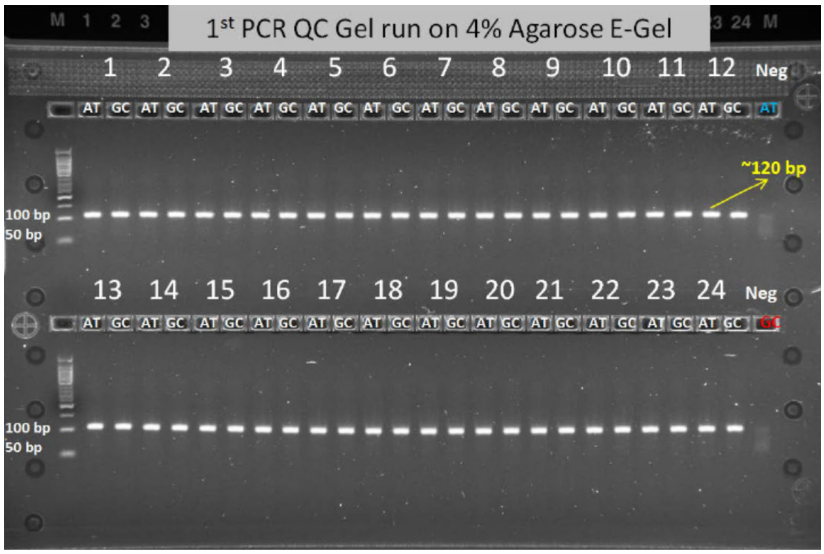


Figure 20 Example of a successful First QC Gel.

Examples of poor-quality gel images

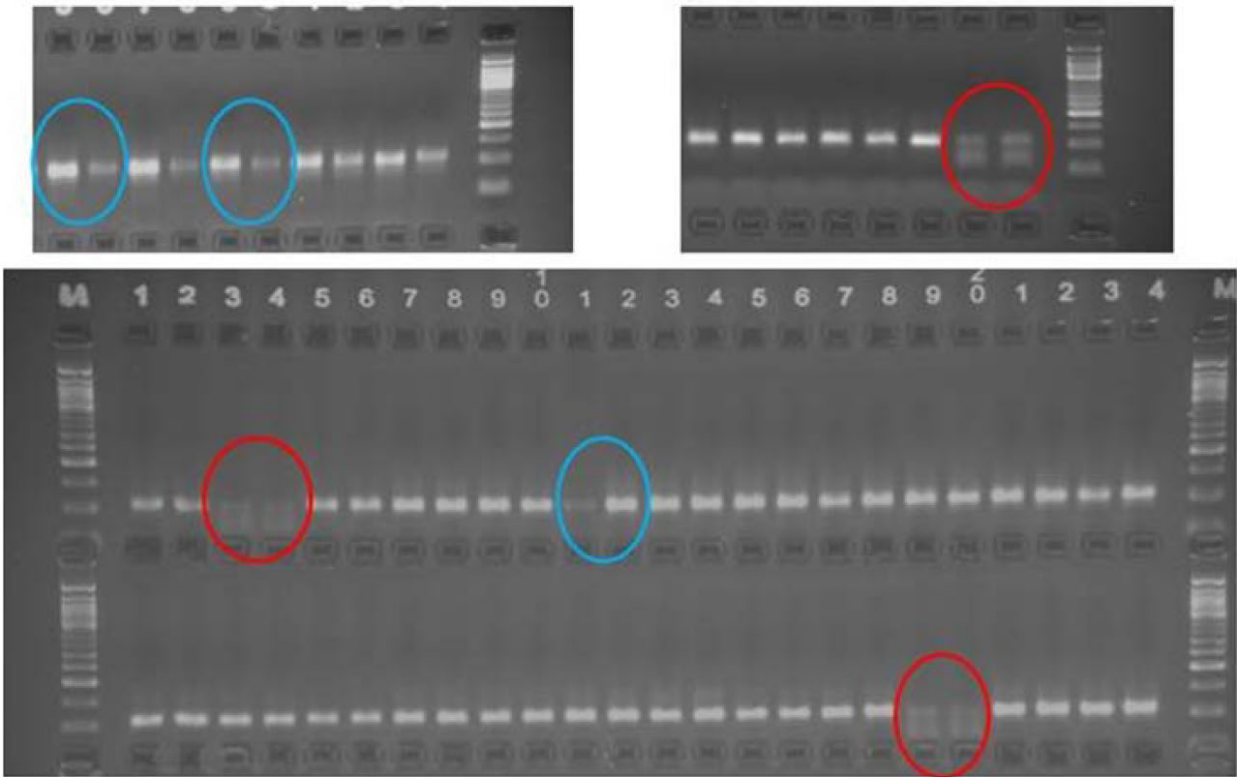


Figure 21 An example of faint or no PCR product visible on the First QC Gel image.

IMPORTANT! Possible issues with the First QC Gel image are:

Blue circles
Gel pattern: Faint or no PCR product visible on the First QC Gel image in the AT or GC channel for a given sample. (Figure 21, blue circles.)

- Possible causes: Gel loading error or mis-pipetting.
- Solution: Eliminate the gel loading error by running the First QC Gel again. If the repeat gel shows the same faint/no PCR band in a given channel, there was likely a mis-pipetting step after the channel split.
- Repeat any samples with this issue from the beginning of the assay. **Do not** proceed with the assay.

Red circles
Gel pattern: Faint or no PCR product visible on the First QC Gel in both the AT and GC channels for a given sample. (Figure 21, red circles.)

- Possible cause: Poor gDNA quality, inaccurate gDNA quantitation, or mis-pipetting the gDNA or MIP probe during the Anneal step.
- Solution: Confirm the quantification of the gDNA sample by using the recommended PicoGreen™ protocol. Ensure the input into the assay was 80 ng of starting material.

  If the input was correct, the issue is likely mis-pipetting during the Anneal step by not adding the input gDNA or the probe mix.
- Repeat any samples with this issue from the beginning of the assay. **Do not** proceed with the assay.

First QC Gel checkpoint

Do one of the following:

- PROCEED:

  If the First QC Gel image matches Figure 20, proceed to the following step. (“Complete the OncoScan 2nd PCR thermal cycler protocol” on page 77.)
- STOP:

  If the First QC Gel image does not match Figure 20, or looks like Figure 21, **STOP!** See Appendix F, “Troubleshooting the OncoScan™ CNV Plus Assay”.

Complete the OncoScan 2nd PCR thermal cycler protocol

1. When the OncoScan 2nd PCR thermal cycler is complete and ramps down to 4°C, do the following:
   a. Remove the **2nd PCR Plate** from the thermal cycler, then place it on a chilled cooling block for 1 minute.
   b. After 1 minute, ensure that the plate is tightly sealed, then vortex the plate for 1 second in all corners and center.
   c. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
   d. Place the **2nd PCR Plate** back on the chilled cooling block.
2. Stop the OncoScan 2nd PCR protocol on the thermal cycler.

IMPORTANT! PROCEED: Immediately proceed to the next stage. (“Stage 4: HaeIII Digest and Second QC Gel” on page 78.)

Stage 4: HaeIII Digest and Second QC Gel

About Stage 4

This stage is performed in the Post-PCR Room. During this stage, the HaeIII digestion reaction is setup using 2nd PCR product from the **2nd PCR Plate**. The OncoScan HaeIII thermal cycler protocol is started.

While the OncoScan HaeIII thermal cycler protocol is running, an aliquot is taken. This aliquot is used for the Second QC Gel which is run to check that the HaeIII digestion occurred, and the correct size DNA fragments were generated.

When the OncoScan HaeIII thermal cycler protocol is complete, and the Second QC Gel has been assessed, the assay can be stopped or continued to Stage 5: Hybridization.

Reagents required

Table 16 Reagents required for Stage 4.

Quantity	Reagent and cap color	Source	
From the OncoScan™ CNV Plus Reagent Kit for Research		**Module**	**Part No.**
1	Buffer B	OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270)	902261
1	HaeIII Enzyme (10 U/µL)		902262
1	Exo I Enzyme (20 U/µL)		902263
QC Gel Materials			**Cat. No.**
1	Invitrogen™ E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4%		G820804
1	1:6 diluted 25-bp DNA Ladder	Previously prepared	
1	1:100 diluted TrackIt™ Cyan/Orange Loading Buffer	Previously prepared	
Other			
1	**2nd PCR Plate**	From Stage 3	

Set up the work area in the Post-PCR Room for Stage 4

1. Refresh the ice bucket with ice, then place 3 cooling blocks on the ice.
2. Thaw the Buffer B at room temperature, then immediately place it on ice.

Prepare and add the HaeIII Master Mix

1. Label a 96-well plate “HAE”, then place it on a chilled cooling block.

   Note: This plate is called the **HAE Plate**.

2. Confirm the **2nd PCR Plate** and the **HAE Plate** are in the same orientation with well A1 at the top left.

3. To the **HAE Plate**, label the AT wells with a blue marker and the GC wells with a red marker as follows:
   - 7-sample protocol: Label wells A1—A7 as AT (Blue) and wells C1—C7 as GC (Red).
   - 9-sample protocol: Label wells A1—A9 as AT (Blue) and wells C1—C9 as GC (Red).
   - 13-sample protocol: Label wells A1—A12, B1 as AT (Blue) and wells C1—C12, D1 as GC (Red).
   - 25-sample protocol: Label wells A1—A12, E1—E12, F1 as AT (Blue) and wells C1—C12, G1—G12, H1 as GC (Red).

4. Cover the **HAE Plate** with an unopened seal to prevent contamination.

Prepare the HaeIII Master Mix

1. Label a 1.5-mL microcentrifuge tube “HAE”, then place it on ice.

2. Label a strip tube “HAE”, then place it on a chilled cooling block. (See Table 17 for the correct strip tube.)

3. Remove the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) from the freezer, then immediately place them in a –25°C to –15°C cooler.

4. Prepare the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL).
   a. Centrifuge for 1 second. Vortex for 1 second, then centrifuge for 3 seconds.

   b. Return the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) to the cooler.

5. Prepare the Buffer B.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.

   b. Place on ice.

6. Prepare the HaeIII Master Mix. Use the appropriate column in the following table. (Table 17.)

Table 17 HaeIII Master Mix.

Reagent and cap color	1 reaction	14 reactions (~20% overage)	18 reactions (~20% overage)	26 reactions (~20% overage)	50 reactions (~20% overage)
Buffer B	19.10 µL	321.0 µL	413.0 µL	596.0 µL	1,146.0 µL
HaeIII Enzyme (10 U/µL)	0.40 µL	6.7 µL	8.6 µL	12.5 µL	24.0 µL
Exo I Enzyme (20 U/µL)	0.50 µL	8.4 µL	10.8 µL	15.6 µL	30.0 µL
Total volume	**20.0 µL**	**336.1 µL**	**432.4 µL**	**624.1 µL**	**1,200.0 µL**
Strip tube wells		**7**	**9**	**12**	**12**
Volume per strip tube well		45.0 µL	45.0 µL	45.0 µL	96.0 µL

7. Add the Buffer B, the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) to the 1.5-mL microcentrifuge tube labeled “HAE”.
   a. Pipet the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) up and down 3 times to rinse the tips.
   b. Between each addition, change tips.
   c. Between each addition, vortex the master mix 3 times for 1 second each, then centrifuge 3 seconds.
   d. Place on ice.
8. Return the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) to the cooler.
9. Complete the HaeIII Master Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.
   b. Place on ice.
10. Aliquot the appropriate volume of HaeIII Master Mix into the chilled strip tube, “HAE”:
    - 7-sample protocol: Add 45.0 µL into each well of a 7-well strip tube.
    - 9-sample protocol: Add 45.0 µL into each well of a 9-well strip tube.
    - 13-sample protocol: Add 45.0 µL into each well of a 12-well strip tube.
    - 25-sample protocol: Add 96.0 µL into each well of a 12-well strip tube.
11. Check that there are no bubbles in the bottom of the strip tube. If bubbles are present, seal, then centrifuge for 3 seconds.
12. Place the strip tube on a chilled cooling block until the HaeIII Master Mix addition step.
13. Place the HaeIII Master Mix tube, “HAE”, on ice. Do not discard.

Add the HaeIII Master Mix to the HAE Plate

Use a multichannel P200 pipette to add 20.0 µL of the HaeIII Master Mix to each empty sample well of the **HAE Plate**. (Figure 22.)

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 20.0 µL of the HaeIII Master Mix from the "HAE" microcentrifuge tube to the Negative Control wells. (Figure 22.)

Note: The current volume in each well is 20.0 µL.

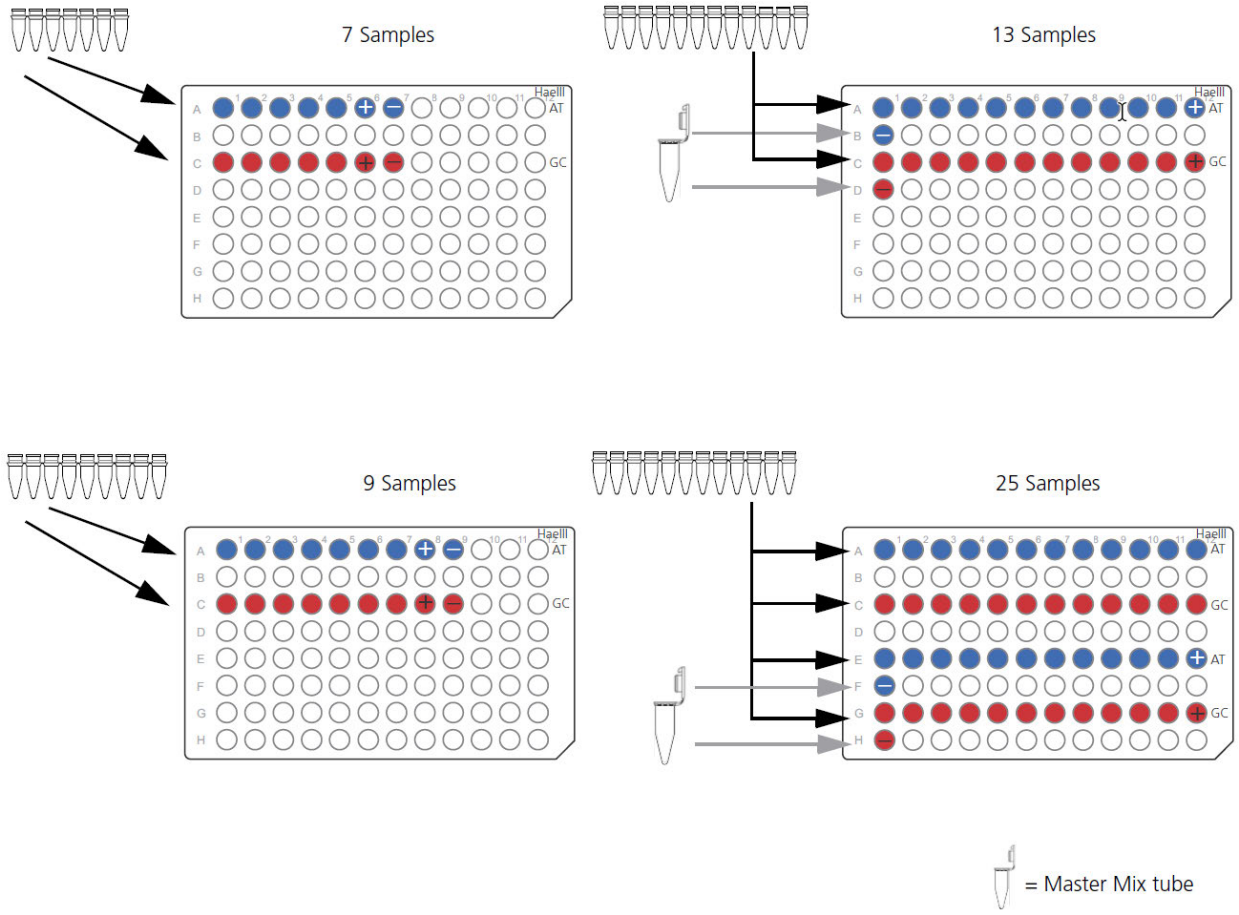


Figure 22 Adding the HaeIII Master Mix to the empty HAE Plate.

Add the 2nd PCR product to the HAE Plate

1. Confirm the **2nd PCR Plate** and the **HAE Plate** are in the same orientation with well A1 at the top left.
2. Remove the seal from the **2nd PCR Plate**, then discard the seal.
3. Remove the unopened seal from the **HAE Plate** and set it aside.
4. Use a multichannel P20 pipette to transfer 10.0 µL of the 2nd PCR product in the **2nd PCR Plate** to the corresponding well of the **HAE Plate**. (Figure 23.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 10.0 µL of the Negative Control 2nd PCR product from the **2nd PCR Plate** to the corresponding wells of the **HAE Plate**. Pipet up and down 3 times to rinse the tip. (Figure 23.)

Note: The final volume in each well is 30.0 µL.

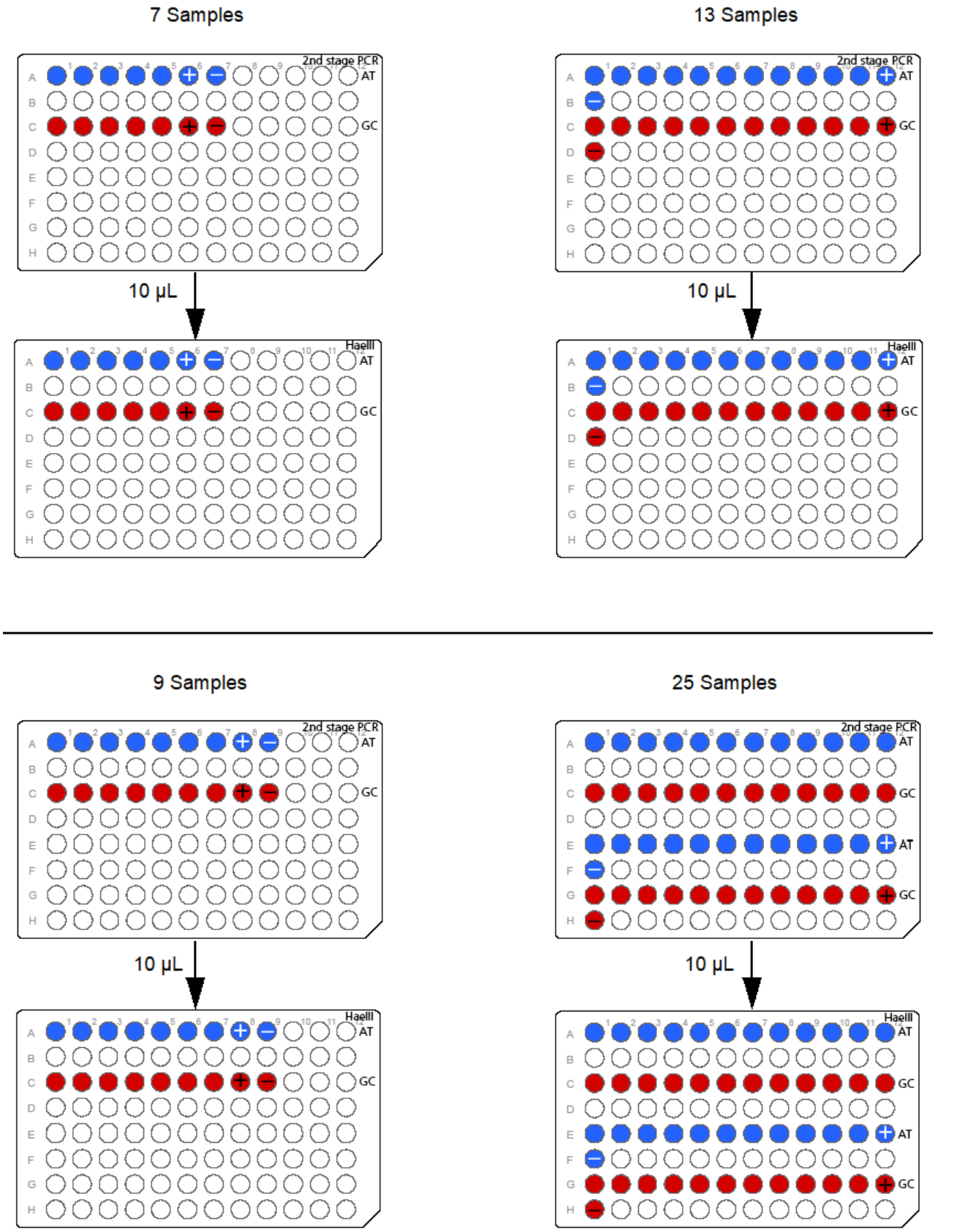


Figure 23 Adding the 2nd PCR product to the HAE Plate.

5. Tightly seal the **HAE Plate** with a new seal, then vortex the plate for 1 second in all corners and center.

6. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.

7. Place the **HAE Plate** back on the chilled cooling block.

Start the OncoScan HaeIII thermal cycler protocol

1. Load the **HAE Plate** into the thermal cycler.

2. Start the OncoScan HaeIII thermal cycler protocol. (Figure 24.)

Note: Ensure that the thermal cycler protocol is running.

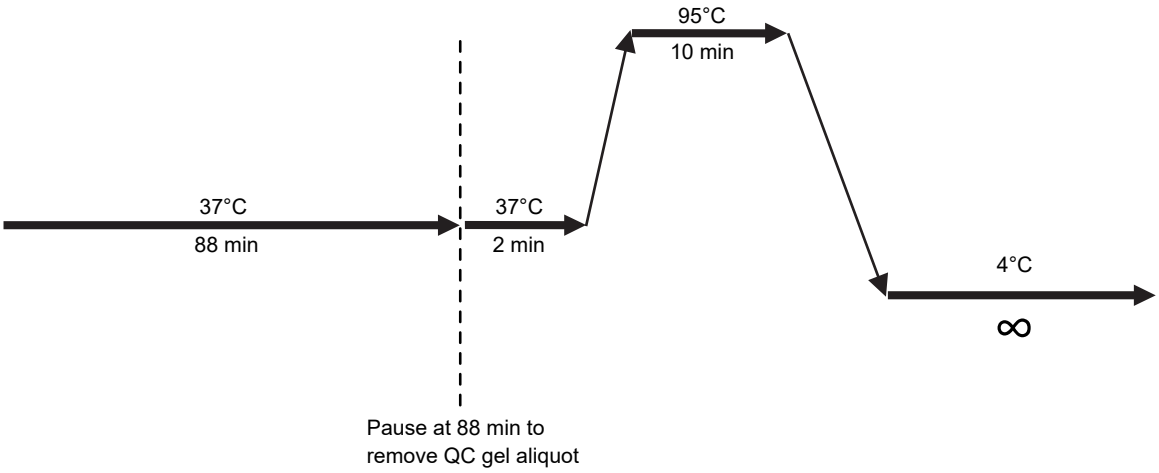


Figure 24 OncoScan HaeIII thermal cycler protocol.

3. Start a timer for 85 minutes.

IMPORTANT! After 85 minutes, return to the thermal cycler to pause the protocol and remove an aliquot for the Second QC Gel. (Figure 24.) The OncoScan HaeIII thermal cycler protocol must be paused and the aliquot taken before the thermal cycler block temperature ramps to 95°C.

Setting the timer for 85 minutes allows enough time to return to the thermal cycler and pause the protocol at 37°C at 88 minutes (2 minutes before the protocol reaches 95°C).

4. Discard the HaeIII Master Mix microcentrifuge tube and strip tube.

5. Return the HaeIII Enzyme (10 U/µL) and the Exo I Enzyme (20 U/µL) to the –25°C to –15°C storage.

6. Tightly seal the **2nd PCR Plate** with a new seal, then place it on a 96-well plate storage rack. Store the plate at –25°C to –15°C until the end of the assay. It can then be discarded.

Setup the area for the Second QC Gel

Set up the work area in the Post-PCR Room

1. Assemble the E-Gel™ Power Snap Plus Electrophoresis System.
2. Place the following items on the benchtop:
   - E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4%
   - 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer (previously prepared)
   - 1:6 diluted 25-bp DNA Ladder (previously prepared)

Note: The OncoScan™ CNV Plus Assay protocol is optimized to be run on E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4% using the Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System.

Prepare the Gel 2 Plate

1. Label a 96-well plate “GEL 2”, then place it on a chilled cooling block.

Note: This plate is called the **Gel 2 Plate**.

2. To the **Gel 2 Plate**, label the AT wells with a blue marker and the GC wells with a red marker as follows:
   - 7-sample protocol: Label wells A1—A7 as AT (Blue) and wells C1—C7 as GC (Red).
   - 9-sample protocol: Label wells A1—A9 as AT (Blue) and wells C1—C9 as GC (Red).
   - 13-sample protocol: Label wells A1—A12, B1 as AT (Blue) and wells C1—C12, D1 as GC (Red).
   - 25-sample protocol: Label wells A1—A12, E1—E12, F1 as AT (Blue) and wells C1—C12, G1—G12, H1 as GC (Red).
3. Add 16.0 µL of 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer to all the labeled wells of the **Gel 2 Plate**.
4. When the timer reaches 85 minutes, return to the thermal cycler.

Remove an aliquot of the HaeIII product

1. When the protocol reaches 88 minutes, and the thermal cycler block temperature is at 37°C, pause the OncoScan HaeIII thermal cycler protocol. (Figure 24.)

Note: Ensure that the thermal cycler protocol is paused.

2. Remove the **HAE Plate** from the thermal cycler, then place it on a chilled cooling block for 1 minute.
3. After 1 minute, ensure that the plate is tightly sealed.
4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge for 2,400 rpm for 30 seconds.

5. Place the **HAE Plate** back on the chilled cooling block.
6. Confirm the **HAE Plate** and the **Gel 2 Plate** are in the same orientation with well A1 at the top left.
7. Remove seal from the **HAE Plate**, then discard the seal.
8. Use a multichannel P20 pipette to transfer 4.0 µL of the HaeIII product in the **HAE Plate** to the corresponding well of the **Gel 2 Plate**.
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: For the 13- or 25-sample protocol, use a single channel pipette to add 4.0 µL of the Negative Control HaeIII product from the **HAE Plate** to the corresponding wells of the **Gel 2 Plate**. Pipet up and down 3 times to rinse the tips.

Note: The final volume in each well is 20.0 µL.

9. Tightly seal the **HAE Plate** with a new seal.

Resume the OncoScan HaeIII thermal cycler protocol

1. Load the **HAE Plate** into the thermal cycler, then resume the OncoScan HaeIII thermal cycler protocol.

Note: Ensure that the thermal cycler protocol has resumed and is running.

2. Tightly seal the **Gel 2 Plate** with a new seal, then vortex the plate for 1 second in all corners and center.
3. Transfer to the plate centrifuge, then centrifuge at 2,400 rpm for 1 minute.
4. Place the **Gel 2 Plate** on a 96-well plate storage rack on the benchtop.

Run the Second QC Gel, capture, and inspect the image

Run the Second QC Gel

1. Power on the E-Gel™ Power Snap Plus Electrophoresis Device.
2. Gently remove the combs and place the gel into the E-Gel™ Adaptor.
3. Starting from the right edge, insert the gel cassette and adaptor into the E-Gel™ Power Snap Plus Electrophoresis Device. Press down on the left side of the cassette to secure it into the device.
4. Remove the seal from the **Gel 2 Plate**, then discard the seal.
5. Load 20.0 µL of each sample from the **Gel 2 Plate** into the gel wells.
6. Load 15.0 µL of the 1:6 diluted 25-bp DNA Ladder into the marker wells.

7. Load 15.0 µL of the 1:100 diluted TrackIt™ Cyan/Orange Loading Buffer into any remaining empty wells.
8. Select the number of wells by selecting **Set up run** ▸ **Category** ▸ **48 wells**.
9. Select **Type** ▸ **E-Gel SYBR 4%**.
10. Select **Duration** and adjust the time using the **+/–** buttons.

    Note: It is suggested to start with 15 minutes. Adjust time as needed to adequately discern a band at 120 bp. DNA separation can be viewed in real time by using the E-Gel™ Power Snap Plus Camera.

11. Select **Start run** to begin running the gel.
12. Discard the **Gel 2 Plate**.

    Note: Any remaining 1:6 25-bp DNA Ladder can be stored at at 2—8°C for up to 1 month.

Capture the image

The gel run stops and beeps after the programmed time has elapsed.

1. On the E-Gel™ Power Snap Plus Electrophoresis Device, select **Done** to end the protocol.
2. Attach the E-Gel™ Power Snap Plus Camera.
3. Select **View Gel** to access the view gel screen and visualize the bands on the gel. Adjust the exposure setting if necessary.
4. Select **Capture** to access the capture screen and save an image to the camera. Adjust the capture settings if necessary.

Inspect the image

1. Select **Gallery** to view the image.
2. Inspect the image and ensure that:
   a. The HAEIII product sample wells have a predominant pattern of double bands at 40 bp and 70 bp. These double bands indicate that the 2nd PCR reaction and the HaeIII digestion were successful. (Figure 25.)

b. The Negative Control wells have a broad smear from approximately 50 bp to 100 bp. This indicates that no sample or cross contamination has occurred.

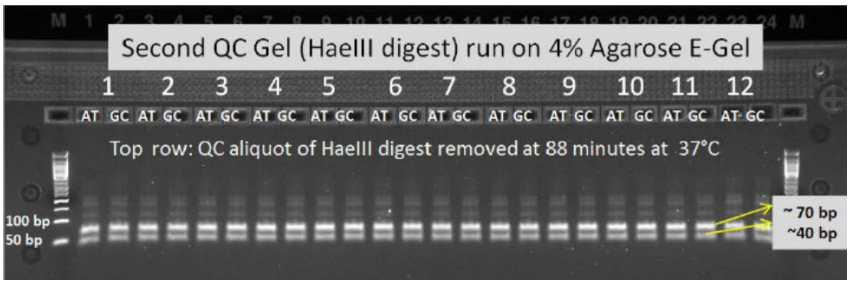


Figure 25 Example of a successful Second QC Gel.

c. If the HaeIII product aliquot was removed after the thermal cycler block reached 95°C, the sample wells will not have a distinct double band pattern at 40 bp and 70 bp. (Figure 26.)

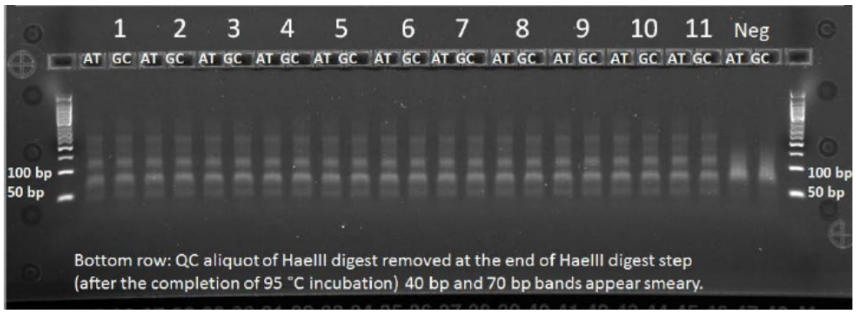


Figure 26 Example of HaeIII aliquot taken after the thermal cycler block ramped to 95°C.

IMPORTANT! Possible issues with the Second QC Gel image are:

Gel Pattern: No sample wells show a double band pattern at 40 bp and 70 bp. All samples show a single band at 120 bp.

- Possible cause: The HaeIII digestion failed.
- Solution: Do not proceed with the assay. Repeat the HaeIII digestion using the **2nd PCR Plate**. Start at "Stage 4: HaeIII Digest and Second QC Gel" on page 78.

Gel Pattern: All samples show a smear around 50—100 bp. No distinct double band pattern at 40 bp and 70 bp is observed. The image looks like Figure 26.

- Possible cause: The HaeIII product aliquot was taken after the thermal cycler ramped to 95°C.
- Solution: If the issue is due to the aliquot taken at the incorrect time, proceed with the assay.

Second QC Gel checkpoint

Do one of the following:

- PROCEED:

  If the Second QC Gel image matches Figure 25, proceed to the following step. (“Complete the OncoScan HAEIII thermal cycler protocol” on page 89).

- STOP:

  If the Second QC Gel image does not match Figure 25 and has a distinct band at 120 bp, **STOP!** Do not proceed to “Stage 5: Hybridization”. See Appendix F, “Troubleshooting the OncoScan™ CNV Plus Assay”.

Complete the OncoScan HAEIII thermal cycler protocol

1. When the OncoScan HAEIII thermal cycler protocol is complete and ramps down to 4°C, do the following:
   a. Remove the **HAE Plate** from the thermal cycler, then place it on a chilled cooling block for 1 minute.
   b. After 1 minute, ensure that the plate is tightly sealed, then vortex the plate for 1 second in all corners and center.
   c. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
   d. Place the **HAE Plate** back on the chilled cooling block.
2. Stop the OncoScan HAEIII thermal cycler protocol.
3. Do one of the following:
   - PROCEED:

     If continuing the assay, proceed to Stage 5. (“Stage 5: Hybridization” on page 91.)
   - STOP:

     If stopping and not proceeding with the assay, perform the “Optional stopping point” on page 89. When resuming the assay, perform the step, “Resume the assay” on page 90.

Optional stopping point

IMPORTANT! Only perform the following steps if stopping the assay and storing the **HAE Plate** for future use.

1. Place the **HAE Plate** on a 96-well plate storage rack.
2. Store the plate at –25°C to –15°C in the Post-PCR Room.

   Note: The plate can be stored for up to 10 days.

Resume the assay

When resuming the assay, perform the following steps in the Post-PCR Room.

1. Fill the ice bucket with ice, then place a cooling block on the ice.
2. Thaw the **HAE Plate** at room temperature (≤30 minutes). Once thawed, immediately place it on the chilled cooling block.
3. Ensure that the plate is tightly sealed, then vortex the plate for 1 second in all corners and center.
4. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
5. Place the **HAE Plate** back on the chilled cooling block.

IMPORTANT! PROCEED: Immediately proceed to “Stage 5: Hybridization” on page 91.

Stage 5: Hybridization

About Stage 5

This stage is performed in the Post-PCR Room. During this stage, a hybridization cocktail is prepared for each reaction. The Hybridization Cocktail is denatured, then loaded into an OncoScan™ CNV Plus Array. Each sample will have 2 arrays, 1 for each channel. The arrays are placed into the hybridization oven, then left to hybridize for 16—18 hours.

When the array hybridization is complete, the assay is continued to Stage 6: Wash and stain the arrays.

Reagents required

Table 18 Reagents required for Stage 5.

Quantity	Reagent and cap color	Module	Part No.
From the OncoScan™ CNV Plus Reagent Kit for Research			
1	◯ Nuclease-Free Water	OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270)	902253
1	◯ Hybridization Mix		902264
Arrays			**Cat. No.**
As needed	OncoScan™ CNV Plus Array	Thermo Fisher Scientific	902292
Other			
1	**HAE Plate**	From Stage 4	

IMPORTANT! Before starting Stage 5: Hybridization:

- Ensure that each sample to be hybridized has passed the First and Second QC Gel criteria.
- Set the hybridization oven to 49°C and 60 rpm. Allow it to warm to the set temperature before use. An accurate hybridization oven temperature is critical to this assay.
- Prepare only enough Hybridization Master Mix for the number of arrays that will be hybridized. The **Hyb Plate** must be prepared fresh before hybridization.
- If samples are not going to be hybridized, do not add Hybridization Master Mix to them. Store them at –25° to –15°C by performing the optional stopping point. See “Optional stopping point” on page 89.
- The number of arrays that can be hybridized at one time is dependent on the number of fluidics stations available. Hybridizations may need to be staggered. If more than 2 batches of arrays will be processed through the fluidics stations, it is recommended to stagger the hybridization start of the arrays by 2 hours. This staggered start prevents the arrays in the third or fourth batch from hybridizing for more than 18 hours.

Preheat the hybridization oven

1. Power on the GeneChip™ Hybridization Oven 645, then set it to 49°C and 60 rpm.
2. Place the oven trays in the hybridization oven.
3. Allow the oven to operate for 30 minutes before arrays are loaded into the oven.

Prepare the arrays

1. Remove 2 arrays for each sample from 4°C storage.

   Note: The Negative Control will not be hybridized.

   - 7-sample protocol: 12 arrays
   - 9-sample protocol: 16 arrays
   - 13-sample protocol: 24 arrays
   - 25-sample protocol: 48 arrays

2. Place the arrays on the benchtop, then allow 15 minutes to equilibrate to room temperature.

Set up the work area in the Post-PCR Room for Stage 5

1. Power on the thermal cycler to preheat the lid.
2. Refresh the ice bucket with ice, then place 3 cooling blocks on the ice.
3. Thaw the following at room temperature, then immediately place them on ice:
   - Nuclease-Free Water
   - Hybridization Mix

Label the arrays

1. Remove the arrays from their pouches, then place them on a clean benchtop close to the thermal cycler.
2. Label the front and back of each array with a designation that identifies which sample will be hybridized to the array.
   a. For example, based on the number of samples to be hybridized, label the front side of the array 1A to 24A (for the AT channel) and 1C to 24C (for the GC channel).

   b. Flip the arrays face down and label the back side of the array with the same designation.

3. Pair the arrays associated with a single sample together. (Figure 27.)

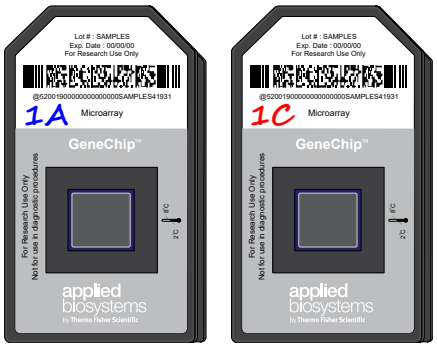


Figure 27 Arrays for sample 1 are paired together. The A (AT channel) is on the left and the C (GC) channel is on the right.

Register the arrays

Each array must be registered using the Applied Biosystems™ GeneChip™ Data Collection Software (GCDC). During registration, the sample and channel (AT or GC) is associated with the array barcode. See Appendix A, “Register samples in GeneChip™ Data Collection Software”.

Prepare the Hyb Plate

1. Label a 96-well plate “HYB”, then place it on a chilled cooling block.

   Note: This plate is called the **Hyb Plate**.

2. To the **Hyb Plate**, label the AT wells with a blue marker and the GC wells with a red marker as follows:
   - 7-sample protocol: Label wells A1—A6 as AT (Blue) and wells C1—C6 as GC (Red).
   - 9-sample protocol: Label wells A1—A8 as AT (Blue) and wells C1—C8 as GC (Red).
   - 13-sample protocol: Label wells A1—A12 as AT (Blue) and wells C1—C12 as GC (Red).
   - 25-sample protocol: Label wells A1—A12, E1—E12 as AT (Blue) and wells C1—C12, G1—G12 as GC (Red).

3. Cover the **Hyb Plate** with an unopened seal to prevent contamination.

Prepare the Hybridization Master Mix

1. Label a 15-mL conical centrifuge tube “HYB”, then place it on ice.

2. Prepare the Hybridization Mix.
   a. Vortex 3 times for 1 second each, then centrifuge for 3 seconds.

   b. Place on ice.

3. Prepare the Hybridization Master Mix. Use the appropriate column in the following table. (Table 19.)

Table 19 Hybridization Master Mix.

Number of samples (includes Positive Control)		6	8	12	24
Reagent and cap color	**1 array**	**12 arrays**	**16 arrays**	**24 arrays**	**48 arrays**
◯ Nuclease-Free Water	30.0 µL	0.504 mL	0.648 mL	0.936 mL	1.80 mL
◯ Hybridization Mix	118.0 µL	1.982 mL	2.549 mL	3.681 mL	7.08 mL
Total volume	**148.0 µL**	**2.486 mL**	**3.197 mL**	**4.617 mL**	**8.88 mL**

4. Add the Nuclease-Free Water and the Hybridization Mix, to the 15-mL conical centrifuge tube labeled “HYB”.

5. Complete the Hybridization Master Mix.
   a. Vortex continuously for 3 seconds.
   b. Repeat the vortex step 2 more times to ensure that the Hybridization Master Mix is homogenous.
   c. Place on ice.

Add the Hybridization Master Mix to the Hyb Plate

Note: Depending on the number of samples, add the Hybridization Master directly to the samples or use a reagent reservoir.

1. If performing the 7- or 9-sample protocol:
   a. Use a single channel P200 to add 148.0 µL of the Hybridization Master Mix to each empty sample well of the **Hyb Plate**. (Figure 28.)
   b. Change tips between additions.

2. If performing the 13- or 25-sample protocol:
   a. Place a reagent reservoir on top of a chilled cooling block.
   b. Pour the Hybridization Master Mix into the reagent reservoir.
   c. Use a multichannel P200 pipette to add 148.0 µL of the Hybridization Master Mix to each empty sample well of the **Hyb Plate**. (Figure 28.)
   d. Change tips between each row.

Note: The current volume in each well is 148.0 µL.

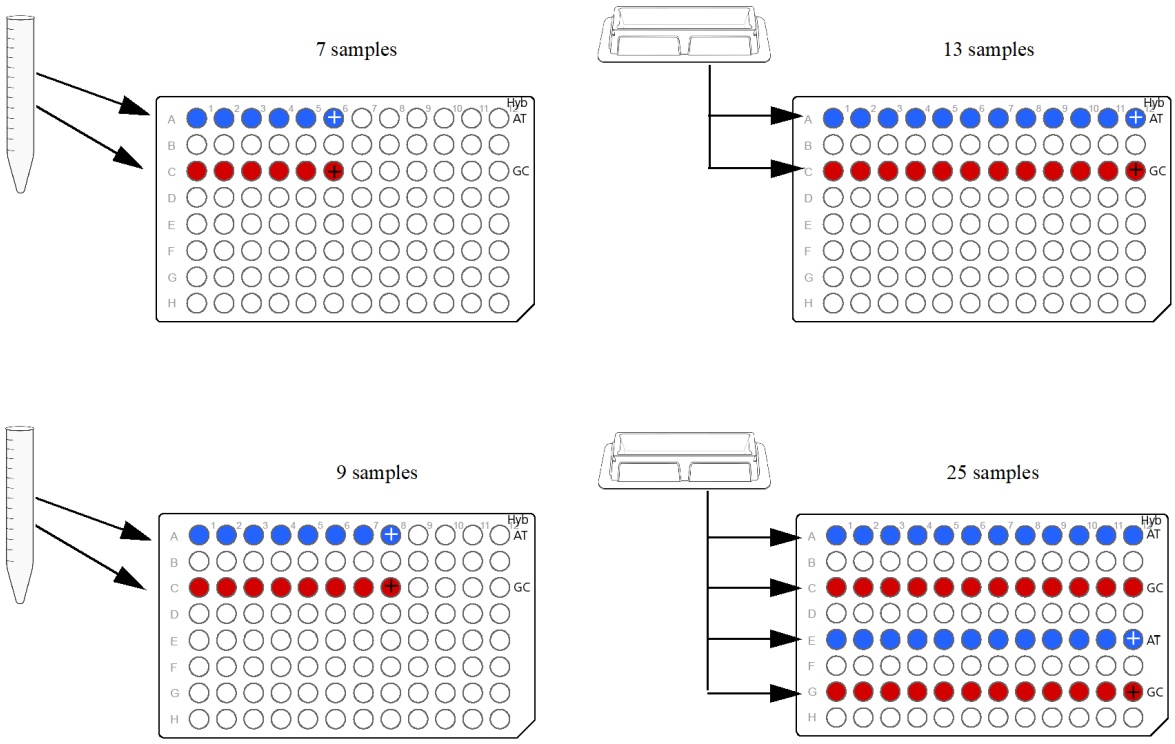


Figure 28 Adding the Hybridization Master Mix to the empty Hyb Plate.

Add the HaeIII product to the Hyb Plate

1. Confirm the **HAE Plate** and the **Hyb Plate** are in the same orientation with well A1 at the top left.
2. Remove the seal from the **HAE Plate**, then discard the seal.
3. Remove the unopened seal from the **Hyb Plate** and set it aside.
4. Use a multichannel P200 pipette to transfer 22.0 µL of the HaeIII product in the **HAE Plate** to the corresponding well of the **Hyb Plate**. (Figure 29.)
   a. Pipet up and down 3 times to rinse the tips.
   b. Change tips between rows.

Note: The final volume in each well is 170.0 µL.

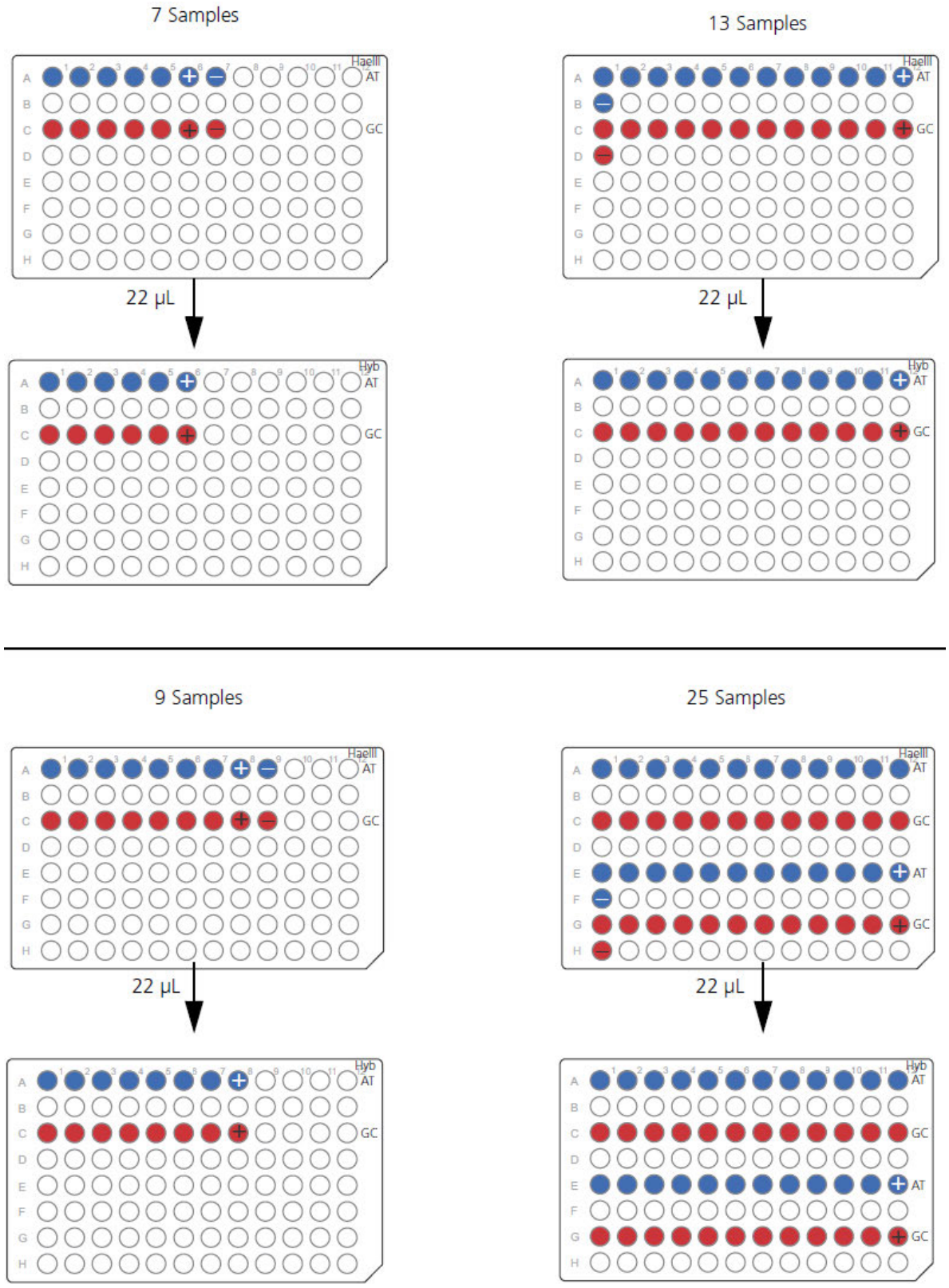


Figure 29 Add the HaeIII product to the Hyb Plate.

5. Tightly seal the **Hyb Plate** with a new seal, then vortex the plate for 1 second in all corners and center.
6. *Repeat* the vortex for 1 second in all corners and center.
7. Transfer to the plate centrifuge on the chilled cooling block, then centrifuge at 2,400 rpm for 1 minute.
8. Place the **Hyb Plate** back on the chilled cooling block.
9. Do one of the following:
   - If all samples in the **HAE Plate** have been added to the **Hyb Plate**, discard the **HAE Plate**.
   - If there are samples that have not been added to the **Hyb Plate**, and will not be hybridized at this step, tightly seal the **HAE Plate** with a new seal. Return to "Stage 4: HaeIII Digest and Second QC Gel", and perform the "Optional stopping point" on page 89.

Start the OncoScan Hyb thermal cycler protocol

1. Load the **Hyb Plate** into the thermal cycler.
2. Start the OncoScan Hybridization thermal cycler protocol. (Figure 30.)

Note: Ensure that the thermal cycler protocol is running.

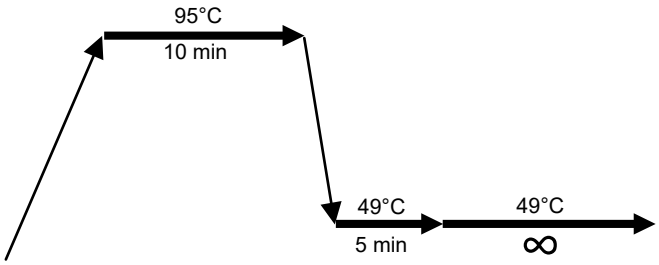


Figure 30 OncoScan Hybridization thermal cycler protocol.

Prepare the arrays

1. While the OncoScan Hybridization thermal cycler protocol is running, apply two ½" Tough-Spots™ label dots on the top edge of each array.
2. Vent the array by inserting a 200 µL pipette tip into the upper right septa of each array. (Figure 31.)

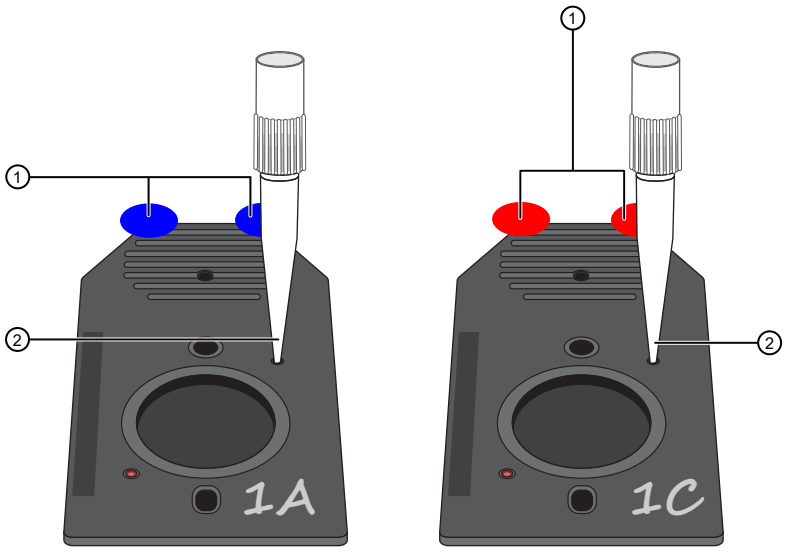


Figure 31 Prepare the arrays by applying Tough-Spots™ label dots and venting the array.

① Tough-Spots™ label dots to cover the septa.
② Pipette tip in septa to vent.

Inject the Hybridization Cocktail into the arrays

Precautions when loading the arrays

IMPORTANT! Follow these precautions when loading the Hybridization Cocktail into the arrays:

- The Hybridization Cocktail is viscous, pipet slowly.
- To prevent cross-contamination and evaporation, leave the remaining wells covered by the cut seal.
- Move quickly to inject the Hybridization Cocktail into an array and load the array into the hybridization oven.
- Inject no more than 4 arrays at a time, then load the arrays into the hybridization oven.
- Do not allow the sample injected arrays to be outside of the hybridization oven for more than 1 minute.
- Within 30 minutes, all samples should be injected into an array and all arrays should be loaded into the hybridization oven.

Inject Hybridization Cocktail into the arrays

1. When the OncoScan Hybridization thermal cycler protocol is complete and in the 49°C ∞ step, do the following:
   a. Open the thermal cycler lid.

b. Keep the **Hyb Plate** in the thermal cycler at 49°C.

c. Use a blade to cut the seal of the **Hyb Plate** between rows. Cut the seal to allow it to be peeled back from the plate.

2. Inject the Hybridization Cocktail into the array.
   a. Peel back the seal on the **Hyb Plate** to expose the wells.

   b. Use a P200 pipette to remove 160.0 µL of a Hybridization Cocktail from the **Hyb Plate**, then immediately inject it into the corresponding array.

   c. Discard the vent tip.

   d. Dry any fluid from around the septa, then cover both septa using the ½" Tough-Spots™ label dots.
      - To prevent evaporation and leakage during hybridization, firmly press the Tough-Spots™ label dots over the septa. The Tough-Spots™ label dots can slightly overlap the center area of the array.
      - Ensure that the label dots are flat. If not, remove the label dots and apply a new one.

3. For the next 3 arrays, repeat step 2.

4. When 4 arrays are complete, do the following:
   a. Leave the thermal cycler lid open.

   b. Cover the open wells of the **Hyb Plate** with the peeled-back seal.

   c. Remove a heated oven tray from the hybridization oven.

   d. Evenly space the 4 arrays in the oven tray.

   e. Immediately place the oven tray into the hybridization oven.

5. Repeat step 2—step 4 until all arrays are injected and loaded into the hybridization oven.

Start array hybridization in the oven

1. Allow the arrays to hybridize for 16—18 hours at 49°C and 60 rpm.

   IMPORTANT! The hybridization time, temperature, and rotational speed are optimized for this assay and must be stringently followed.

2. PROCEED: After 15.5 hours of hybridization, proceed to “Stage 6: Wash and stain the arrays” on page 101, then start the step, “Set up the work area in the Post-PCR Room for Stage 6” on page 101

Clean the work area

1. Discard the **Hyb Plate**, the Hybridization Master Mix conical centrifuge tube, and the reagent reservoir.
2. Return the Nuclease-Free Water and Hybridization Mix to the –25°C to –15°C storage.
3. Empty the ice bucket, then place the cooling blocks in the refrigerator.

Stage 6: Wash and stain the arrays

About Stage 6

This stage is performed in the Post-PCR Room. During this stage, the arrays are removed from the hybridization oven, then washed and stained on the fluidics station.

When the array wash and stain is complete, the assay is continued to Stage 7: Scan the arrays.

Reagents required

Table 20 Reagents required for Stage 6.

Quantity	Reagent and cap color	Module	Part No.
From the OncoScan™ CNV Plus Reagent Kit for Research			
1	Stain 1	OncoScan™ CNV Plus Stain Reagents (Part No. 902271)	902265
1	Stain 2		902266
1	Array Holding Buffer		901733
1	Wash A	Individual bottles	901680
1	Wash B		901681
Other			
As necessary	Deionized (DI) water	—	

IMPORTANT! The OncoScan™ CNV Plus Arrays are sensitive to residual SAPE-antibody complex present in the fluidics station tubing and needles. To obtain the best data, perform the fluidics station bleach protocol weekly. See “Fluidics station Bleach protocol” on page 159 .

Set up the work area in the Post-PCR Room for Stage 6

Note: After 15.5 hours of hybridization, begin preparing the stains and priming the fluidics station. Have everything prepared before removing arrays from the hybridization oven at 16—18 hours.

IMPORTANT! Arrays *should not* be in the hybridization oven longer than 18 hours.

1. Place full bottles of Wash A and Wash B on the fluidics station.
2. Fill the Water Bottle with deionized (DI) water, then place it on the fluidics station.
3. Empty the Waste Bottle, then place it on the fluidics station.

   IMPORTANT! Ensure that all the bottles are correctly positioned and the appropriate wash lines are placed in the correct bottle.

4. Place a tube storage rack on the benchtop.
5. For each array, place one of the following on the tube storage rack:
   - Amber colored 1.5-mL microcentrifuge tube.
   - Clear colored 1.5-mL microcentrifuge tube.
   - Blue colored 1.5-mL microcentrifuge tube.
6. Place the following on the benchtop:
   - Stain 1
   - Stain 2
   - Array Holding Buffer

Prepare the stains

1. Mix Stain 1, Stain 2, and Array Holding Buffer by gently inverting 10 times.
2. For each array, aliquot the following reagents into the appropriate-colored 1.5-mL microcentrifuge tube:
   a. Aliquot 500 µL Stain 1 into an amber-colored 1.5-mL microcentrifuge tube.

      Note: Stain 1 is light sensitive, use an amber colored tube.

   b. Aliquot 500 µL Stain 2 into a clear-colored 1.5-mL microcentrifuge tube.

   c. Aliquot 1,000 µL Array Holding Buffer into a blue-colored 1.5-mL microcentrifuge tube.

Prime the fluidics station

Note: The GeneChip™ Fluidics Station 450 is used to wash and stain the arrays. It is operated using the GCDC software. Priming the fluidics station helps ensure that the lines are filled with the appropriate buffers and the instrument is ready to run the fluidics station protocols. Priming should be performed:

- When the fluidics station is first powered on.
- When wash solutions are changed.
- The fluidics station is to be used again after a **Shutdown** protocol has been performed.
- When a fluidics station module LCD window indicates that the module is **Not Primed**.

1. Power on the fluidics station.
2. Place 3 empty 1.5-mL microcentrifuge tubes in the stain holders of each module that will be primed. Leave the needle lever in the raised position. Ensure that Wash A, Wash B, and the water bottles are filled and the corresponding tube from the fluidics is inserted into the appropriate bottle. Empty the waste bottle if needed.
3. Start the GCDC Launcher, then click the **Fluidics** button.

   The **Fluidics Control** window appears.

Figure 32 GCDC Fluidics Control window.

4. From the **Fluidics Control Master Control** pane, select the following:
   - **Probe Array Type**: (Leave empty)
   - **Protocol Type**: *Maintenance Protocols*
   - **Protocol**: *PRIME_450*
5. From the **Fluidics Control Station Control** pane, select the boxes of the fluidics station modules to prime.
6. Click the **Copy to Selected Modules** button. The selected protocol (PRIME_450) is applied to the selected stations and modules.
7. To initiate the **Prime** protocol, do one of the following:
   - Click the **Station Configure** button then click the **Start** button to start an individual module.
   - Click the **Run All** button to start all modules.
8. Monitor the prompts in the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window.
9. Lower the needle levers, then engage the Wash Blocks.
10. When the **Prime_450** protocol is complete, the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window display the message **REMOVE ALL VIALS**.
11. Raise the needle levers, then discard the used 1.5-mL microcentrifuge tubes.
    The **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window display the message **PRIME DONE, READY**.
12. Eject the Wash Block to avoid a sensor time out.

Start a fluidics run

1. Before beginning, ensure that:
   - Full bottles of Wash A and Wash B are on the fluidics station.
   - The Water bottle is full of deionized (DI) water and on the fluidics station.
   - The Waste bottle is empty and on the fluidics station.
   - The appropriate wash lines are placed in the correct bottle.
   - The fluidics station has been primed.
2. Start the GCDC Launcher, then click the **Fluidics** button.
   The **Fluidics Control** window appears.
3. From the **Fluidics Control Master Control** pane, select the following:
   - **Probe Array Type**: OncoScan.Universal
   - **Protocol Type**: Compatible Protocols
   - **GeneChip IVT Labeling Kit or GeneChip WHS Kit**: (Do not select)
   - **Protocol**: OncoScan
4. From the **Fluidics Control Station Control** pane, select the boxes of the primed fluidics station modules to wash and stain an array.
5. Click the **Copy to Selected Modules** button.
   The selected protocol (OncoScan) is applied to the selected stations and modules.
6. For each module, enter information for the array that will be washed and stained on the module. Do one of the following:
   - Select the **Sample File Name**.
   - Enter the **Array Barcode**.
7. To initiate the fluidics protocol, do one of the following:
   - Click the **Start** button to start an individual module.
   - Click the **Run All** button to start all modules.
8. Follow the instructions on the **Fluidics Control Fluidics Status** pane or the fluidics station module LCD window.
   a. Eject the Wash Block to avoid a sensor time out.
   b. Remove any previously loaded empty vials.
   c. When prompted to **Load vials 1-2-3**, load the stains in the stain holders of each module.
      - Load the amber-colored vial containing 500 µL Stain 1 in position 1.
      - Load the clear-colored vial containing 500 µL Stain 2 in position 2.
      - Load the blue-colored containing 1,000 µL Array Holding Buffer in position 3.

Remove arrays from the hybridization oven then load arrays in the fluidics station

IMPORTANT! After the arrays are removed from the hybridization oven, ***do not*** delay loading them into the fluidics station and starting the fluidics protocol. Delays will impact data quality.

1. After 16—18 hours of hybridization, do the following:
   a. Remove only the number of arrays that will be washed at this time from the hybridization oven. Leave the remaining arrays in the hybridization oven at 49°C until the fluidics station is ready for another run.
   b. Remove the Tough-Spots™ label dots from the arrays.
   c. *Immediately* insert the arrays into the designated module for that array on the fluidics station.
2. Engage the Wash Block of each module.
3. Lower the needle lever of each module to start the fluidics protocol. The Fluidics protocol will automatically start.

   Note: Sensors in the fluidics station detect when the array has been loaded in the Wash Block and the needles have been lowered into Vials 1–2–3. The fluidics protocol automatically proceeds from this point, and takes approximately 1.5 hours to complete.
   The **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window displays the progress of the fluidics protocol.
4. Repeat step 1—step 3 until all arrays are out of the oven and in the fluidics station.

Remove arrays from the fluidics station, check for bubbles, complete the fluidics run

1. When the fluidics protocol is complete:
   a. The **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window display the message **Eject & Inspect Cartridge**.
   b. Disengage the Wash Block of each module.
   c. Remove the arrays from the fluidics station.

      Note: After removing an array from the Wash Block, the fluidics station module LCD window displays the message **ENGAGE WASHBLOCK**. Do not perform this step until the array has been inspected for bubbles. When engaged, the instrument automatically performs a **Cleanout** procedure.
   d. Inspect each array window for bubbles.
2. If no bubbles are present, proceed to step 7.

3. If bubbles are present in the array window:
   a. Return the array to the fluidics station and engage the Wash Block.
   b. Follow the instructions on the fluidics station module LCD window to perform the debubbling procedure. Remove Vial 3, add additional Array Holding Buffer, then lower the needle levers to start the protocol.
4. When the debubbling procedure is complete, do the following:
   a. Disengage the Wash Block of the module.
   b. Remove the array from the fluidics station.
   c. Inspect the array window for bubbles.
5. If no bubbles are present, proceed to step 7.
6. If bubbles are still present after performing the debubbling procedure, manually remove and add Array Holding Buffer to the array.
   a. Insert a 200-µL pipette tip into the upper right septa.
   b. Set a P200 pipette to 200 µL. Remove half of the buffer in the array.
   c. Fill the array with Array Holding Buffer until there are no bubbles.
7. When all arrays have been removed from the fluidics station, follow the instructions on the **Fluidics Control Fluidics Status** pane or the fluidics station module LCD window.
   a. Engage the Wash Block.
   b. Remove the Vials 1-2-3, then discard the used tubes.
   c. When prompted, place 3 empty 1.5-mL microcentrifuge tubes in the stain holders of each module. Lower the needle levers. The **Cleanout** procedure automatically starts.

      Note: The **Cleanout** procedure takes approximately 10 minutes to complete. Once complete, the fluidics station is ready to perform a wash and stain procedure on another array. If not changing the buffer type, the fluidics station does not need to be primed again.

   d. The **REMOVE VIALS** message indicates the **Cleanout** procedure is complete.
8. If more arrays are in the hybridization oven, perform the following steps until all arrays are out of the oven and have been washed and stained.
   a. “Start a fluidics run” on page 104.
   b. “Remove arrays from the hybridization oven then load arrays in the fluidics station” on page 105.
   c. “Remove arrays from the fluidics station, check for bubbles, complete the fluidics run” on page 105.

Prepare the arrays for scanning

1. Prepare arrays for scanning.
   a. Clean the glass surface of the array with a non-abrasive tissue. Do not use alcohol to clean the glass surface.

   b. Dry any fluid from around the septa.

2. Cover both septa of the array with the 3/8" Tough-Spots™ label dots. (Figure 33.)
   - To prevent leakage, firmly press the Tough-Spots™ label dots over the septa. The the Tough-Spots™ label dots **must not** overlap the center area of the array.
   - Ensure that the label dots are flat. If not, remove the label dot and apply a new one.

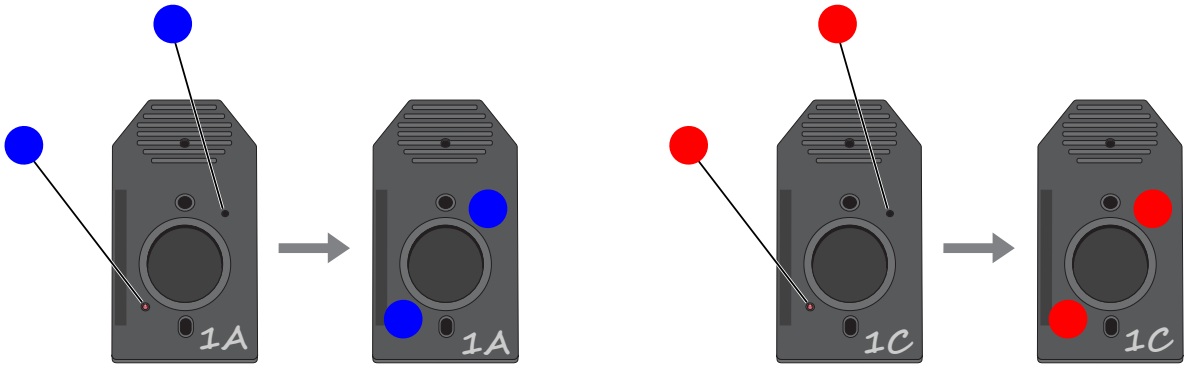


Figure 33 Applying Tough-Spots™ label dots to an array.

3. Proceed to "Stage 7: Scan the arrays" on page 108.

Note: If the arrays can not be placed into the scanner and scanned, store them at 4°C in the dark. When they can be loaded into the scanner, remove them from the 4°C storage and allow them to warm to room temperature.

Power off the hybridization oven and shut down the fluidics station

Perform the following procedures after all the arrays have been processed in the hybridization oven and fluidics station.

Power off the hybridization oven

1. Place the oven trays in the hybridization oven.

2. Power off the hybridization oven.

Shut down the fluidics station

Perform the **Shutdown_450** protocol when the instrument will be shut down. This protocol removes buffer from the fluidics station tubing and needles and replaces it with deionized (DI) water. See "Fluidics station Shutdown protocol" on page 157.

Stage 7: Scan the arrays

This stage is performed in the Post-PCR Room. During this stage, the arrays are scanned. The stage ends when all arrays have been scanned.

Prepare the scanner

Power on the scanner.

Note: The scanner takes approximately 10 minutes to warm the laser before arrays can be scanned. During this time both the yellow and green lights illuminate and the scanner enters the laser warm-up state. During the warm-up time, the green light turns off and the yellow light remains illuminated. After 10 minutes, the laser warm-up is complete and stable. A solid green light indicates the scanner is ready to scan.

Prepare the arrays for scanning

Note: If arrays have been stored at 4°C in the dark, remove them from storage and allow them to warm to room temperature.

---

1. Check that there are no bubbles in the array window. If bubbles are present, manually remove them and completely fill the array with Array Holding Buffer.
2. Ensure that the Tough-Spots™ label dots are flat and ***do not*** overlap the center area of the array. If not, remove the label dots and apply new ones.

Scan the arrays

1. From the GCDC Launcher window, click the **Scanner** button  .
   The **Scan Control** window appears.

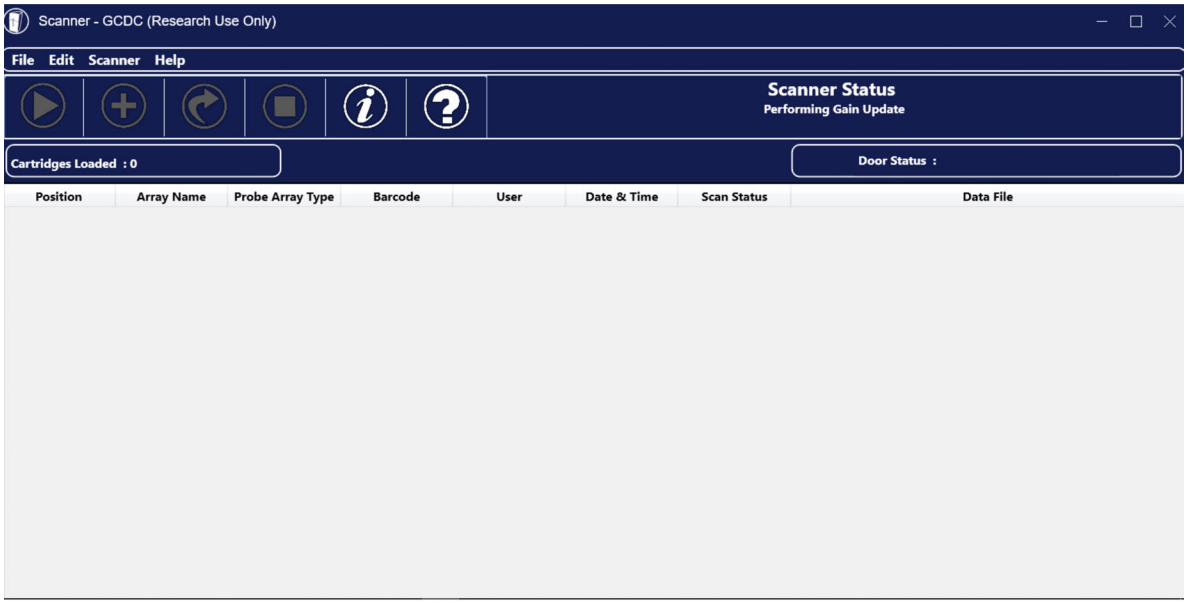


Figure 34 GCDC Scan Control window.

2. Load all the arrays into the GeneChip™ AutoLoader carousel, then close the AutoLoader door.

3. In the **Scan Control** window, click **Start**.

4. In the **Scanner Start Options** dialog window that appears, do the following:
   a. Select the checkbox **Arrays in carousel positions 1–4 at room temperature**.

      Note: If the arrays are not at room temperature, do not select this option. If not selected, the scanner waits 10 minutes before scanning starts to allow the arrays to reach room temperature.

   b. Do not select the **Allow Rescans** option. Only 1 scan per array is required.

5. Click **OK** to start the scanning run.
   - The GeneChip™ AutoLoader blue indicator light turns on and the AutoLoader door locks.
   - The carousel homes and inventories the position and number of arrays loaded in the AutoLoader carousel.
   - The scanning run starts. During the scan, the scanner green light flashes, and the yellow light is off.
   - The scanner performs an autofocus on each array before it is scanned. This takes approximately 2—3 minutes.
   - The scanning run stops when the last array has been scanned, or if **Stop** is clicked in the **Scan Control** window.

Add arrays during an AutoLoader run

1. In the GCDC **Scanner Control** window, click **Add Chips**.

   If an array is already being scanned, the scanning continues for that array until complete. When ready, the AutoLoader blue indicator light turns off and the AutoLoader door unlocks.

2. Add the arrays to the AutoLoader, then close the door.

3. Click **Resume**.

4. The **Scanner Start Options** dialog window appears. Select the checkbox **Arrays in carousel positions 1–4 at room temperature**.

5. Click **OK** to resume the run.
   - The GeneChip™ AutoLoader blue indicator light turns on and the AutoLoader door locks.
   - The carousel homes and inventories the position and number of arrays loaded in the AutoLoader carousel.
   - The carousel proceeds to the next array position from the previously scanned array. The scanner resumes the run.

Complete the scan

When the GCDC **Scan Control** dialog box displays the message **Scan Complete** for all arrays:

1. Open the AutoLoader door, then remove the arrays from the carousel.

2. Arrays can be discarded after DAT or CEL file inspection.

Shut down the scanner

Note: This is the recommended way to power off the scanner laser.

1. Close the GCDC software.

2. Power off the scanner.

A Register samples in GeneChip™ Data Collection Software

Sample naming conventions

During the OncoScan™ CNV Plus Assay, each sample is split into 2 reactions. This is called the Channel Split. One channel has AT dNTPs added, and the other channel has GC dNTPs added. The 2 channels remain separate reactions through the assay, and each is hybridized onto a separate array. After hybridization, each array is washed, stained, and scanned. When scanning is complete, each sample will have two intensity files (.cel files).

The Chromosome Analysis Suite (ChAS) is a software used to combine the 2 intensity files (.cel files) and generate a single analysis file (.oschp file). The analysis file is interpreted using ChAS.

Correctly naming the sample files during GCDC array registration is critical to the analysis in ChAS. The Analysis file name generated by ChAS is based on the Intensity file names entered when registering the arrays in GCDC. (Table 21).

When registering arrays use the following naming convention:

- For each sample, determine a name.
- The sample name will be used for both of the arrays during registration.
- An additional designation is added at the end of the name for each array. The designation is “A” for the AT channel, and “C” for the GC channel.

Table 21 Example of sample name, array name, and resulting files.

Target preparation	GCDC array registration		Intensity files		ChAS Analysis file
Sample name	AT array name	GC array name	AT channel file	GC channel file	Analysis file name
Sample 1	Sample 1_A	Sample 1_C	Sample 1_A.cel	Sample 1_C.cel	Sample 1.oschp
Sample 2	Sample 2_A	Sample 2_C	Sample 2_A.cel	Sample 2_C.cel	Sample 2.oschp
PosControl	PosControl_A	PosControl_C	PosControl_A.cel	PosControl_C.cel	PosControl.oschp

Register samples GCDC batch registration

Batch registration enables multiple sample to be registered simultaneously. A .xlsx or .tsv file is created then uploaded into the **Batch Registration** window of GCDC.

Create a Batch Registration spreadsheet

1. Create a spreadsheet that contains information about each array. The first 2 columns are required to be ***Name*** and ***Barcode***. Additional columns can be added to include optional attribute information. (Figure 35.)
   - The ***Name*** column should follow the sample naming convention. For each sample, there are 2 arrays. The ***Name*** column is the name of each array and will be used to name of the Intensity file (.cel) and the Analysis File (.oschp).
   - The ***Barcode*** column is the barcode of the array that the sample will be hybridized to. Use a barcode reader to enter the barcode.
   - Additional columns can be added. They are optional and user defined. For example: The ***Plate Position*** details where the sample is located during target preparation. The ***Sample Type*** describes the type of sample. ***Source*** can be sample source information.
2. Save the spreadsheet as a .xlsx or .tsv file.

	A	B	C	D	E	F
1	**Name**	**Barcode**	**Plate Position**	**Sample Type**	**Source**	
2	00965_ChRCC_A	@52078000936022063015420433546645	A1	ChRCC	00965	
3	00965_ChRCC_C	@52078000936022063015420433546655	C1	ChRCC	00965	
4	10236_Liver_A	@52078000936043063015420433524593	A2	Liver	10236	
5	10236_Liver_C	@52078000936060063015420480327964	C2	Liver	10236	
6	10559_Brain_A	@52078000936036063015420433523918	A3	Brain	10559	
7	10559_Brain_C	@52078000936053063015420480327917	C3	Brain	10559	
8	22210_CCRCC_A	@52078000936055063015420480348654	A4	CCRCC	22210	
9	22210_CCRCC_C	@52078000935494063015420480350036	C4	CCRCC	22210	
10	56544_ChRCC_A	@52078000935721022821438785326685	A5	ChRCC	56544	
11	56544_ChRCC_C	@52078000935703022821438785323470	C5	ChRCC	56544	
12	PC_20230613_A	@52078000935703022821438785323445	A6	Positive Control	OncoScan CVN Plus Kit	
13	PC_20230613_C	@52078000935710022821438785309365	C6	Positive Control	OncoScan CNV Plus Kit	
14						

Figure 35 Example of a Batch Registration spreadsheet.

Register the arrays

Note: The following instructions are an abbreviated version from the Applied Biosystems™ *GeneChip™ Data Collection Software (GCDC) User Guide* (Pub. No. MAN0026726).

1. Start the GCDC Launcher, then double-click the **Array Registration** button .
   The **Array Registration** window opens.
2. Select the **Batch Registration** tab.
3. From the **Batch Registration** window tab, click **Import**.
   An Explorer window opens.

4. Navigate to the Batch Registration spreadsheet location, select the file, then click **Open**.
   The following will appear in the Batch Registration window (Figure 36):
   - The file path of the Batch Registration file appears in the **Batch File** field.
   - The information from the Batch Registration file.
   - The **Array Type** column has the array type based on the barcode entered in the Batch Registration file.

Figure 36 The Batch Registration file has been imported.

5. Click **Register Samples**.
   An “All samples registered successfully!” message window appears.

6. Click **OK**.
   A “Sample registered successfully!” notification appears in the **Status** column. (Figure 37.)

Figure 37 All samples have been successfully registered to an array.

7. Close the GCDC **Array Registration** window.
8. Return to “Stage 5: Hybridization” on page 91. Start at “Prepare the Hyb Plate” on page 93 .

B

Thermal cycler protocols

Thermal cycler room and protocol setup

The thermal cycler protocols listed in the following tables are used in the OncoScan™ CNV Plus Assay. Program these protocols on the appropriate thermal cycler in the Pre-PCR Room and the Post-PCR Room.

Table 22 Pre-PCR Room.

Number of thermal cyclers required	Thermal cycler protocol name
1	OncoScan Anneal
	OncoScan Gap Fill
	OncoScan 1st PCR

Table 23 Post-PCR Room.

Number of thermal cyclers required	Thermal cycler protocol name
1	OncoScan 2nd PCR
	OncoScan HaeIII
	OncoScan Hybridization

The thermal cycler protocols are multi-stepped and include pausing the protocol, performing a step, then resuming the protocol. Depending on the thermal cycler used, the ability to pause a protocol may differ. This user guide provides different protocol programs based on the thermal cycler. The

protocol steps are the same, but additional information can be programed to aid pausing and resuming a specific protocol.

- "OncoScan™ CNV Plus Assay thermal cycler protocols for non-Veriti™ thermal cyclers" on page 116.
- "OncoScan™ CNV Plus Assay thermal cycler protocols for Veriti™ thermal cyclers with firmware less than v2.0.4" on page 123.
- "OncoScan™ CNV Plus Assay thermal cycler protocols for Veriti™ thermal cyclers with firmware v2.0.4 or greater" on page 131.

OncoScan™ CNV Plus Assay thermal cycler protocols for non-Veriti™ thermal cyclers

Use the following settings and protocols for non-Veriti™ thermal cyclers.

Thermal cycler ramp speed configuration requirements

Note: For all protocols, set the thermal cycler to maximum ramp rate.

1. For the Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block), set the ramp speed to "Max" when starting a protcocol.
2. For the Applied Biosystems™ ProFlex™ 96-well PCR System, the instrument can be run in standard mode or "GeneAmp PCR System 9700" simulation mode. With either mode, ensure that the ramp rate is set to the maximum value.
3. For the Bio-Rad™ T100 Thermal Cycler, set the ramp rate to the maximum ramp rate of 4°C/second.

OncoScan Anneal thermal cycler protocol (for non-Veriti™ thermal cyclers)

Reaction volume: 10 µL

Temperature	Time	Step	Manually pause during assay
95°C	5 minutes	Denature	—
58°C	16—18 hours	Anneal	Pause 1 minute into 58°C, chill plate, resume
58°C	∞	Hold	—

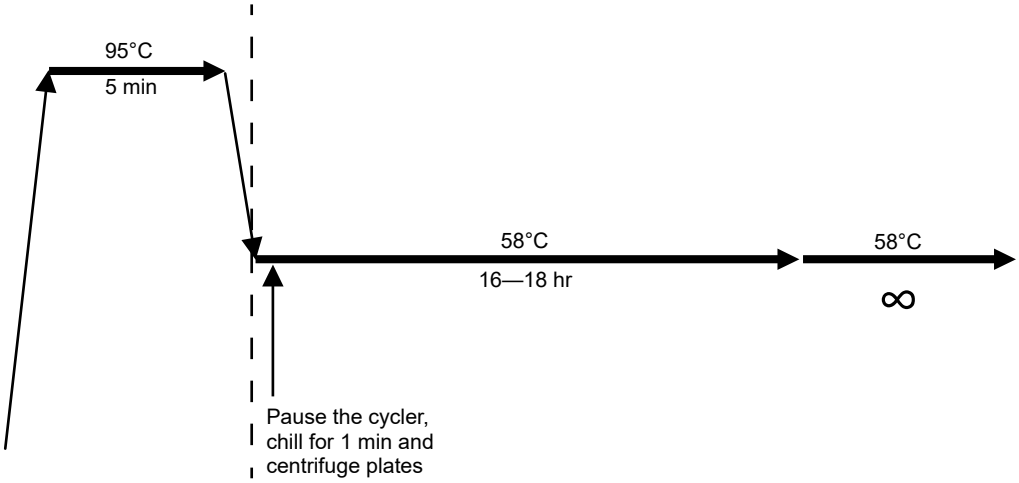


Figure 38 OncoScan Anneal thermal cycler protocol.

OncoScan Gap Fill thermal cycler protocol (for non-Veriti™ thermal cyclers)

IMPORTANT! Do not use this protocol for the Veriti™ Thermal Cycler. Use one of the following:

- "OncoScan Gap Fill thermal cycler protocol (Veriti™ firmware less than v2.0.4)" on page 126.
- "OncoScan Gap Fill thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)" on page 134.

Reaction volume: 42 µL

Temperature	Time	Step	Manually Pause During Assay
	0 minutes	—	Pause when block reaches 58°C, load plate, resume to start protocol
58°C	11 minutes	Gap Fill	Pause at 11 minutes, add AT/GC Mix, resume
58°C	11 minutes	AT/GC Ligation	Pause at 11 minutes, remove plate, resume with no plate
37°C	20 minutes	Exonuclease	Pause at 37°C, add Exo Mix, resume
95°C	10 minutes	Denature	—
37°C	15 minutes	Cleavage	Pause at 37°C, add Cleavage Mix, resume
95°C	15 minutes	Denature	—
4°C	∞	Hold	—

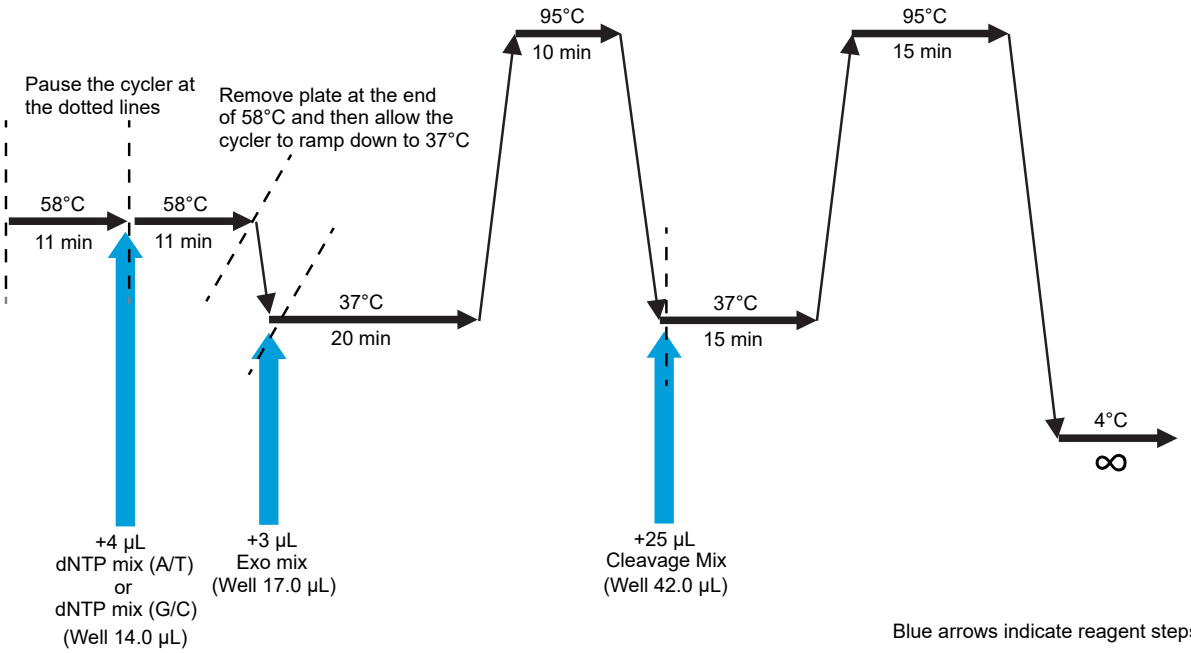


Figure 39 OncoScan Gap Fill thermal cycler protocol.

OncoScan 1st PCR thermal cycler protocol (for non-Veriti™ thermal cyclers)

Note: This reaction is performed in the Pre-PCR Room, then transferred to the Post-PCR Room. Do not remove the seal from the **1st PCR Plate** in the Pre-PCR Room.

Reaction volume: 67 µL

Temperature	Time	Cycles	Step
60°C	0 minute	—	Pause when block reaches 60°C, load plate, resume to start protocol
60°C	30 seconds	1X	PCR Reaction Start
95°C	1 minute	1X	Template Denaturation
95°C	20 seconds	20X	Denaturation
60°C	10 seconds		Anneal
72°C	10 seconds		Extension
72°C	5 minutes	1X	Extension
4°C	∞	—	Hold

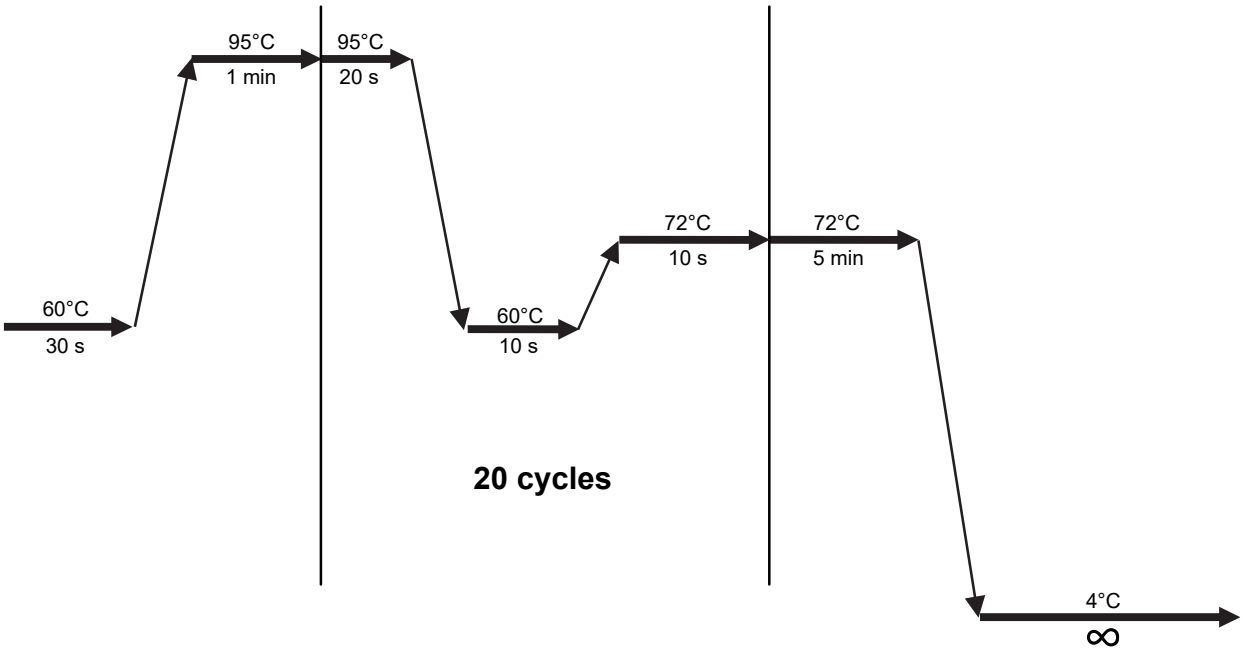


Figure 40 OncoScan 1st PCR thermal cycler protocol.

OncoScan 2nd PCR PCR thermal cycler protocol (for non-Veriti™ thermal cyclers)

Reaction volume: 27 µL

Temperature	Time	Cycles	Step
60°C	0 seconds	—	Pause when block reaches 60°C, load plate, resume to start protocol
60°C	30 seconds	1X	PCR Reaction Start
95°C	1 minute	1X	Template Denaturation
95°C	20 seconds	15X	Denaturation
60°C	10 seconds		Anneal
72°C	10 seconds		Extension
72°C	5 minutes	1X	Extension
4°C	∞	—	Hold

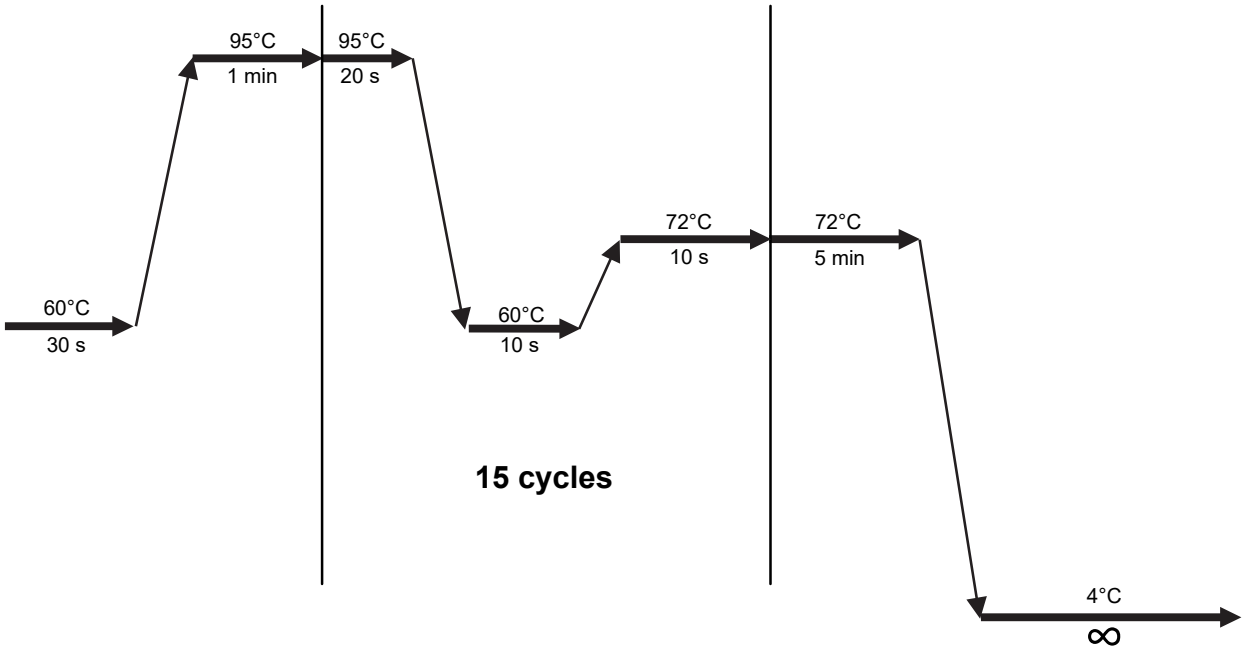


Figure 41 OncoScan 2nd PCR PCR thermal cycler protocol.

OncoScan HaeIII thermal cycler protocol (for non-Veriti™ thermal cyclers)

Reaction volume: 30 µL

Temperature	Time	Step	Manually Pause During Assay
37°C	88 minutes	Digestion	Pause at 88 minutes, remove gel aliquot, resume
37°C	2 minutes	Digestion	—
95°C	10 minutes	Deactivation	—
4°C	∞	Hold	—

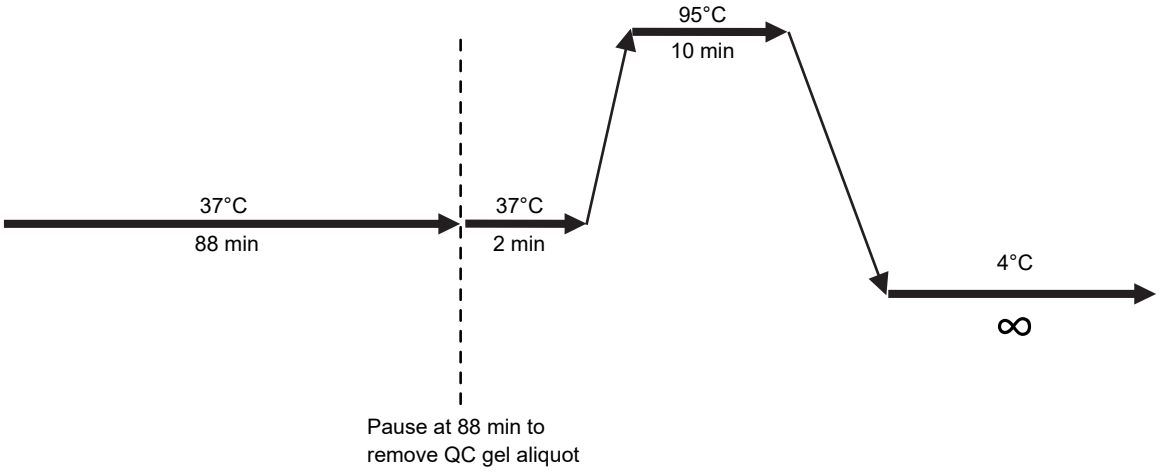


Figure 42 OncoScan HaeIII thermal cycler protocol.

OncoScan Hybridization thermal cycler protocol (for non-Veriti™ thermal cyclers)

Reaction volume: 170 µL

Temperature	Time	Step
95°C	10 minutes	Denature
49°C	5 minutes	Hybridization Temperature Equilibration
49°C	∞	Hybridization Temperature Hold

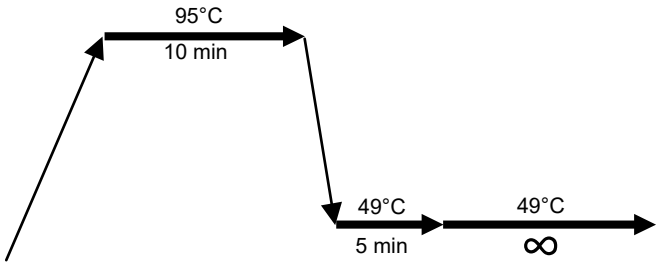


Figure 43 OncoScan Hybridization thermal cycler protocol.

OncoScan™ CNV Plus Assay thermal cycler protocols for Veriti™ thermal cyclers with firmware less than v2.0.4

Use the following settings and protocols for Veriti™ thermal cyclers with firmware less than v2.0.4.

Thermal cycler ramp speed configuration requirements

For the Veriti™ thermal cycler, set the ramp speed to "9700-Max-Mode" during programming of each protocol. Use the "Convert Method" feature to create a protocol on the Veriti™ thermal cycler to simulate the GeneAmp™ PCR System 9700 "Max mode".

Convert Method for the Veriti™ Thermal Cycler

1. In the **Main Menu** screen, touch **Tools Menu**.
2. In the **Tools Menu** screen, touch **Convert a Method** to open the Convert Method wizard.
3. Touch the check box next to the run method's original format "9700 Max Mode", then touch **Next**.
4. In the second page, enter the protocol. Touch **Next**.
5. In the **Save Run Method** page, enter a name of the protocol, then select a folder for the run method. Names are limited to 16 characters. By default, the name begins with "9700-Max-Mode".
6. Enter the reaction volume, and set the heated lid cover to 105.0°C.
7. When finished select **Save & Exit**.

About the Veriti™ thermal cycler firmware (less than v2.0.4)

It has been observed that the touch screen may not respond when **Pause Run** or **Resume Run** functions are selected. Make sure that the screen is only tapped once, firmly. ***Do not*** tap twice.

When the touch screen is tapped twice to perform the **Pause Run**, the program momentarily pauses, then immediately resumes. This can create assay interruptions and possibly lead to assay failures.

To reduce issues, perform the following steps during thermal cycler setup, program entry, and use.

Set up the thermal cycler for Veriti™ firmware version less than v2.0.4

1. In the **Main Menu** screen, touch **Settings Menu**.
2. In the **Settings Menu** screen, touch **Set Time-Outs**.
3. In the **Set Time-Outs** screen, touch **Time-out**, then enter the duration.
    a. **Default Standby Time-Out** sets the duration that the instrument is idle before the touchscreen is turned off. Enter a duration of ***00:00:00*** to prevent the touchscreen from turning off.

b. **Default Pause Duration** sets the duration the instrument pauses after touching **Pause** during a run. Set to ***24:00:00*** to prevent the pause from stopping.

4. Touch **Done** to save the changes, then touch **OK** to return to the **Settings Menu** screen.

Thermal cycler operation (Veriti™ firmware less than v2.0.4)

When pausing the thermal cycler, pay attention to the screen and ensure the following:

- When pausing the thermal cycler, use a timer to help track the necessary steps as required in the protocol.
- Only tap the **Pause Run** button once. ***Do not*** tap it twice. (Figure 44.)
- When the protocol is paused, the **Time Remaining** display stops.
- The protocol resumes when **Resume Run** is tapped. The **Time Remaining** display counts down again.

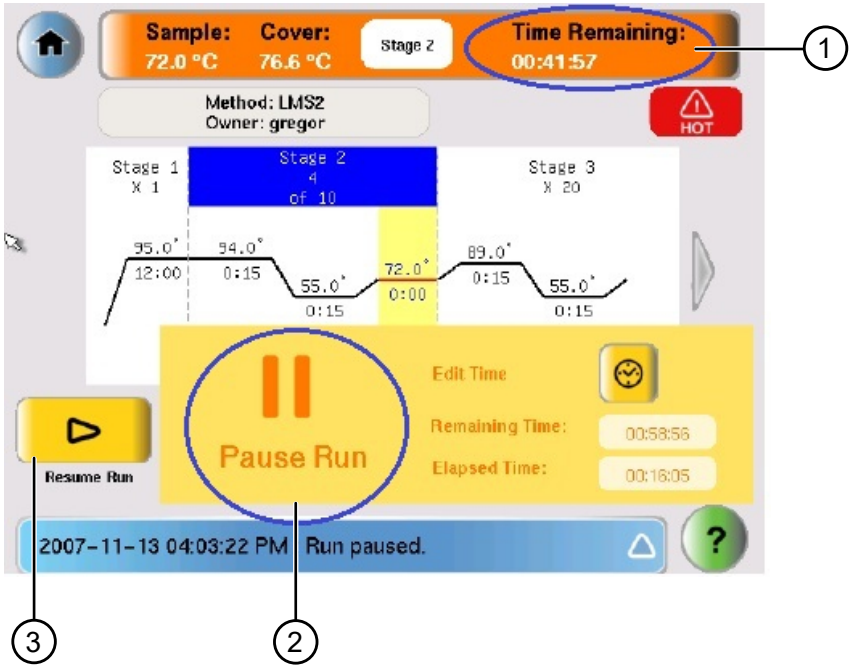


Figure 44 Example of a Veriti™ Thermal Cycler touch screen.

① When the protocol is paused, the clock stops in the **Time Remaining** display.
② The protocol pauses when **Pause Run** is touched.
③ The protocol resumes when **Resume Run** is touched.

OncoScan Anneal thermal cycler protocol (Veriti™ firmware less than v2.0.4)

Reaction volume: 10 µL

Temperature	Time	Step	Manually pause during assay
95°C	5 minutes	Denature	—
58°C	16—18 hours	Anneal	Pause 1 minute into 58°C, chill plate, resume
58°C	∞	Hold	—

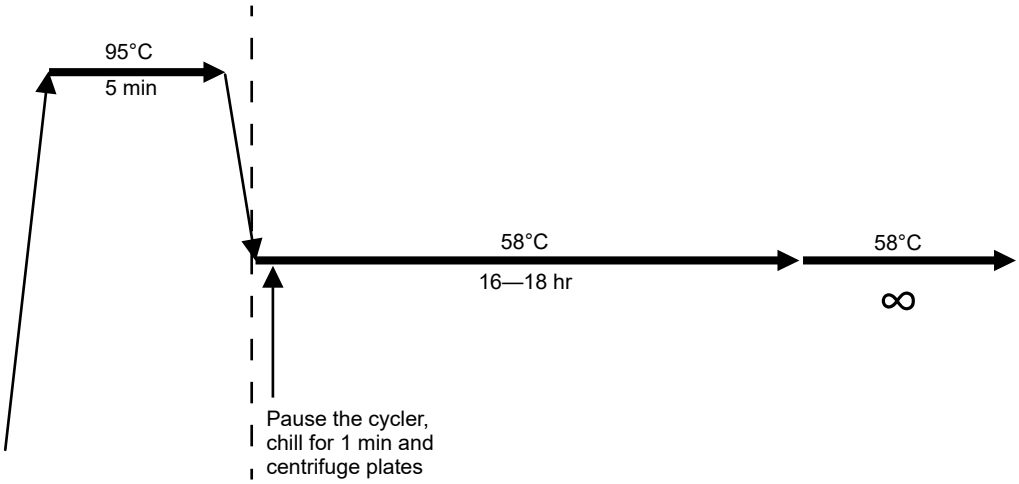


Figure 45 OncoScan Anneal thermal cycler protocol.

OncoScan Gap Fill thermal cycler protocol (Veriti™ firmware less than v2.0.4)

IMPORTANT! This protocol combines the first two 58°C incubations into one 22 minutes stage. (Figure 46.)

This change is recommended because it has been noticed that the touchscreen does not consistently respond to the **Pause Run** function when paused between the 2 stages of 58°C. (The touch screen may not pause at the end of the 58°C at 11 minutes and will move on to the next 58°C stage). This will ruin the Gap Fill reaction, leading to assay failure.

Reaction volume: 42 µL

Temperature	Time	Step	Manually pause during assay
58°C	0 minutes	—	Pause when block reaches 58°C, load plate, resume to start protocol
58°C	22 minutes	Gap Fill and AT/GC Ligation	• Pause at 11 minutes, add AT/GC Mix, resume • Pause at 22 minutes, remove plate, resume
37°C	20 minutes	Exonuclease	Pause at 37°C, add Exo Mix, resume
95°C	10 minutes	Denature	—
37°C	15 minutes	Cleavage	Pause at 37°C, add Cleavage Mix, resume
95°C	15 minutes	Denature	—
4°C	∞	Hold	—

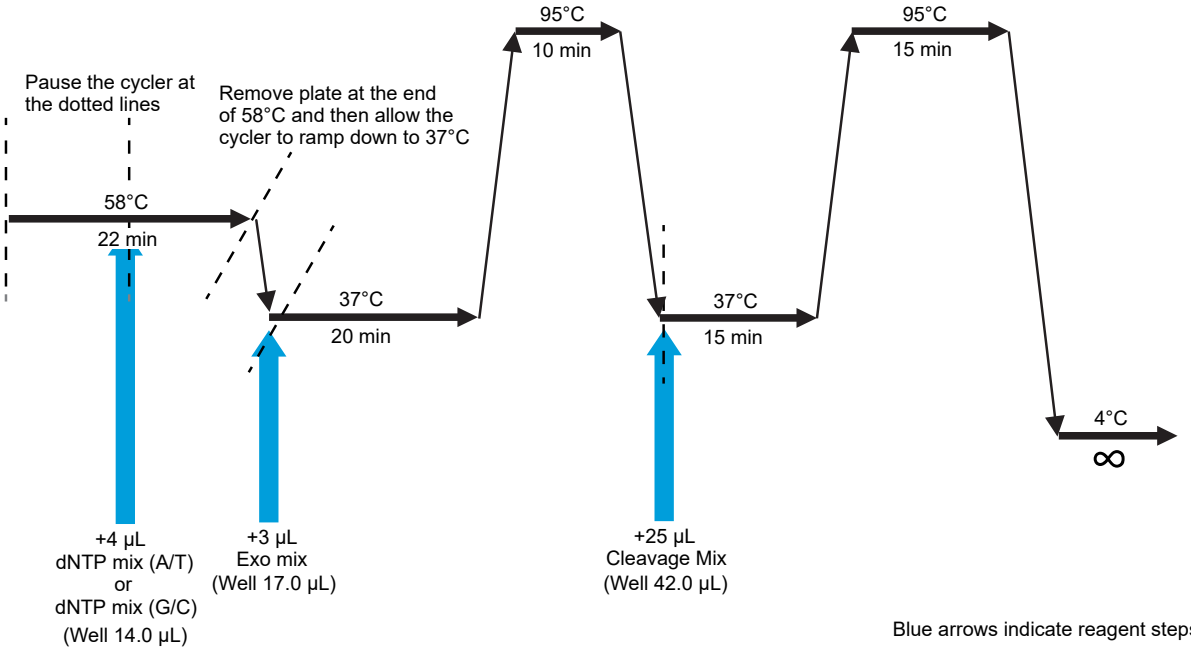


Figure 46 OncoScan Gap Fill thermal cycler protocol (Veriti™ firmware less than v2.0.4).

OncoScan 1st PCR thermal cycler protocol (Veriti™ firmware less than v2.0.4)

Note: This reaction is performed in the Pre-PCR Room, then transferred to the Post-PCR Room. Do not remove the seal from the **1st PCR Plate** in the Pre-PCR Room.

Reaction volume: 67 µL

Temperature	Time	Cycles	Step
60°C	0 minute	—	Pause when block reaches 60°C, load plate, resume to start protocol
60°C	30 seconds	1X	PCR Reaction Start
95°C	1 minute	1X	Template Denaturation
95°C	20 seconds	20X	Denaturation
60°C	10 seconds		Anneal
72°C	10 seconds		Extension
72°C	5 minutes	1X	Extension
4°C	∞	—	Hold

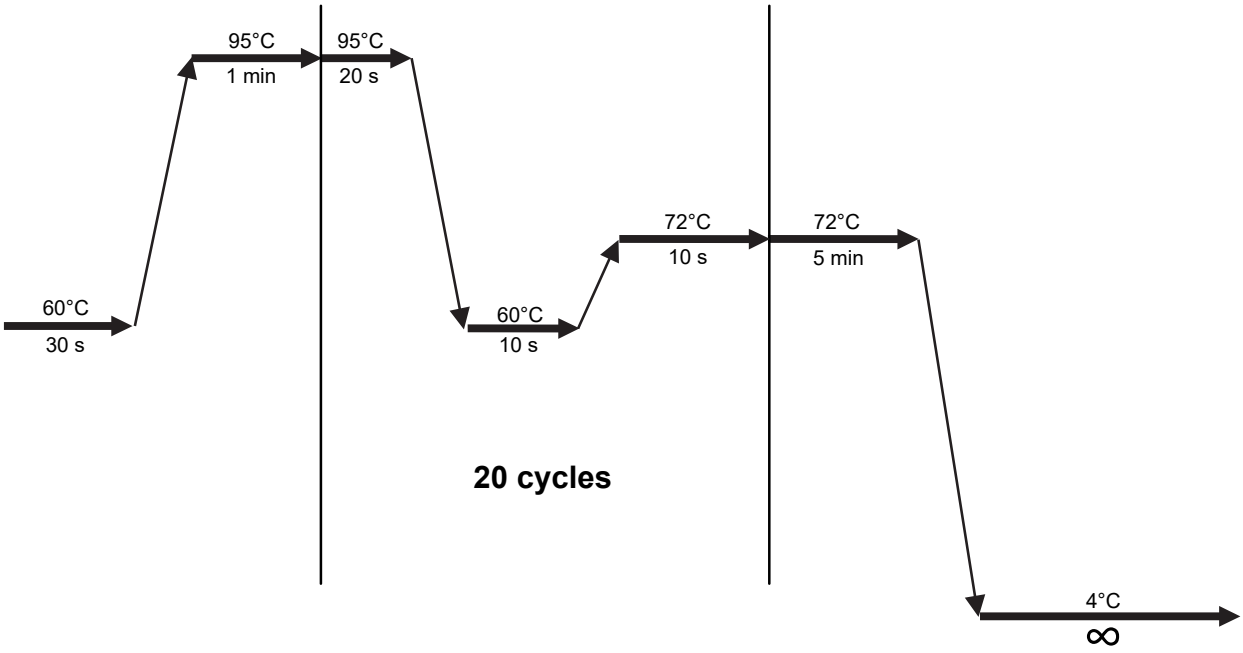


Figure 47 OncoScan 1st PCR thermal cycler protocol.

OncoScan 2nd PCR PCR thermal cycler protocol (Veriti™ firmware less than v2.0.4)

Reaction volume: 27 µL

Temperature	Time	Cycles	Step
60°C	0 seconds	—	Pause when block reaches 60°C, load plate, resume to start protocol
60°C	30 seconds	1X	PCR Reaction Start
95°C	1 minute	1X	Template Denaturation
95°C	20 seconds	15X	Denaturation
60°C	10 seconds		Anneal
72°C	10 seconds		Extension
72°C	5 minutes	1X	Extension
4°C	∞	—	Hold

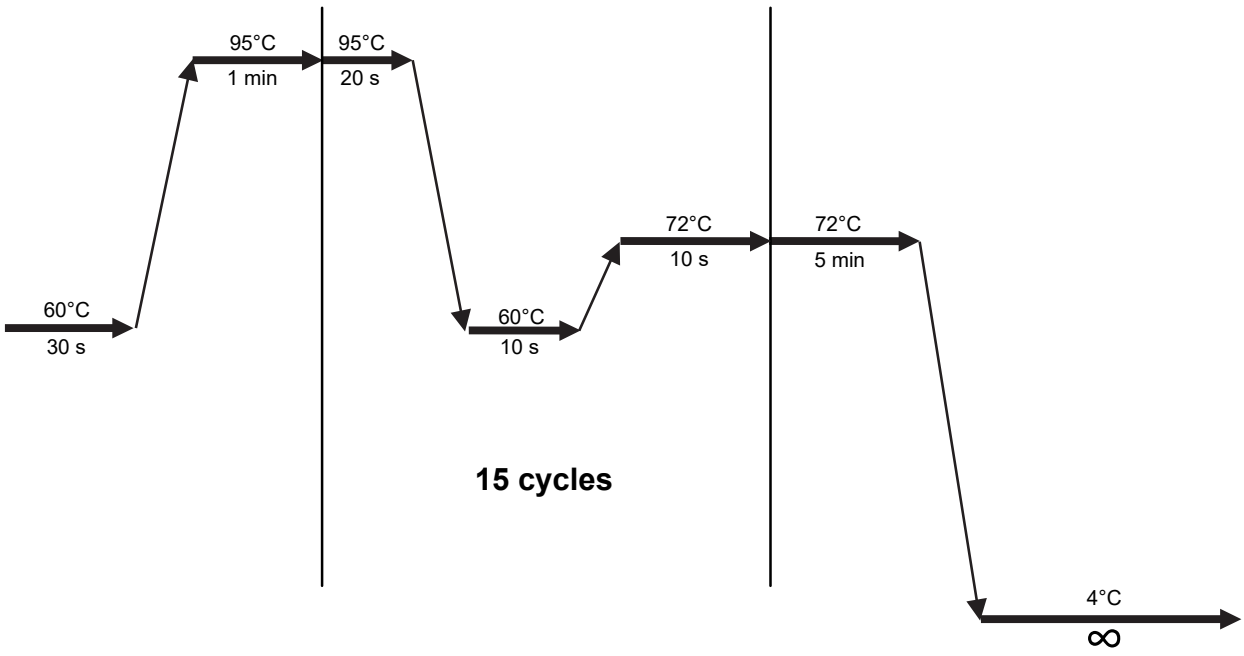


Figure 48 OncoScan 2nd PCR PCR thermal cycler protocol.

OncoScan HaeIII thermal cycler protocol (Veriti™ firmware less than v2.0.4)

Reaction volume: 30 µL

Temperature	Time	Step	Manually Pause During Assay
37°C	88 minutes	Digestion	Pause at 88 minutes, remove gel aliquot, resume
37°C	2 minutes	Digestion	—
95°C	10 minutes	Deactivation	—
4°C	∞	Hold	—

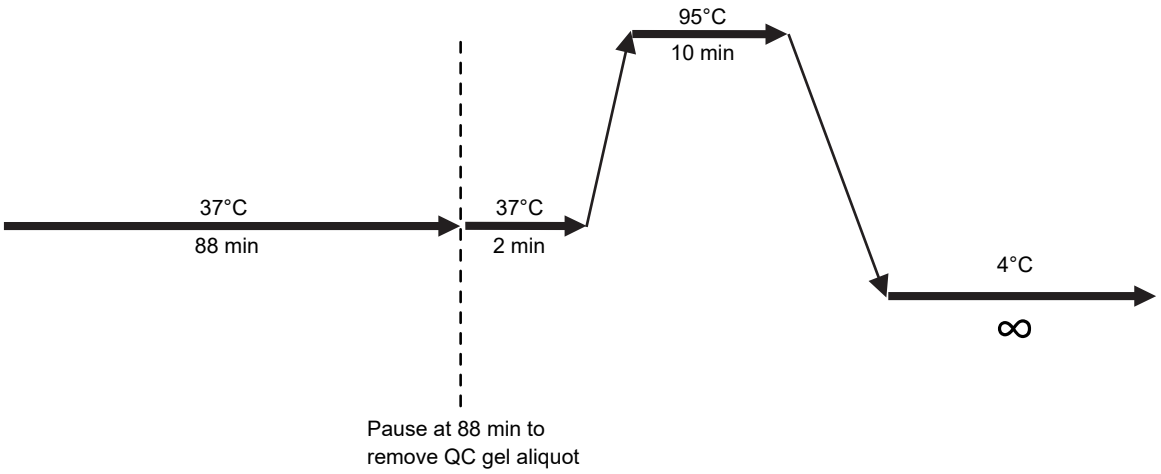


Figure 49 OncoScan HaeIII thermal cycler protocol.

OncoScan Hybridization thermal cycler protocol (Veriti™ firmware less than v2.0.4)

Reaction volume: 170 µL

Temperature	Time	Step
95°C	10 minutes	Denature
49°C	5 minutes	Hybridization Temperature Equilibration
49°C	∞	Hybridization Temperature Hold

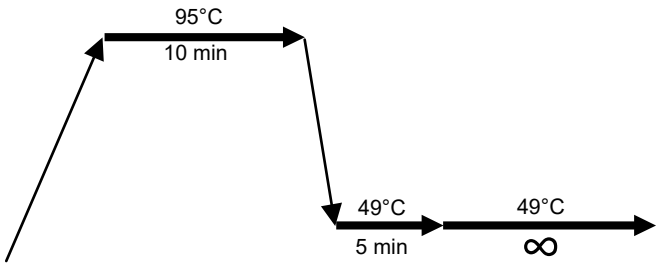


Figure 50 OncoScan Hybridization thermal cycler protocol.

OncoScan™ CNV Plus Assay thermal cycler protocols for Veriti™ thermal cyclers with firmware v2.0.4 or greater

Use the following settings and protocols for Veriti™ thermal cyclers with firmware v2.0.4 or greater.

Thermal cycler ramp speed configuration requirements

For the Veriti™ thermal cycler, set the ramp speed to “9700-Max-Mode” during programming of each protocol. Use the “Convert Method” feature to create a protocol on the Veriti™ thermal cycler to simulate the GeneAmp™ PCR System 9700 “Max mode”.

Convert Method for the Veriti™ Thermal Cycler

1. In the **Main Menu** screen, touch **Tools Menu**.
2. In the **Tools Menu** screen, touch **Convert a Method** to open the Convert Method wizard.
3. Touch the check box next to the run method’s original format “9700 Max Mode”, then touch **Next**.
4. In the second page, enter the protocol. Touch **Next**.
5. In the **Save Run Method** page, enter a name of the protocol, then select a folder for the run method. Names are limited to 16 characters. By default, the name begins with “9700-Max-Mode”.
6. Enter the reaction volume, and set the heated lid cover to 105.0°C.
7. When finished select **Save & Exit**.

About the Veriti™ thermal cycler firmware (v2.0.4 or greater)

It has been discovered that some thermal cyclers with firmware v2.0.4 or greater will not pause when the **Pause Run** button is tapped. The **Time Remaining** display continues to count down time. This can create assay interruptions and possibly lead to assay failures.

To reduce issues, perform the following steps during thermal cycler setup, program entry, and use.

Setup the thermal cycler for Veriti™ firmware version v2.0.4 or greater

The protocols in this section incorporate infinity (∞) hold steps at every step that requires a pause. To add an infinity (∞) hold step, follow the instructions below.

1. In the **Edit Run Method** screen, touch anywhere in the **Step** column to select it. When the step is selected, the column turns red.
2. Touch **Add**. A copy of the selected step is added to the run method.
3. Touch the time field to select it. The text editor opens.
4. In the text editor, touch **Symbols**, then touch infinity (∞) to select an infinity hold.
5. Touch **Done** to close the editor, then touch **Save** to save the method.

Thermal cycler operation (Veriti™ firmware v2.0.4 or greater)

1. Use a timer to help perform the necessary steps as required in the protocol.
2. When an infinity (∞) hold step occurs, the **Time Remaining** display stops. Perform the required assay step during the hold. (Figure 51.)

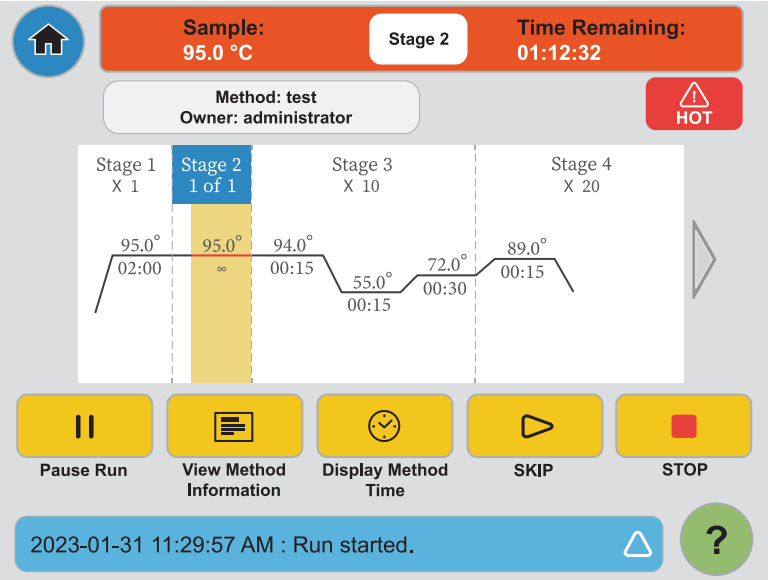


Figure 51 Infinity hold step.

3. When the assay step is complete, touch **SKIP** ▷ to resume the protocol.
   The **Time Remaining** display counts down again.
4. When prompted to confirm skipping the infinity (∞) hold step, touch **OK**.

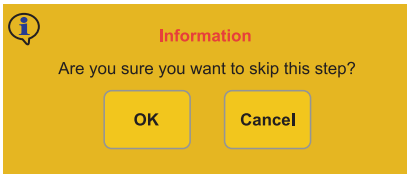


Figure 52 Infinity hold skip confirmation.

5. Ensure that the thermal cycler protocol resumes and the **Time Remaining** display counts down again. (Figure 53.)

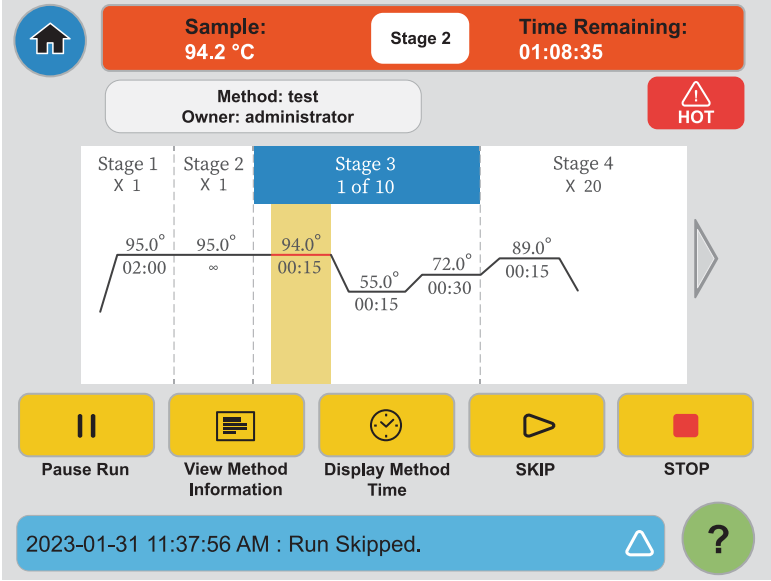


Figure 53 Protocol resumes after skip.

OncoScan Anneal thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)

Reaction volume: 10 µL

Temperature	Time	Step	Manually pause during assay
95°C	5 minutes	Denature	—
58°C	16—18 hours	Anneal	Incubate plate 1 minute at 58°C, chill plate, SKIP to resume protocol
58°C	∞	Hold	—

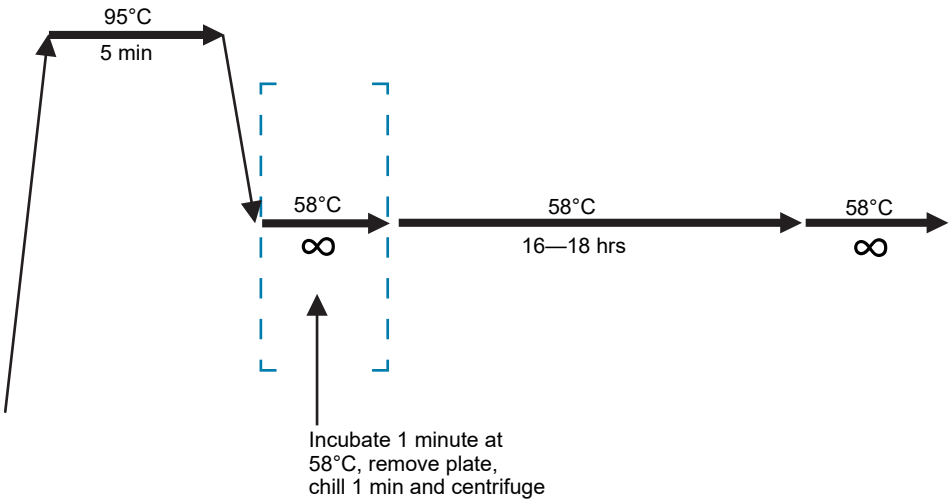


Figure 54 OncoScan Anneal thermal cycler protocol.

OncoScan Gap Fill thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)

Reaction volume: 42 µL

Temperature	Time	Step	"SKIP" after the ∞ pause
58°C	∞	—	Load plate, SKIP to start protocol
58°C	11 minutes	Gap Fill	—
58°C	∞	—	Add AT/GC Mix, SKIP to resume protocol
58°C	11 minutes	AT/GC Mix Ligation	—
58°C	∞	—	Remove plate, SKIP to resume protocol with no plate
37°C	∞	—	Add Exo Mix, SKIP to resume protocol
	20 minutes	Exonculease	—
95°C	10 minutes	Denature	—
37°C	∞	—	Add Cleavage Mix, SKIP to resume protocol
	15 minutes	Cleavage	—
95°C	15 minutes	Denature	—
4°C	∞	Hold	—

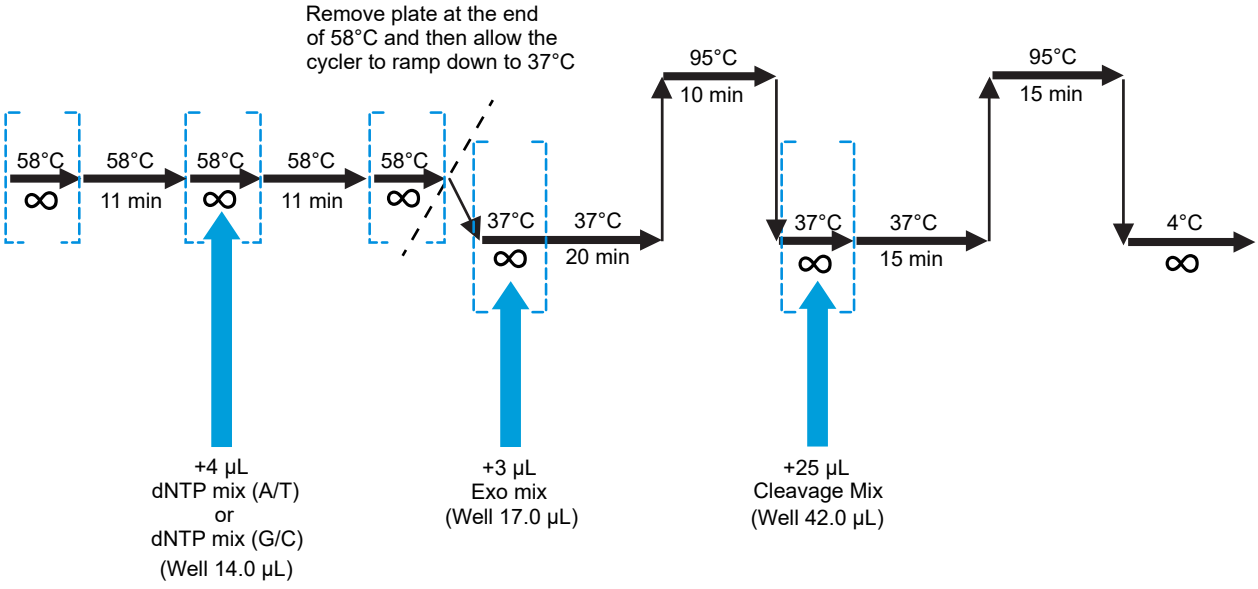


Figure 55 OncoScan Gap Fill thermal cycler protocol (Veriti™ firmware v2.0.4 or greater).

OncoScan 1st PCR thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)

Note: This reaction is performed in the Pre-PCR Room, then transferred to the Post-PCR Room. Do not remove the seal from the **1st PCR Plate** in the Pre-PCR Room.

Reaction volume: 67 µL

Temperature	Time	Cycles	Step
60°C	∞	—	Load plate, SKIP to start protocol
60°C	30 seconds	1X	PCR Reaction Start
95°C	1 minute	1X	Template Denaturation
95°C	20 seconds	20X	Denaturation
60°C	10 seconds		Anneal
72°C	10 seconds		Extension
72°C	5 minutes	1X	Extension
4°C	∞	—	Hold

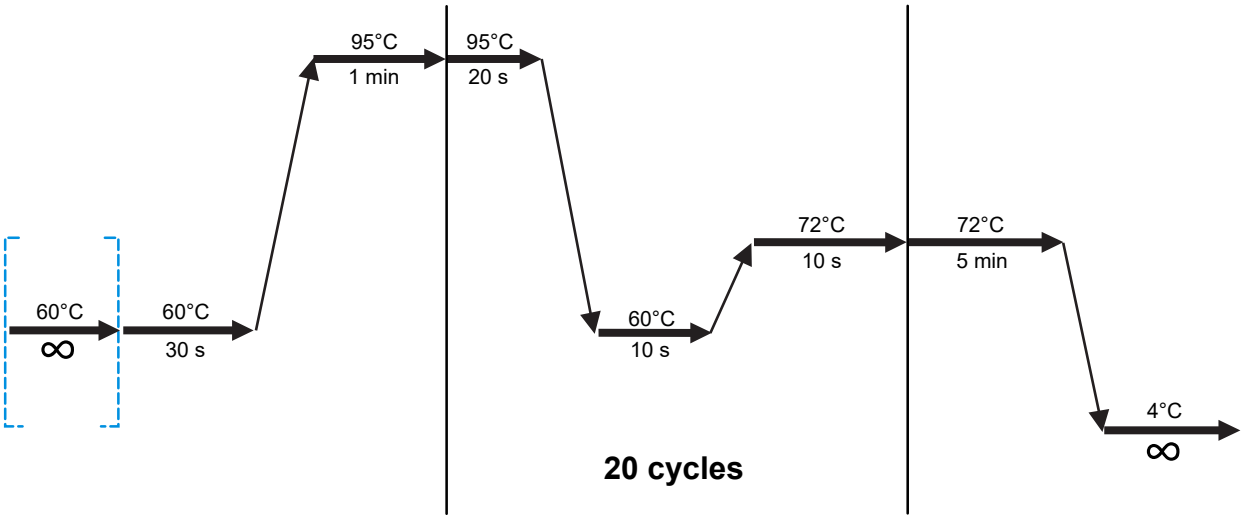


Figure 56 OncoScan 1st PCR thermal cycler protocol (Veriti™ firmware v2.0.4 or greater).

OncoScan 2nd PCR PCR thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)

Reaction volume: 27 µL

Temperature	Time	Cycles	Step
60°C	∞	—	Load plate, SKIP to start protocol
60°C	30 seconds	1X	PCR Reaction Start
95°C	1 minute	1X	Template Denaturation
95°C	20 seconds	15X	Denaturation
60°C	10 seconds		Anneal
72°C	10 seconds		Extension
72°C	5 minutes	1X	Extension
4°C	∞	—	Hold

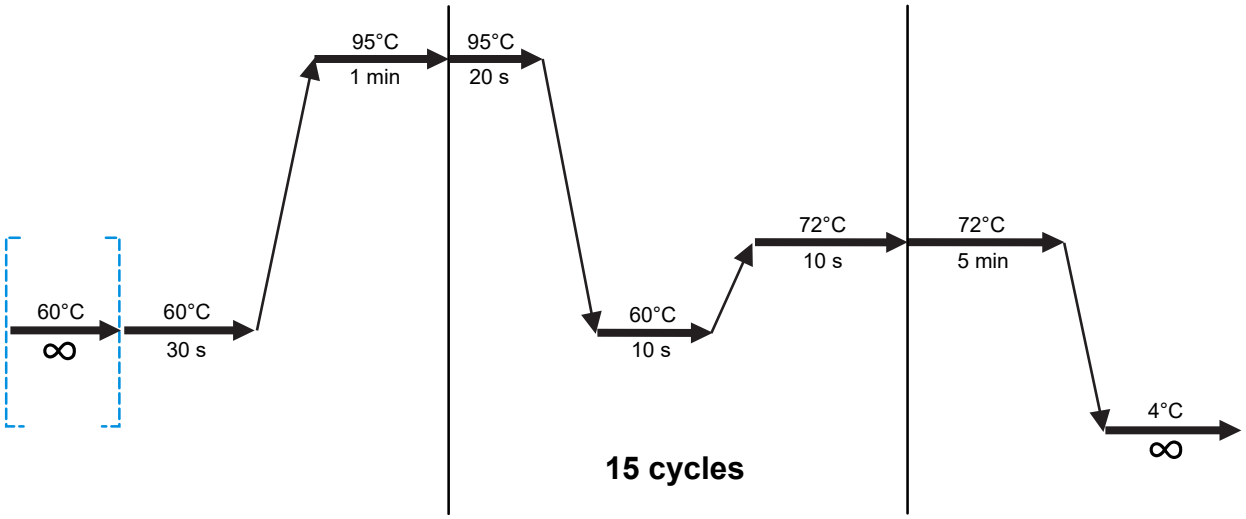


Figure 57 OncoScan 2nd PCR PCR thermal cycler protocol (Veriti™ firmware v2.0.4 or greater).

OncoScan HaeIII thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)

Reaction volume: 30 µL

Temperature	Time	Step	Manually Pause During Assay
37°C	88 minutes	Digestion	—
37°C	∞	—	Remove gel aliquot, SKIP to resume protocol
37°C	2 minutes	Digestion	—
95°C	10 minutes	Deactivation	—
4°C	∞	Hold	—

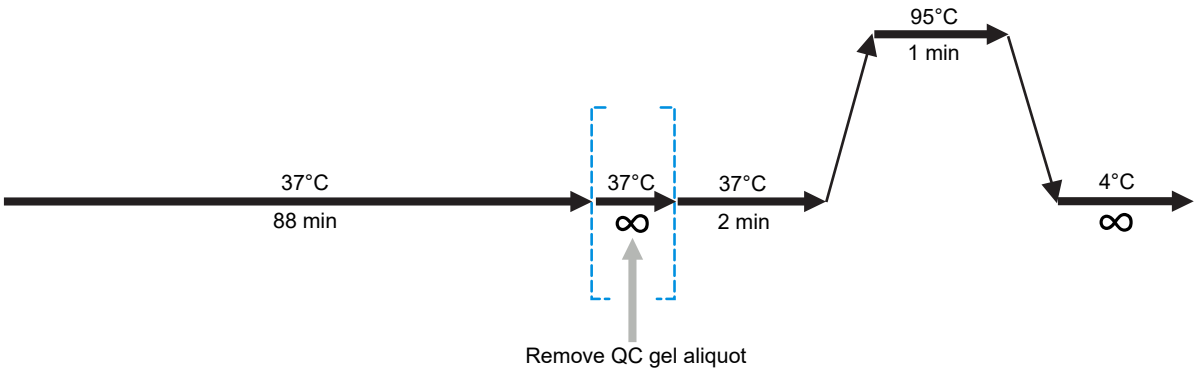


Figure 58 OncoScan HaeIII thermal cycler protocol (Veriti™ firmware v2.0.4 or greater).

OncoScan Hybridization thermal cycler protocol (Veriti™ firmware v2.0.4 or greater)

Reaction volume: 170 µL

Temperature	Time	Step
95°C	10 minutes	Denature
49°C	5 minutes	Hybridization Temperature Equilibration
49°C	∞	Hybridization Temperature Hold

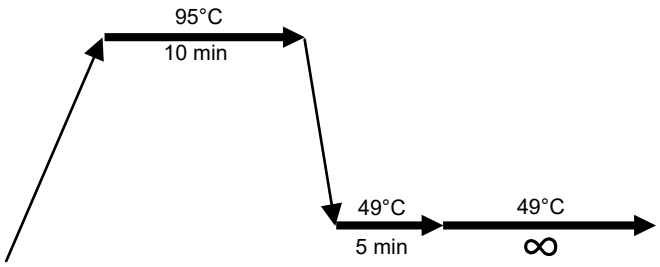


Figure 59 OncoScan Hybridization thermal cycler protocol.

C Equipment, consumables, and reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Thermo Fisher Scientific materials required

Equipment and software required

The OncoScan™ CNV Plus Array can be processed on the GeneChip™ System 3000 and the GeneChip™ System 3000Dx v2.

Table 24 GeneChip™ System 3000 equipment and software required.

Item	Source
Equipment	
GeneChip™ System 3000 • GeneChip™ Scanner 3000 7G System with the Workstation and AutoLoader • GeneChip™ Fluidics Station 450 (2 or more units required)	 • 00-0218 • 00-0079
GeneChip™ Hybridization Oven 645	00-0331
Tubing, silicone peristaltic for the GeneChip™ Fluidics Station 450 (Quantity = 1 tube)	400110
Software	
Applied Biosystems™ GeneChip™ Data Collection Software (GCDC) (Version 1.0 or higher) or Applied Biosystems™ GeneChip™ Command Console™ (GCC) (Version 6.1.3 or higher)	611403
Applied Biosystems™ Chromosome Analysis Suite software (Version 4.1 or higher)	901394

Table 25 GeneChip™ System 3000Dx v2 equipment and software required.

Item	Source
Equipment	
GeneChip™ System 3000Dx v2 Instrument System • GeneChip™ Scanner 3000Dx v2 with GeneChip™ AutoLoader Dx and GeneChip™ Fluidics Station 450Dx v2	00-0334
GeneChip™ Hybridization Oven 645	00-0331
GeneChip™ Fluidics Station 450Dx v2 (Optional)	00-0335
Tubing, silicone peristaltic for the GeneChip™ Fluidics Station 450 (Quantity = 1 tube)	400110
Software	
Applied Biosystems™ GeneChip™ Data Collection Software Dx (GCDC Dx) (Version 1.0 or higher) or Affymetrix™ Molecular Diagnostics Software (AMDS) (Version 1.1 or higher)	611403 or 610449
Applied Biosystems™ Chromosome Analysis Suite software (Version 4.1 or higher)	901394

Thermo Fisher Scientific reagents and array required

Table 26 OncoScan™ CNV Plus reagents and array kits.

Item	Source
OncoScan™ CNV Plus Reagent Kit for Research	902294
OncoScan™ CNV Plus Array (12 pack)	902292
OncoScan™ CNV Plus Assay for Research, consists of: • OncoScan™ CNV Plus Reagent Kit for Research • OncoScan™ CNV Plus Array (48 arrays)	902293
OncoScan™ CNV Plus Assay Training Kit, consists of: • OncoScan™ CNV Plus Reagent Kit for Research • OncoScan™ CNV Plus Array (36 arrays) • Training controls	902305

OncoScan™ CNV Plus Reagent Kit for Research

Table 27 OncoScan™ CNV Plus Reagent Kit for Research (Cat. No. 902294).

Component and cap color	Part No.	Storage
OncoScan™ CNV Plus Somatic Mutation Probe Mix 1.0 (Part No. 902272)		
Somatic Mutation Probe Mix 1.0	902247	–25°C to –15°C
OncoScan™ CNV Plus Copy Number Probe Mix 1.0 & Controls (Part No. 902268)		
Positive Control (12 ng/µL)	902249	–25°C to –15°C
Negative Control	902250	
Copy Number Probe Mix 1.0	902248	
Buffer A	902246	
OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269)		
Buffer A	902246	–25°C to –15°C
Gap Fill Enzyme Mix	902252	
SAP, Recombinant (1 U/µL)	902251	
dNTP Mix (A/T)	902254	
dNTP Mix (G/C)	902255	
Nuclease-Free Water	902253	
Exo Mix	902256	
Cleavage Buffer	902257	
Cleavage Enzyme (2 U/µL)	902258	
PCR Mix	902259	
Taq Polymerase (5 U/µL)	902260	
OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270)		
PCR Mix	902259	–25°C to –15°C
Taq Polymerase (5 U/µL)	902260	
Buffer B	902261	
HaeIII Enzyme (10 U/µL)	902262	
Exo I Enzyme (20 U/µL)	902263	
Nuclease-Free Water	902253	
Hybridization Mix	902264	

Table 27 OncoScan CNV Plus Reagent Kit for Research (Cat. No. 902294). *(continued)*

Component and cap color	Part No.	Storage
OncoScan™ CNV Plus Stain Reagents (Part No. 902271)		
Stain 1	902265	2—8°C
Stain 2	902266	
Array Holding Buffer	901733	
Individual bottles		
Wash A	901680	15–30°C
Wash B	901681	

OncoScan™ CNV Plus Array

Component	Cat. No.	Storage
OncoScan™ CNV Plus Array (12 pack)	902292	2—8°C

Recommended dsDNA quantitation kits and equipment

The success of the OncoScan™ CNV Plus Assay requires accurate quantification of the input gDNA using a dsDNA specific quantification method. Two methods that have been verified for use are the Quant-iT™ PicoGreen™ Assay and the Qubit™ dsDNA Quantification Assay. Sample concentration determined by UV absorbance or NanoDrop™ spectrophotometer ***must not*** be used in this assay.

Table 28 PicoGreen™ reagents and equipment required.

Item	Source
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P7589
Required: Fluorometer Recommended: Varioskan™ LUX Multimode Microplate Reader	VL0000D0

Table 29 Qubit™ reagents and equipment required.

Item	Source
Qubit™ dsDNA HS Assay Kit	Q32851
Qubit™ fluorometer	Q33327 or Q33238
Qubit™ assay tubes specific to the Qubit™ instrument • Qubit™ 4 Fluorometer: Qubit™ Assay Tubes • Qubit™ Flex Fluorometer: Qubit™ Flex Assay Tube Strips	 • Q32856 • Q33252

Other reagents

Item	Source
TE Buffer, 1X Solution pH 8.0, Low EDTA	J75793.AE
Nuclease-Free Water (from different source)	10977015
Applied Biosystems™ 25-bp DNA Ladder	931343
Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer	10482028
Invitrogen™ E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4%	G820804
Bleach, Sodium Hypochlorite solution without additives	**MLS**

Equipment required but not provided

Pre-PCR Room

Table 30 Pre-PCR Room—required equipment not provided.

Item	Source
If assay is to be performed in 1 room: • Laminar flow cabinet, 6 foot, or • PCR cabinet	**MLS**
Required markers: • 1 blue permanent marker, extra-fine tip • 1 red permanent marker, extra-fine tip	**MLS**
Required: Rectangular ice bucket, large, 9 L (16 × 13 in; 41 × 33 cm) Recommended: Corning™ Rectangular Ice Pan, Maxi 9L	Fisher Scientific™, 07-210-093
Required: Cooling block with the capacity to hold 96-well plates with a maximum volume capacity of 330 µL. Recommended: Quantity = 4, Electron Microscopy Sciences CoolSafe™ Cooling Chamber for 0.2 mL tubes	Fisher Scientific™, 50-334-22
Required: Vortexer, 600—3,200 rpm, cup head or 3' head. Recommended: Fisherbrand™ Analog Vortex Mixer	Fisher Scientific™, 02-215-414
Required: Microfuge (for tubes and strip tubes) Recommended: Fisherbrand™ Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey	Fisher Scientific™, 12-006-901

Table 30 Pre-PCR Room—required equipment not provided. *(continued)*

Item	Source
Pipettors: • 12-channel, 2 µL to 20 µL • 12-channel, 20 µL to 200 µL • Single-channel, 2 µL to 20 µL • Single-channel, 20 µL to 200 µL • Single-channel, 100 µL to 1,000 µL **Note:** Electronic multichannel pipettes are not recommended.	**MLS**
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. The use of a compatible compression pad is recommended throughout the assay. Recommended: One of the following verified thermal cyclers. • Applied Biosystems™ ProFlex™ 96-well PCR System • Applied Biosystems™ Veriti™ 96-Well Thermal Cycler, 0.2 mL,[1] • Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block) • Applied Biosystems™ 2720 Thermal Cycler • Bio-Rad™ T100 Thermal Cycler	• 4484075 • 4375786 • 4314878 • 4359659 • 1861096
Required: Centrifuge, plate, multipurpose, 330 µL capacity with swinging buckets Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Ventilated) with swinging bucket rotor and unsealed buckets (Set of 2) or Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Refrigerated) with swinging bucket rotor and unsealed buckets (Set of 2)	75007200 with 75005706 and 75005723 or 75007203 with 75005706 and 75005723
Refrigerator, 2—8°C, 6 cu ft	**MLS**
Freezer, –25°C to –15°C; deep freeze; manual defrost; 17 cu ft	**MLS**
Required: Storage racks, tube Recommended: Thermo Scientific™ 4-Way Flipper™ Rack	Fisher Scientific™, 21-402-27
Required: PCR tube storage rack, 96-well, with cover Recommended: Axygen™ PCR Tube Storage Rack	Fisher Scientific™, 14-222-396

Table 30 Pre-PCR Room—required equipment not provided. *(continued)*

Item	Source
Required: Benchtop cooler, with the capacity to hold 8—12 tubes (1.5 mL) Recommended: Thermo Scientific™ Benchtop Cooler	Fisher Scientific™, 15-350-61
Required: Adhesive film applicator (hard plastic) Recommended: MicroAmp™ Adhesive Film Applicator	Fisher Scientific™, 4333183

[1] The Veriti™ *Fast* 96-Well Thermal Cycler, Cat. No. 4375305 and the and the Eppendorf™ Mastercycler™ pro S, Cat. No. 950030020 are not compatible with this assay.

Post-PCR Room

Table 31 Post-PCR Room—required equipment not provided.

Item	Source
Required markers: • 1 blue permanent marker, extra-fine tip • 1 red permanent marker, extra-fine tip	**MLS**
Required: Rectangular ice bucket, large, 9 L (16 × 13 in; 41 × 33 cm) Recommended: Corning™ Rectangular Ice Pan, Maxi 9L	Fisher Scientific™, 07-210-093
Required: Cooling block with the capacity to hold 96-well plates with a maximum volume capacity of 330 µL. Recommended: Quantity 3, Electron Microscopy Sciences CoolSafe™ Cooling Chamber for 0.2 mL tubes	Fisher Scientific™, 50-334-22
Required: Vortexer, 600–3,200 rpm, cup head or 3' head. Recommended: Fisherbrand™ Analog Vortex Mixer	Fisher Scientific™, 02-215-414
Required: Microfuge (for tubes and strip tubes) Recommended: Fisherbrand™ Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey	Fisher Scientific™, 12-006-901
Pipettors: • 12-channel, 2 µL to 20 µL • 12-channel, 20 µL to 200 µL • Single-channel, 2 µL to 20 µL • Single-channel, 20 µL to 200 µL • Single-channel, 100 µL to 1,000 µL **Note:** Electronic multichannel pipettes are not recommended.	**MLS**

Table 31 Post-PCR Room—required equipment not provided. *(continued)*

Item	Source
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. The use of a compatible compression pad is recommended throughout the assay. Recommended: One of the following verified thermal cyclers. • Applied Biosystems™ ProFlex™ 96-well PCR System • Applied Biosystems™ Veriti™ 96-Well Thermal Cycler, 0.2 mL,[1] • Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block) • Applied Biosystems™ 2720 Thermal Cycler • Bio-Rad™ T100 Thermal Cycler	 • 4484075 • 4375786 • 4314878 • 4359659 • 1861096
Required: Centrifuge, plate, multipurpose, 330 µL capacity with swinging buckets Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Ventilated) with swinging bucket rotor and unsealed buckets (Set of 2) or Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Refrigerated) with swinging bucket rotor and unsealed buckets (Set of 2)	 75007200 with 75005706 and 75005723 or 75007203 with 75005706 and 75005723
Required: Electrophoresis supplies Recommended for electrophoresis and image capture: • E-Gel™ Power Snap Plus Electrophoresis Device • Safe Imager™ Viewing Glasses • E-Gel™ agarose gel cassette • E-Gel™ Power Snap Plus Camera	 • G9110 • S37103[2] • —[2] • G9200
Additional options for electorphoresis and image capture: • Invitrogen™ Mother E-Base™ Device • Invitrogen™ Daughter E-Base™ Device • Invitrogen™ iBright™ CL750 Instrument	 • EBM03 (Mother) • EBD03 (Daughter) • A44116
Refrigerator, 2–8°C, 6 cu ft	**MLS**
Freezer, –25°C to –15°C; deep freeze; manual defrost; 17 cu ft	**MLS**
Required: Storage racks, tube Recommended: Thermo Scientific™ 4-Way Flipper™ Rack	Fisher Scientific™, 21-402-27
Required: PCR tube storage rack, 96-well, with cover Recommended: Axygen™ PCR Tube Storage Rack	Fisher Scientific™, 14-222-396

Table 31 Post-PCR Room—required equipment not provided. *(continued)*

Item	Source
Required: Benchtop cooler, with the capacity to hold 8–12 tubes (1.5 mL) Recommended: Thermo Scientific™ Benchtop Cooler	Fisher Scientific™, 15-350-61
Required: Adhesive film applicator (hard plastic) Recommended: MicroAmp™ Adhesive Film Applicator	4333183
Required: Microtube storage rack, capacity to hold 96 tubes (1.5 mL) Recommended: Thermo Scientific™ 96-Well Flipper™ Microtube Rack	21-402-18
Fluidics station recommended bottles: • Media Bottle, SQ, 500 mL • Media Bottle, SQ, 1,000 mL	 • 400118 • 400119

[1] The Veriti™ *Fast* 96-Well Thermal Cycler, Cat. No. 4375305 and the and the Eppendorf™ Mastercycler™ pro S, Cat. No. 950030020 are not compatible with this assay.
[2] Included with E-Gel™ Power Snap Plus Electrophoresis Device.

Labware and consumables required

Table 32 Consumables required but not provided.

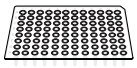
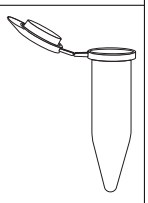
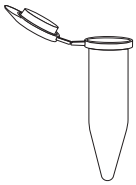
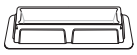
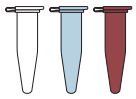
Labware	Source	Room	Image
Required: Plates, 96-well unskirted PCR with a maximum volume of 330 µL • Recommended: Amplifyt™ 96-Well PCR Plate, Semi-Skirted • Alternative: Applied Biosystems™ MicroAmp™ Optical 96-Well Reaction Plate • Alternative: Bio-Rad™ Multiplate™ 96-Well PCR Plates, high profile, unskirted, clear	• Thomas Scientific™, 1148A74 • N8010560 • Bio-Rad™, MLP9601	Both	
Required: Adhesive films, clear, PCR-certified, 96-well plates Recommended: Applied Biosystems™ MicroAmp™ Clear Adhesive Film	4306311	Both	
Pipette tips with aerosol barriers, 20 µL, 200 µL, and 1,000 µL	**MLS**	Both	—
Tube strips, nuclease-free, 8-well or 12-well, 0.2 mL polypropylene	**MLS**	Both	
Optional: 8-tube or 12-tube strip caps	**MLS**	Both	—
Microcentrifuge tubes, 1.5 mL, non-stick, polypropylene, DNAse and RNAse-free **Note:** Required for all sample workflows.	**MLS**	Both	

Table 32 Consumables required but not provided. *(continued)*

Labware	Source	Room	Image
Microcentrifuge tubes, 2.0 mL, non-stick, polypropylene, DNAse and RNAse-free **Note:** Only required if running the 25-sample workflow.	**MLS**	Post	
Conical centrifuge tubes, nuclease-free, sterile, non-stick, 15 mL	**MLS**	Post	
Required: Reagent reservoir, 25 mL Recommended: Matrix™ Reagent Reservoirs	8093	Post	
Microcentrifuge tubes, nuclease-free, non-stick, 1.5 mL natural polypropylene	**MLS**	Post	
Microcentrifuge tubes, nuclease-free, non-stick, 1.5 mL blue polypropylene	**MLS**	Post	
Microcentrifuge tubes, nuclease-free, non-stick, 1.5 mL amber, polypropylene	**MLS**	Post	
Adhesive label dot (Tough-Spots™ labels), 1/2-inch roll and 3/8-inch roll	**MLS**	Post	—

D FFPE DNA extraction protocol for the OncoScan™ CNV Plus Assay

This appendix includes instructions to extract DNA from FFPE blocks using a modified protocol and the QIAamp™ DNA FFPE Tissue Kit. This modified protocol was developed and tested by Thermo Fisher Scientific and found to generate good quality genomic DNA for the OncoScan™ CNV Plus Assay. It includes steps to improve the tissue digestion process and release more DNA from the tissue sections.

This procedure should be performed in a clean room such as the OncoScan™ CNV Plus Assay Pre-PCR Room. Use caution to not contaminate the gDNA with OncoScan™ CNV Plus Assay PCR 1 and PCR 2 amplicons, or other gDNA sources.

The standard QIAamp™ DNA FFPE Tissue Kit protocol can be found on the QIAGEN™ web page.

https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-dna-ffpe-tissue-kit/

Equipment, consumables, and reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Reagents required

Table 33 Reagents required for the FFPE DNA Extraction protocol.

Item	Source
QIAamp™ DNA FFPE Tissue Kit • QIAamp™ MinElute Columns • Collection Tubes, 2 mL • Buffer ATL • Proteinase K • Buffer AL • Buffer AW1 • Buffer AW2 • Buffer ATE	QIAGEN™, 56404
Xylene	Sigma-Aldrich™, 534056-500ML
Absolute Ethanol, 200 proof, Molecular Biology Grade	T038181000 (1L)
RNase A	QIAGEN™, 19101
Optional elution reagent: TE Buffer, 1X Solution pH 8.0, Low EDTA	J75793.AE

Pre-PCR Room equipment required not provided

Table 34 Pre-PCR Room equipment required not provided.

Item	Source
Required: Microtome Recommended: Epredia™ HM 340E Electronic Rotary Microtome	Fisher Scientific™, 23-900-670
Required: Vortexer, 600—3,200 rpm, cup head or 3' head. Recommended: Fisherbrand™ Analog Vortex Mixer	Fisher Scientific™, 02-215-414
Required: Microfuge (for tubes and strip tubes) Recommended: Fisherbrand™ Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey	Fisher Scientific™, 12-006-901
Required: Microcentrifuge (with rotor for 2 mL tubes) Recommended: Thermo Scientific™ Sorvall™ Legend™ Micro 17 Microcentrifuge	Fisher Scientific™, 75-002-431
Required: Thermal mixer with block for 24 x 1.5 mL Tubes (Quantity required: 2) Recommended: Thermo Scientific™ Thermal Mixer with 24 x 1.5 mL microtube block	13687717

Table 34 Pre-PCR Room equipment required not provided. *(continued)*

Item	Source
Pipettors: • Single-channel, 20 µL to 200 µL • Single-channel, 100 µL to 1,000 µL **Note:** Electronic multichannel pipettes are not recommended.	**MLS**

Pre-PCR Room labware and consumables

Table 35 Pre-PCR Room labware and consumables required.

Item	Source
Epredia™ Ultra Disposable Microtome Blades	Fisher Scientific™, 31-537-35
Eppendorf™ Safe-Lock™ Microcentrifuge Tubes, 1.5 mL	VWR™, 022363204
Invitrogen™ Nonstick, RNase-free Microfuge Tubes, 1.5 mL	AM12450
Pipette tips with aerosol barriers, 200 µL, and 1,000 µL	**MLS**
Required: Storage racks, microtube Recommended: Thermo Scientific™ 96-Well Flipper™ Microtube Rack	Fisher Scientific™, 21-402-18

Set up the work area in the Pre-PCR Room or clean room

1. Power on the thermal mixers.
   - Set the first thermal mixer to 98°C, then program it to run for 15 minutes and shake at 1,400 rpm for 15 seconds every 1 minute.
   - Set second thermal mixer to 56°C, then program it to run for 3.5 hours and shake at 1,400 rpm for 15 seconds every 1 minute.
   - Allow both thermal mixers to reach temperature before use.
2. Set a tube storage rack on the benchtop.
3. Place the Proteinase K and RNase A on the tube storage rack.
4. Place the Buffer ATE on the benchtop.
5. Place the xylene and ethanol on the benchtop.

Prepare buffers

1. Prepare Buffer ATL and Buffer AL.
   a. Check whether precipitate has formed in Buffer ATL or Buffer AL.
   b. If necessary, dissolve precipitate by heating to 70°C with gentle agitation.
2. Prepare Buffer AW1.
   a. Add 25 mL ethanol (96—100%) to the bottle containing 19 mL Buffer AW1 concentrate.
   b. Mark the bottle to indicate that the ethanol has been added.
   c. Store at room temperature up to 1 year.
   d. Before using in the procedure, mix thoroughly by shaking.
3. Prepare Buffer AW2.
   a. Add 30 mL ethanol (96—100%) to the bottle containing 13 mL Buffer AW2 concentrate.
   b. Mark the bottle to indicate that the ethanol has been added.
   c. Store at room temperature up to 1 year.
   d. Before using in the procedure, mix thoroughly by shaking.
4. Place the buffers on the benchtop.

Perform deparaffinization

Note: This procedure is written to process one sample. Multiple samples can be processed simultaneously.

1. From a FFPE block, prepare 10 µm thick slicks. If the block surface has been exposed to air, discard the first 2-3 sections. Place 5 slices in a 1.5-mL Eppendorf™ Safe-Lock™ Tube.
2. Immediately add 1 mL of xylene to the sample tube.
3. Vortex the sample tube at maximum speed for 10 seconds.
4. Centrifuge the sample tube at full speed (~14,000 rpm) for 5 minutes.
5. Without disturbing the pellet, use a P1000 pipette to remove the xylene.

   Note: Dispose of the xylene properly.
6. Add 1 mL of ethanol to the sample tube.
7. Vortex the tube at maximum speed for 10 seconds.

8. Centrifuge the sample tube at full speed (~14,000 rpm) for 5 minutes.
9. Without disturbing the pellet, use a P1000 pipette to remove the ethanol.

   Note: Dispose of the ethanol properly. It contains residual xylene.

10. Repeat step 6 through step 9.
11. Centrifuge the sample tube at full speed (~14,000 rpm) for 3 minutes.
12. Without distrubing the pellet, use a P200 to completely remove any residual ethanol.
13. Place the sample tube on the tube storage rack.
14. Open the cap of the sample tube and incubate for 10 minutes at room temperature to allow any remaining ethanol to evaporate.

Perform tissue lysis

1. Add 180 µL of Buffer ATL to the sample tube.
2. Vortex at full speed for 10 seconds, then briefly centrifuge to remove any solution from the cap of the tube.
3. Place the sample tube onto the first thermal mixer set to 98°C.
   a. Incubate at 98°C for 15 minutes with a 15 second mix at 1,400 rpm every 1 minute.

   Note: Ensure that the thermal mixer is programmed to shake for 15 seconds after each minute at 1,400 rpm.

4. After 15 minutes, stop the first thermal mixer program, then power off the thermal mixer.
5. Allow the sample tube to cool for 5 minutes in the first thermal mixer *before* carefully removing it. Cooling helps prevent the cap from popping open due to high temperature and pressure.
6. Remove the sample tube slowly from the first thermal mixer, then place it on the tube storage rack.
7. Incubate for 10 minutes at room temperature to allow the sample tube to cool, then briefly centrifuge.
8. Add 20 µL of Proteinase K to the sample tube.
9. Vortex at full speed for 10 seconds, then briefly centrifuge to remove any solution from the cap of the tube.
10. Place the sample tube onto the second thermal mixer set to 56°C.
    a. Incubate at 56°C for 3.5 hours with a 15 second mix at 1,400 rpm every 1 minute.

Note: Ensure that the thermal mixer is programmed to shake for 15 seconds after each minute at 1,400 rpm.

11. After 3.5 hours, visually verify that all tissue has lysed.

Note: If tissue remains, incubate the sample an additional amount of time at 56°C. The additional time will vary depending on the amount of input material and tissue type.

If tissue still remains, add 20 µL of Proteinase K to the sample, and incubate an additional hour or more.

12. Power on the first thermal mixer and set it to 90°C.
13. Briefly centrifuge the sample tube to remove any solution from the cap of the tube.
14. Place the sample tube onto the first thermal mixer set to 90°C.
    a. Incubate at 90°C for 1 hour with a 15 second mix at 1,400 rpm every 1 minute.

Note: Ensure that the thermal mixer is programmed to shake for 15 seconds after each minute at 1,400 rpm.

15. After 1 hour, remove the sample tube from the first thermal mixer and place it on the tube storage rack. Allow it to cool for 10 minutes to reach room temperature.
16. Briefly centrifuge the sample tube to remove any solution from the cap of the tube.
17. Add 2 µL of RNase A to the sample tube.
18. Vortex the sample tube at maximum speed for 10 seconds, then briefly centrifuge.
19. Incubate for 2 minutes at room temperature.

Purify the DNA

1. Remove a QIAamp™ MinElute™ column (in a 2-mL collection tube) from the refrigerator. Allow it to warm to room temperature for 15 minutes. Label the column cap with the sample name
2. Add 200 µL of Buffer AL to the sample tube, vortex at maximum speed for 10 seconds, then briefly centrifuge.
3. Immediately add 200 µL of ethanol to the sample tube, vortex at maximum speed for 10 seconds, then briefly centrifuge.

IMPORTANT! The Buffer AL and ethanol must be mixed immediately.

   a. If processing multiple samples, Buffer AL and ethanol can be premixed and added together in 1 step.

   b. A precipitant may form at this step, which does not affect the DNA yield.

4. Carefully transfer the entire lysate from the sample tube to the QIAamp™ MinElute™ column (in a 2-mL collection tube). Close the cap of the QIAamp™ MinElute™ column, then centrifuge at 8,000 rpm for 1 minute.

   Note: Ensure that all the lysate has moved through the column. If lysate is still in the column, centrifuge again at a higher speed for 1 minute.

5. Place the QIAamp™ column into a new 2.0-mL collection tube, then discard the collection tube containing the flow-through.

6. Open the QIAamp™ MinElute™ column and add 500 µL of Buffer AW1, then close the cap.

7. Load the QIAamp™ MinElute™ column (in a 2-mL collection tube) into the centrifuge, then spin at 8,000 rpm for 1 minute.

   Note: Ensure that all the Buffer AW1 has moved through the column. If Buffer AW1 is still in the column, centrifuge again at a higher speed for 1 minute.

8. Place the QIAamp™ MinElute™ column into a new 2.0-mL collection tube, then discard the collection tube containing the flow-through.

9. Open the QIAamp™ MinElute™ column and add 500 µL of Buffer AW2, then close the cap.

10. Load the QIAamp™ MinElute™ column (in a 2-mL collection tube) into the centrifuge, then spin at 8,000 rpm for 1 minute.

    Note: Ensure that all the Buffer AW2 has moved through the column. If Buffer AW2 is still in the column, centrifuge again at a higher speed for 1 minute.

11. Place the QIAamp™ MinElute™ column into a new 2.0-mL collection tube, then discard the collection tube containing the flow-through.

12. Load the QIAamp™ MinElute™ column (in a 2-mL collection tube) into the centrifuge, then spin at full speed (~14,000 rpm) for 3 minutes to dry the membrane completely.

Elute the DNA

IMPORTANT! DNA must be eluted using the ATE buffer provided in the QIAamp™ DNA FFPE Tissue Kit. DNA ***must not*** be eluted in water.

Alternatively, 1X TE Buffer with low EDTA at pH 8.0 (with 10 mM Tris at pH 8.0 and 0.1 mM EDTA) can be used as the elution reagent.

1. Label a Nuclease-free 1.5-mL microcentrifuge tube with the sample name.
2. Place the QIAamp™ MinElute™ column into the labeled 1.5-mL microcentrifuge tube, then discard the collection tube containing the flow-through.
3. Add 50 µL of Buffer ATE to the center of the QIAamp™ MinElute™ column membrane.
4. Close the cap of the QIAamp™ MinElute™ column and incubate for 5 minutes at room temperature.
5. Load the the QIAamp™ MinElute™ column (in a 1.5-mL microcentrifuge tube) into the centrifuge, then spin at full speed (~14,000 rpm) for 1 minute to elute the DNA.

   Note: Caps for 1.5-mL microcentrifuge will not close at this step due to the column. If processing multiple samples, load the QIAamp™ MinElute™ column (in a 1.5-mL microcentrifuge tube) in alternating positions in the centrifuge. Position the 1.5-mL microcentrifuge tube caps to the right to keep them from breaking during the spin.

6. Remove the QIAamp™ MinElute™ column (in a 1.5-mL microcentrifuge tube) from the centrifuge and place it on a tube storage rack.
7. Discard the QIAamp™ MinElute™ column.

   Note: Do not discard the 1.5-mL microcentrifuge tube which contains the purified DNA. The purified gDNA is now called Stock Test gDNA sample.

Quantitation of eluted DNA

The success of the OncoScan™ CNV Plus Assay requires accurate quantification of the DNA by using a dsDNA specific quantification method. It is recommended to use 1 of these 2 methods:

1. Invitrogen™ Quant-iT™ PicoGreen™ dsDNA Assay Kit (Cat. No. P7589)
2. Invitrogen™ Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851)

Store the Stock Test gDNA sample

Select an option below to store the Stock Test gDNA sample:

- For short-term storage, refrigerate for up to 2 weeks.
- For long-term storage, freeze at ≤ –20°C.

E GeneChip™ Fluidics Station 450 care and maintenance

General fluidics station care

- Use a surge protector on the power line to the fluidics station.
- Always use deionized (DI) water to prevent contamination of the lines. Change buffers with freshly prepared buffer at each system startup.
- Always run a **Shutdown** protocol before powering off the instrument. This prevents salt crystals from forming within the fluidics system.
- Perform a **Bleach** protocol to keep the instrument free of contaminants that will impact data quality.
- Leave the sample needles in the lowered position when not using the instrument. Each needle should be lowered into an empty vial. This precaution protects them from accidental damage.
- Have a Thermo Fisher Scientific Service Engineer perform periodic maintenance to help ensure the proper functioning of the instrument.

WARNING! Turn off power to the fluidics station to avoid injury in case of a pump or electrical malfunction before performing any maintenance.

Fluidics station Shutdown protocol

Note: Performing the **Shutdown** protocol helps ensure that the fluidics station needles are washed and that the wash buffer is removed from the lines and replaced with deionized (DI) water. This protocol must be performed on the GeneChip™ Fluidics Station 450 before it can be used to process a different array type, or before it can be powered off.

Perform the **Shutdown** protocol on the fluidics station when:

- Switching to a different array type that requires different wash buffers.
- Before powering off the fluidics station.
- After performing the **Bleach** protocol.

1. Place 3 empty 1.5-mL microcentrifuge tubes in the stain holders of each module. Leave the needle levers in the raised position.
2. Remove the Wash A and Wash B bottles from the fluidics station.
3. Empty the Waste bottle, then place it back on the fluidics station. Place the Waste line in the bottle.
4. Fill the Water bottle with deionized (DI) water, then place it on the fluidics station.
5. Place the Water, Wash A, and Wash B lines in the Water bottle.
6. From the **Fluidics Control Master Control** pane, select the following:
   - **Probe Array Type**: (Leave empty)
   - **Protocol Type**: *Maintenance Protocols*
   - **Protocol**: *SHUTDOWN_450*
7. From the GCDC **Fluidics Control Station Control** pane, select the boxes of the fluidics station modules to shutdown.
8. Click the **Copy to Selected Modules** button.
   The selected protocol (***SHUTDOWN_450***) is applied to the selected stations and modules.
9. Click the **Run All** button.
10. Monitor the prompts in the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window.
11. Lower the needle levers to start the protocol.
12. When the message **Shutdown done, Not Primed** appears in the fluidics station module LCD window, the ***SHUTDOWN_450*** protocol is complete.
13. Empty the Waste bottle, then place it back on the fluidics station. Place the Waste line in the bottle.
14. The Water bottle can stay on the fluidics station. Keep the Water, Wash A and Wash B lines immersed in the Water bottle.
15. Power off the fluidics station.

Shutdown storage suggestions for the GeneChip™ Fluidics Station 450

If:	Then do this:
Not planning to use the system immediately.	Power off the fluidics station.
Not planning to use the system for an extended period (longer than 1 week).	Discard the DI water, then perform a "dry shutdown". Perform the **Shutdown** procedure again, but place the wash lines in an empty bottle. A "dry shutdown" removes water from the fluidics station and helps prevent unwanted microbial growth in the tubing and wash lines. Power off the fluidics station.

Fluidics station Bleach protocol

It is recommended to run the **Bleachv3_450** protocol to clean the fluidics station. This eliminates residual SAPE-antibody complex present in the fluidics station tubing and needles. The protocol consists of 2 cycles, a Bleach Cycle and a Rinse Cycle. The Bleach Cycle runs a bleach solution through the system. The Rinse Cycle runs deionized (DI) water through the system. This protocol takes approximately 1 hour and 40 minutes to complete. Please contact a Thermo Fisher Scientific technical support representative for guidance on the frequency of fluidics station bleaching.

Prepare the bleach solution

IMPORTANT!

- Use commonly purchased sodium hypochlorite bleach that is no more than 6 months old.
- Prepare fresh bleach solution before running the protocol.
- Each fluidics station requires 500 mL of 0.525% sodium hypochlorite solution.

1. Check the sodium hypochlorite percentage on the label of the stock bottle of bleach. Prepare the 0.525% solution based on the stock percentage.

   For example, if the stock solution is 8.25%: In a 1-L graduated cylinder, combine 32.0 mL of 8.25% sodium hypochlorite commercial bleach with 468.0 mL of (DI) water.

2. Pour the solution into a 500-mL plastic bottle.

3. Cap the bottle and mix well by inverting 10 times.

Prepare the fluidics station

IMPORTANT! To avoid carryover, or cross contamination, from the **Bleach** protocol, it is recommended to use dedicated bottles for bleach and DI water. Additional bottles can be obtained from Thermo Fisher Scientific. (Table 31.)

1. Place the 500-mL bottle of freshly prepared bleach (0.525% sodium hyopchlorite solution) on the fluidics station. Place the Wash A, Wash B, and Water lines into the Bleach bottle. (Figure 60.)
2. Fill a 1-L DI Water bottle with deionized (DI) water, then place it on the fluidics station.

   Note: Do not place a line in the DI Water bottle at this time.
3. Empty the Waste bottle, then place it on the fluidics station. Place the Waste line into the Waste bottle.

   IMPORTANT! Ensure that all the bottles are correctly positioned and the appropriate wash lines are placed in the correct bottle.
4. Engage the Wash Blocks.
5. Place 3 empty 1.5-mL microcentrifuge tubes in the stain holders of each module. Leave the needles in the raised position.

Figure 60 Place Wash and Water lines in the Bleach bottle.

Start the Bleach Cycle

1. Start the GCDC Launcher, then click the **Fluidics** button.
   The **Fluidics Control** window appears.
2. From the **Fluidics Control Master Control** pane, select the following:
   - **Probe Array Type**: (Leave empty)
   - **Protocol Type**: *Maintenance Protocols*
   - **Protocol**: *Bleachv3_450*
3. From the **Fluidics Control Station Control** pane, select the boxes of the fluidics station modules to bleach.
4. Click the **Copy to Selected Modules** button. The selected protocol (***Bleachv3_450***) is applied to the selected stations and modules.
5. Click the **Run All** button.
6. Monitor the prompts in the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window.
7. Lower the needle levers to start the protocol.

   Note: The Bleach Cycle takes approximately 30 minutes to complete.
8. When the Bleach Cycle is complete, the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window indicates that the Rinse Cycle can be performed.

Start the Rinse Cycle

Note: When the Bleach Cycle has finished, a Rinse Cycle is performed. This step is essential to remove all traces of bleach from the system. Failure to complete the Rinse Cycle can result in poor data quality.

1. Follow the prompts in the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window.
   a. Raise the needle levers, then discard the used 1.5-mL microcentrifuge tubes.
   b. Place 3 empty 1.5-mL microcentrifuge tubes in the stain holders of each module. Leave the needle levers in the raised position.

c. Remove the Wash A, Wash B, and Water lines from the Bleach bottle, then place them in the DI Water bottle. (Figure 61.)

Note: There is no need to be concerned about bleach remaining in the lines.

Figure 61 Place the wash and water lines in the DI Water bottle.

2. Lower the needle levers to begin the Rinse Cycle.

Note: The Rinse Cycle takes approximately 1 hour to complete.

3. When the Rinse Cycle is complete, the **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window display the message **REMOVE ALL VIALS**.

4. Raise the needle levers, then discard the used 1.5-mL microcentrifuge tubes.
The **Fluidics Control Fluidics Status** pane and the fluidics station module LCD window display the message **BLEACH DONE**.

5. Place 3 empty 1.5-mL microcentrifuge tubes in the stain holders of each module. Lower the needle levers.

Prime storage suggestions for the GeneChip™ Fluidics Station 450

If:	Then do this:
Planning to use the system immediately.	After running the **Bleach** protocol, discard the DI water, then perform a **Prime** protocol using fresh DI water, Wash A and Wash B. Perform a **Prime** protocol before performing a wash and stain protocol on hybridized arrays. Failure to run a **Prime** protocol will result in poor data.
Not planning to use the system immediately.	Power off the fluidics station.
Not planning to use the system for an extended period (longer than 1 week).	Discard the DI water, then perform a “dry shutdown”. Perform the **Shutdown** protocol again, but place the wash lines in an empty bottle. A “dry shutdown” removes water from the fluidics station and helps prevent unwanted microbial growth in the tubing and wash lines. Power off the fluidics station.

Troubleshooting the OncoScan™ CNV Plus Assay

Troubleshooting assay performance

The OncoScan™ CNV Plus Assay includes QC steps to assess the performance of the assay and the data generated from the array. This section details problems, causes, and possible solutions. Contact a Thermo Fisher Scientific technical support representative for additional guidance.

The assay includes 2 in-process QC steps to determine if the sample preparation is working and the QC products meet requirements. If there is an issue, determine if it is sample, equipment, or process related. Decide if the run should continue, or if a sample should not be hybridized.

The In-process QC steps are:

- First QC Gel: This gel confirms the correct size PCR 1 product has been generated. Individual PCR products from both AT and GC channels are run on a gel in adjacent wells. A single band should be visible around 120 bp.
- Second QC Gel: This gel confirms the PCR 2 product has been digested by Hae III. Successful digestion is confirmed by the presence of a double band at 40 bp and 70 bp.

After the sample has been hybridized to an array, the array has 3 Array–QC metrics to determine if the data generated is acceptable. The Array-QC metrics are:

- ndSNPQC: ndSNPQC is a measure of how well normal diploid markers (that is those that have been determined to have Copy Number = 2 in the sample) are resolved in the array data. Larger ndSNPQC values are better.
- MAPD: Median of the Absolute Values of all Pairwise Differences (MAPD) is a global measure of the variation of all microarray probes across the genome. It represents the median of the distribution of changes in log2 ratio between adjacent probes. Since it measures differences between adjacent probes, it is a measure of short-range noise in the microarray data. Lower MAPD values are better.
- ndWavinessSD: Normal Diploid Waviness Standard Deviation (ndWavinessSD) is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. ndWavinessSD is computed on normal diploid markers.

  ndWavinessSD should be used along with Low Diploid Flag, ndCount (the actual number of diploid markers identified) BAFs, and log2 ratio to assess if the log2 ratio is centered correctly. ndWavinessSD can thus help assess if log2 ratios need to be re-centered.

  When ndWavinessSD is high, the log2 ratios should be examined for clear breakpoints as opposed to a gradual drift of the log2 ratio. When the latter is observed small aberrations should be examined carefully. When breakpoints are sharp and the ndCount is large, a high ndWavinessSD can be ignored.

Observation	Possible cause	Recommended action
Faint or no PCR product visible on the 1st PCR QC gel image in both AT and GC channel for a given sample.	Low input starting material.	Quantitate the input gDNA using a PicoGreen™ or Qubit™ assay to ensure 80 ng of starting material.
	Failed anneal reaction.	Mis-pipetting in the Anneal step by not adding the probe mix or the input DNA can result in no PCR bands. Repeat Assay from the beginning.
	Sample type.	Check to see if this sample might contain chemical or enzymatic inhibitors. If so, try cleaning sample over a column and starting the assay from the beginning.
Faint or no PCR product visible on the 1st PCR QC gel image in AT or GC channel for a given sample.	Pipetting error during Gel QC.	Run the first PCR from both channels again to ensure there was not a pipetting error in loading the gel.
	Pipetting error in Pre-PCR step.	If the repeat gel shows the same faint/no PCR band in a given channel, there was likely a mis-pipetting step after the Channel Split in the Pre-PCR stage. Repeat this sample from the beginning of the assay.
Smeared or multiple bands in PCR Gel.	Low input starting material.	Quantitate the input gDNA using a PicoGreen™ or Qubit™ assay to ensure 80 ng of starting material.
PCR product in the negative control.	Reagents or equipment contaminated with amplified product.	Always use filter pipette tips. Clean the Pre-PCR Room and equipment thoroughly using 10% bleach. Decontaminate the pipettes following manufacturer's recommendation. Retrain personnel on Pre-PCR Room best practices. Repeat the assay using fresh reagents and sample. ***Do not open*** the seal of the amplified **1st PCR Plate** in the Pre-PCR Room. Do not store the **1st PCR Plate** in the Pre-PCR Room.

Observation	Possible cause	Recommended action
Hae III gel smeared, no distinct double bands.	Forgot to pause the thermal cycler at 88 minutes and remove an aliquot for the for Gel QC.	Check your gel against the example gel in which the aliquot was taken after the 95°C incubation.
Hae III gel does not show a double band pattern at 40 and 70 bp, only a single band at 120 bp.	The HaeIII digestion failed.	Do not hybridize the samples.
		Repeat the HaeIII reaction using the **2nd PCR Plate**. If the Second QC Gel shows a double band pattern, proceed.
Dim array.	Fluidics stations needs the bleach protocol run.	Dim arrays (low signal on the array) might indicate that the fluidics stations need the bleach maintenance performed. We recommend bleaching the fluidics station once a week. The peristaltic tubing needs to be changed every 5—6 weeks.
The .cel file is not generated.	Signal from corner checkerboards is dim.	Ensure that the GeneChip™ Hybridization Oven 645 is calibrated and set to the correct temperature. Confirm that Stain 1 and Stain 2 are placed in the correct order on the fluidics station. Ensure that Stain Buffer 1 is stored in the dark when not in use. Use only those reagents provided by Thermo Fisher Scientific.
	One or more of the sub-grids in the .dat file image were not gridded by the software.	Follow the GCDC manual griding procedure to fix the grids and regenerate the .cel file.

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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, and so on). To obtain SDSs, visit **thermofisher.com/support**.

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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number	Description
OncoScan™ CNV Plus Assay Site Preparation Guide	MAN0027851	Provides guidance on reagents, instruments, and supplies required to run the OncoScan™ CNV Plus Assay workflow.
OncoScan™ CNV Plus Assay—7 Samples Quick Reference	MAN0029389	An abbreviated reference for the assay protocol steps of the OncoScan™ CNV Plus Assay workflow for 7, 9, 13, and 25 samples. These quick reference documents are intended for experienced users.
OncoScan™ CNV Plus Assay—9 Samples Quick Reference	MAN0029622	
OncoScan™ CNV Plus Assay—13 Samples Quick Reference	MAN0029623	
OncoScan™ CNV Plus Assay—25 Samples Quick Reference	MAN0027126	
GeneChip™ Data Collection Software (GCDC) User Guide	MAN0026726	This user guide provides instructions on using GeneChip™ Data Collection Software (GCDC) used to control GeneChip™ instrument systems. GeneChip™ Data Collection Software provides an intuitive set of tools for instrument control and data management used in the processing of Applied Biosystems™ cartridge arrays.
Chromosome Analysis Suite User Guide	MAN0027798	This user guide provides instructions on using the Chromosome Analysis Suite Software (ChAS) for in-depth CN result exploration. The software enables cytogenetic analysis to view and summarize chromosomal aberrations, including copy number gain or loss, loss of heterozygosity segments, or variant data, across the genome.

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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

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.