Quick Reference Card

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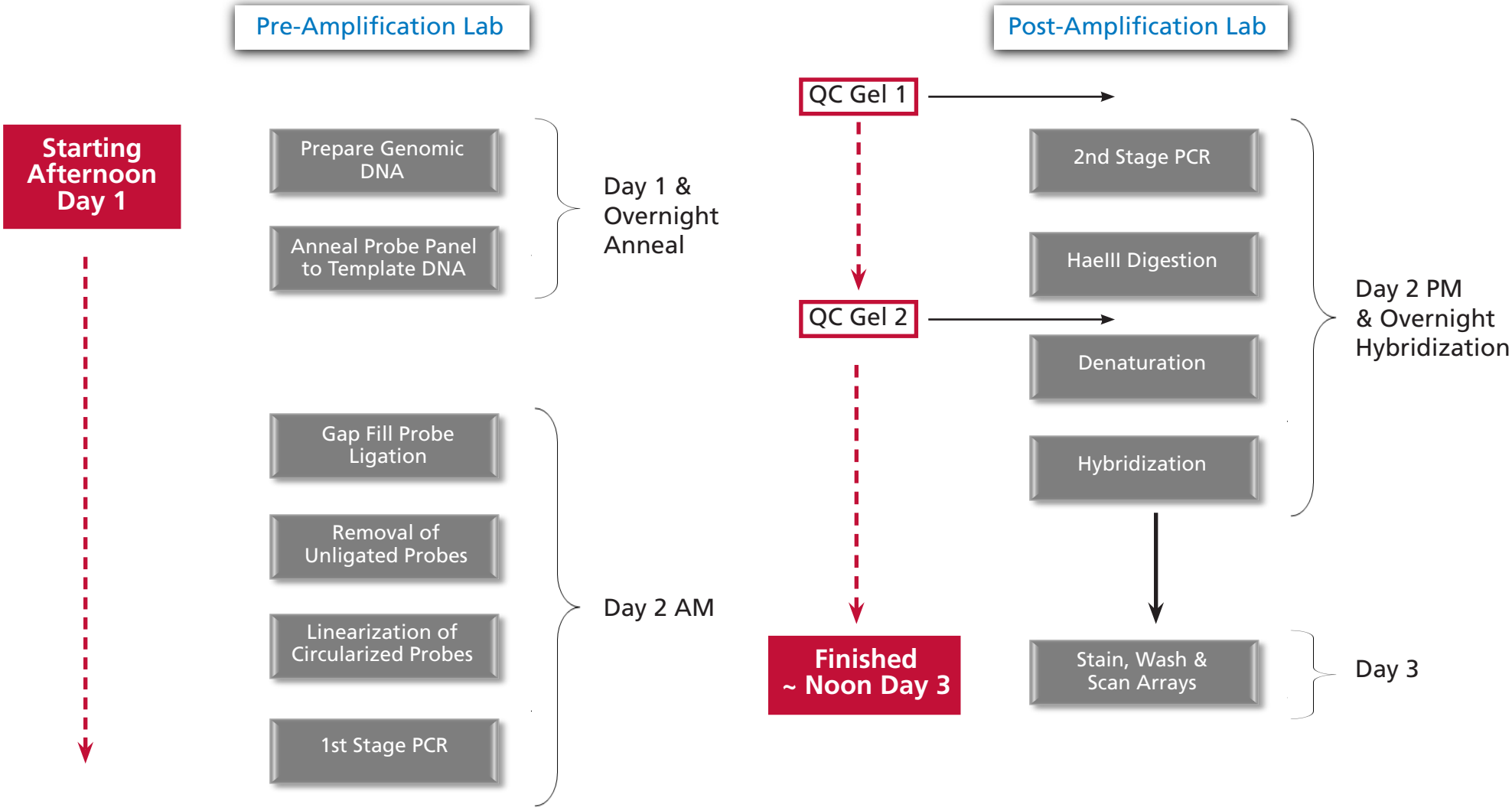
OncoScan™ FFPE Assay Kit – 25 Samples

Workflow Overview

The Affymetrix® OncoScan™ FFPE Assay Kit protocol is optimized for processing 1 to 23 samples at a time to obtain whole genome copy number and Somatic Mutation information from FFPE samples using Affymetrix® OncoScan™ Tag Arrays. The OncoScan™ FFPE Assay Kit QRCs support processing of as few as 7 samples, two of which are a positive and negative control. This QRC is for 25 samples (including 23 FFPEs, 1 pos ctrl, 1 neg ctrl). This protocol is intended for research use only and is not intended for diagnostic purposes.

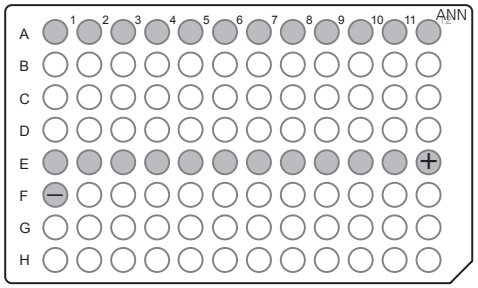


General Procedures

- The Negative Control for the 23 sample QRC is in a different row from the samples and positive control. When pipetting the reagents for this negative control well, use reagents from the master mix tube and not from the strip tubes.
- Vortex Plate after reagent addition: Always vortex plates at max speed for 5 seconds using 5-point plate vortexing.
- Spin down plate after reagent addition: Always spin at 2400 rpm for 1 min.
- Spin down plate after 1 min chill step: Always spin at 2400 rpm for 30 sec.
- Vortex Enzymes: Always vortex for 1 second at max speed.
- Spin down enzymes: Quick spin down.
- Vortex Master Mix tubes: Vortex at max speed for 3 seconds.
- Spin down Master Mixes: Quick spin down.
- Plate handling before reagent addition: Reagents are always added to chilled plates. Chill the plate on a cold block for 1 min, then spin down at 2400 rpm for 60 sec.
- When pipetting, always pipette up and down 3 times to rinse the tips.
- When removing enzymes from the –20 °C freezer, use a cooler to transfer the tube to and from the bench.
- Always remove plate seals slowly and carefully as to not introduce any cross contamination from well-to-well.
- Ensure both plates are at the correct orientation (well A1 at the top left position) during plate-to-plate transfers.
- Program all of the thermal cyclers programs in "9700 Max mode" setting.

Sample Plate (Pre-Amp)

1. Add 6.6 µL FFPE gDNA at 12 ng/µL to wells marked A1 through A12, and E01-E11 in the plate diagram.
2. Add 6.6 µL of the positive control from the OncoScan FFPE Assay Kit to well marked E12.
3. Add 6.6 µL of TE (negative control) to the well marked F1 in the plate diagram.
4. Seal, vortex and spin the plate. Place plate on cold block if continuing assay. Otherwise freeze at –20 °C.

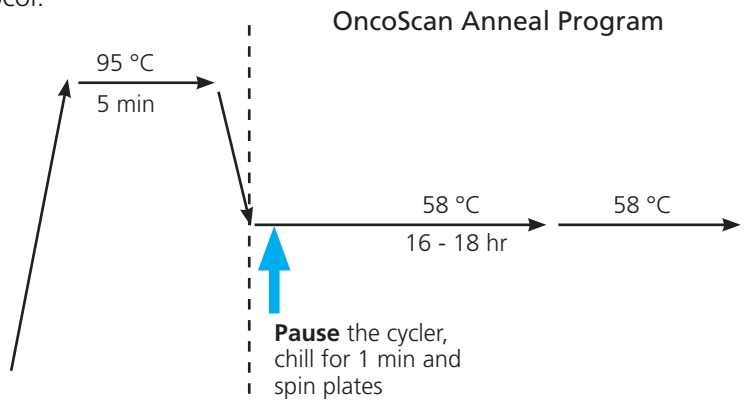


Stage 1 – Anneal (Pre-Amp)

1. Turn on the thermal cycler.
2. Thaw reagents listed in the table at room temperature.
3. Once thawed, vortex, spin down and place on ice.
4. Label a 1.5 mL Eppendorf tube as *Ann* and place on ice.
5. Place 12-tube strip in cold block.
6. To the *Ann* tube, add the reagents listed in the table below.

Reagent	1 Reaction	25 Reactions (~60% overage)
Buffer A	1.53 µL	61.2 µL
Copy Number Probe Mix 1.0	1.37 µL	54.8 µL
Somatic Mutation Mix 1.0	0.5 µL	20.0 µL
Total Volume	**3.40 µL**	**136.0 µL**
Volume per Tube for Tube Strip		10.0 µL 12-Tube Strip

7. Vortex and spin down ANN Master Mix.
8. Aliquot 5.0 µL of ANN Master Mix to each strip tube.
9. Transfer 3.4 µL of ANN Master Mix from the strip tube to wells A1-12, E1-12 and F1. Pipet up and down 3X.
10. Seal, vortex, and spin plate.
11. Place the plate on the thermal cycler and start the *OncoScan Anneal* program.
12. After 6 minutes, **Pause** the program at 58 °C.
13. Remove the plate and place on a chilled cold block for 1 minute.
14. Spin down the plate. Place plate back on the thermal cycler and **Resume** the protocol.



Blue arrows indicate reagent addition steps.

Stage 2 — Gap Fill Through 1st PCR (Pre-Amp)

Thaw Buffer A, dNTPs (AT and GC) Cleavage Buffer, Water and PCR Buffer at room temperature. Vortex, Spin down and keep on ice.

Prepare the AT Mix and GC Mix: Always Make 24-Reaction Master Mix

1. Label two 1.5 mL Eppendorf tubes, one **AT** in **blue** and the other **GC** in **red**. Place on ice.
2. Place two 12-tube strips in a cold block, label one **AT** in **blue**, the other **GC** in **red**.
3. To the AT tube, add the reagents listed in the table below. Pipet 3X. Vortex, spin, place on ice.

Reagent	1 Reaction	25 Reactions (~20% overage)
Water	3.93 µL	118 µL
dNTPs (A/T)	0.07 µL	2.2 µL
Total Volume	**4.0 µL**	**120 µL**
Volume per Tube for Tube Strip		9.6 µL 12-Tube Strip

4. Aliquot 9.6 µL of the AT Mix to each tube in the strip.
5. Spin down the strip and keep in cold block.
6. Change gloves.
7. Repeat steps 3-5 above for the GC dNTPs.

Reagent	1 Reaction	25 Reactions (~20% overage)
Water	3.93 µL	118 µL
dNTPs (G/C)	0.07 µL	2.2 µL
Total Volume	**4.0 µL**	**120 µL**
Volume per Tube for Tube Strip		9.6 µL 12-Tube Strip

8. Change gloves again after completing the GC Master Mix.

Prepare the GAP Fill Mix

1. Label a 1.5 mL Eppendorf tube and one strip of 12 PCR tubes with the letter *G*.
2. Place tube on ice and strip tubes in cold block.
3. To the *G* tube, add the reagents in the table below. Pipet up and down 3X.

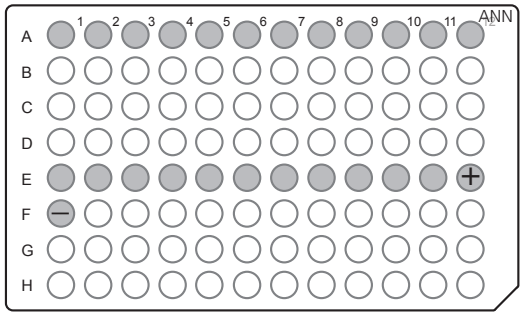
Reagent	1 Reaction	25 Reactions (~20% overage)
Water	10.58 µL	318 µL
Buffer A	1.18 µL	35.3 µL
SAP, Recombinant (1U/µL)	0.84 µL	25.2 µL
Gap Fill Enzyme	1.40 µL	42.0 µL
Total Volume	**14.0 µL**	**420 µL**
Volume per Tube for Tube Strip		33 µL 12-Tube Strip

4. Vortex, spin down, and place on ice.
5. Aliquot 16 µL of the Gap Fill Master Mix to each tube in the strip.
6. Spin down and place on a chilled cold block.

Addition of Gap Fill Mix

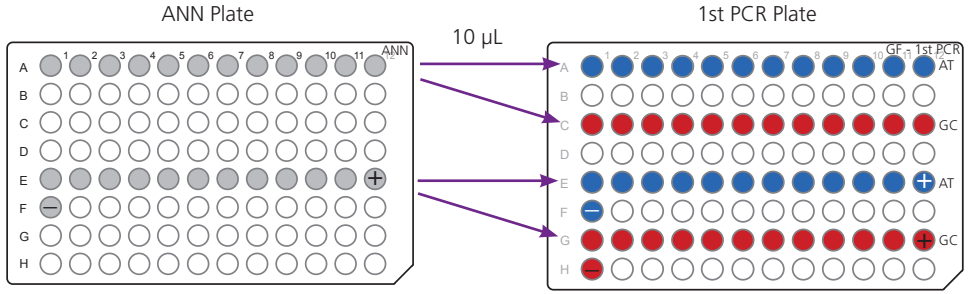
1. Remove the *ANNEAL* Plate from the thermal cycler, place in a cold block on ice for 1 min.
2. Stop the *OncoScan Anneal* program
3. Start the *OncoScan Gap Fill*, **Pause** once cycler reaches 58 °C.
4. After the 1 min incubation, spin the *ANNEAL* Plate and return to cold block.
5. Add 14.0 µL Gap Fill (*G*) Mix to each well. Pipet up and down 3X.
6. Seal the plate, vortex and spin.
7. Keep the *ANNEAL* Plate on the cold block.

25 Samples
10 µL + 14 µL = 24 µL

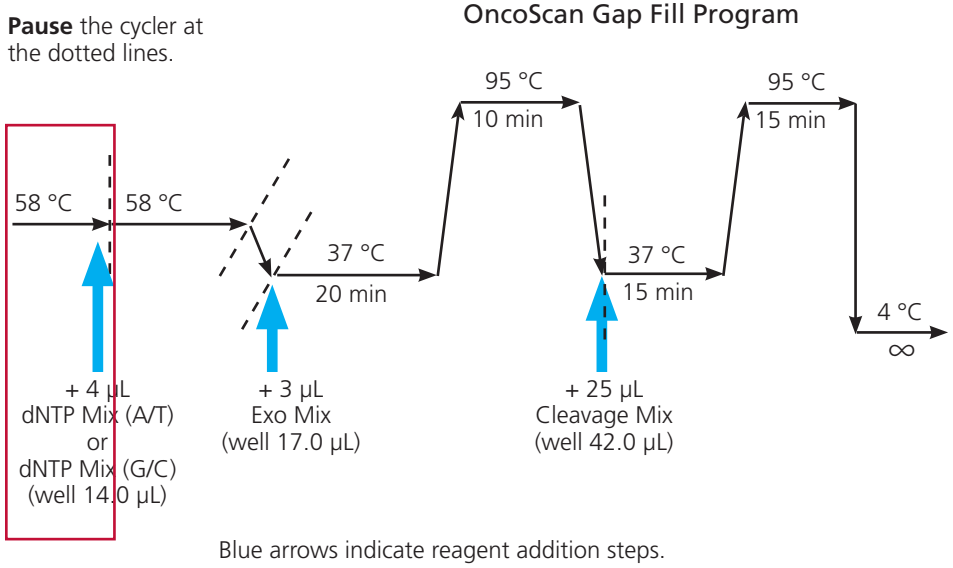


Channel Split

1. Label a new 96-well half-skirt PCR plate '*1st PCR*'.
2. Clearly mark the **AT** (**blue**) and **GC** (**red**) rows (see Figure below).
3. Transfer 10.0 µL each well from the *ANN* Plate to the *1st PCR* Plate, as shown.



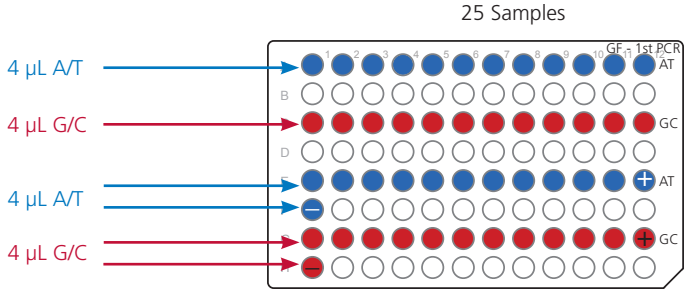
4. Seal and spin down the 1st PCR Plate.
5. Load the plate on the thermal cycler, close the lid, and **Resume** the *OncoScan Gap Fill* program.
6. After 11 min at 58 °C, press **Pause**.
7. Remove the *1st PCR* Plate and place on a cold block for 1 min.
8. Spin down and return the *1st PCR* Plate to a cold block.
9. Move directly to the next section: **Addition of dNTPs**.

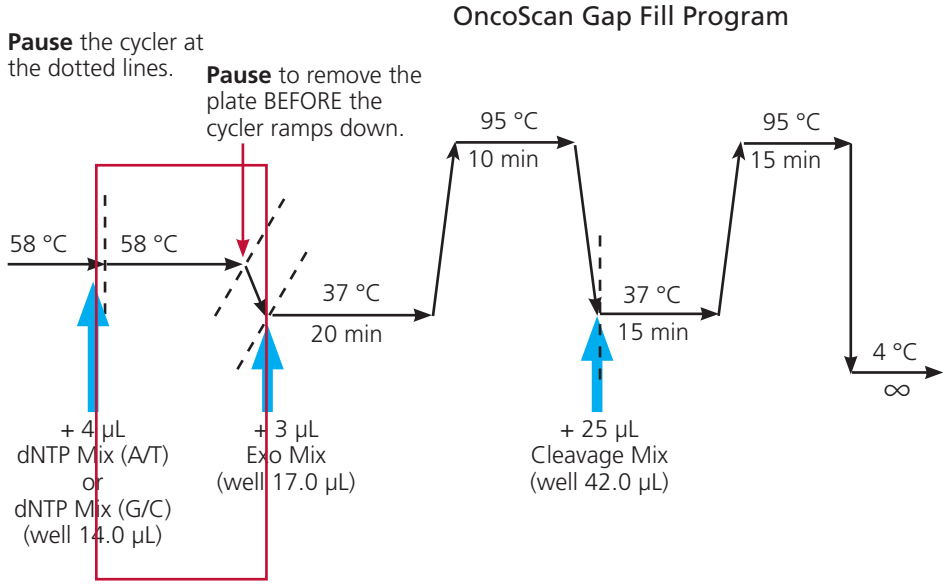


Blue arrows indicate reagent addition steps.

Addition of dNTPs

1. Add 4 µL of AT Mix to samples in rows A and E and well F1 on the *1st PCR* Plate. Pipet up and down 3X.
2. Add 4 µL of GC Mix to samples in rows C and G and well H1 on the *1st PCR* Plate. Pipet up and down 3X.
3. Seal, vortex, and spin the plate.
4. Place the *1st PCR* Plate back on the thermal cycler, press **Resume**.
5. Set a timer for 10 min to be back to **Pause** the program at the end of the 58 °C.
6. During this 11 min incubation, prepare the Exo Mix described in the next section: **Aliquot and Add the Exo Mix**.

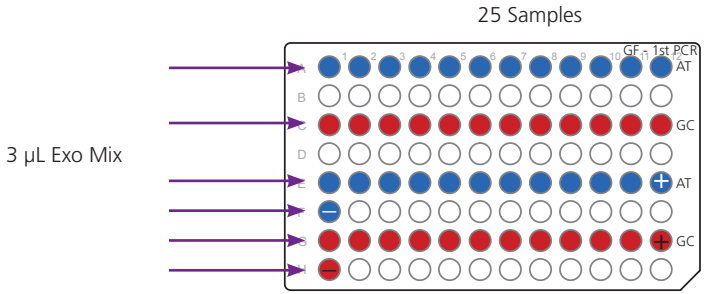


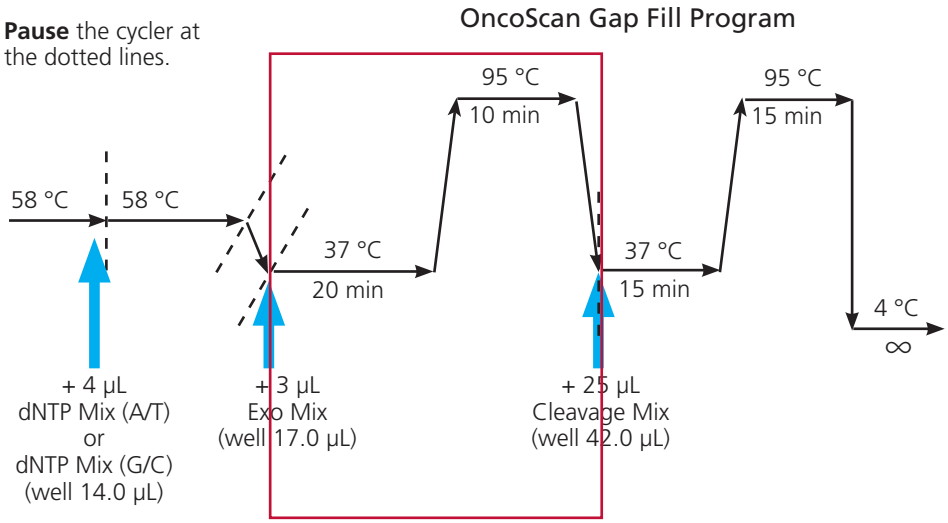


Blue arrows indicate reagent addition steps.

Aliquot and Add the Exo Mix

1. Remove Exo Mix from –20 °C (keep cold). Vortex Exo Mix and keep on ice.
2. Label one strip of 12 tubes with the letter *E*. Aliquot 16.0 µL of the Exo Mix directly into each strip tube. Spin to remove bubbles.
3. At the end of the 58 °C incubation, press **Pause** and place the *1st PCR* Plate in a cold block for 1 min.
4. **Resume** the program (no plate) and **Pause** thermal cycler once it has ramped down to 37 °C.
5. After the 1 min on ice, spin down *1st PCR* Plate, return to cold block.
6. Add 3 µL Exo Mix to each reaction. Pipet up and down 3X.
7. Seal, vortex, and spin the *1st PCR* Plate.
8. Place the *1st PCR* Plate back on the thermal cycler.
9. Press **Resume**.
10. Set a timer for 25 min to be back in order to prepare the Cleavage Mix as described in the next section: **Prepare and Add the Cleavage Master Mix**.





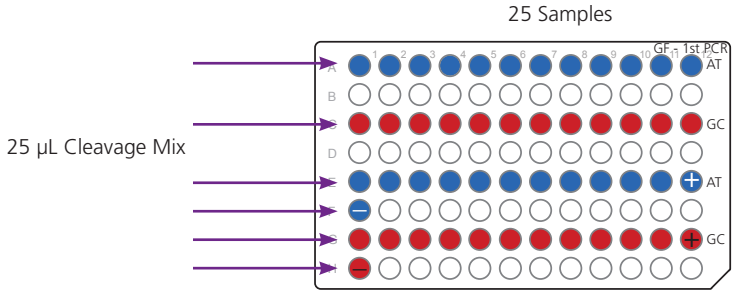
Blue arrows indicate reagent addition steps.

Prepare and Add the Cleavage Master Mix

1. Place 12-tube strip in a cold block and a 1.5 mL Eppendorf tube labeled *CM* on ice.
2. Remove Cleavage Mix from –20 °C (keep cold). Vortex, spin and keep on ice.
3. Vortex and spin down the thawed Cleavage Buffer, return to ice.
4. Add the Cleavage Buffer and Cleavage Enzyme to the *CM* tube. See table below.

Reagent	1 Reaction	50 Reactions (~25% overage)
Cleavage Buffer	25.0 µL	1563 µL
Cleavage Enzyme	0.2 µL	12.5 µL
Total	**25.2 µL**	**1575.0 µL**
Volume per Tube for Tube Strip		120 µL 12-Tube Strip

5. Pipet up and down 3X.
6. Vortex and spin down the *CM* tube briefly. Keep on ice.
7. Aliquot 60 µL Cleavage Master Mix from CM tube to 12 tubes of the strip tube.
8. Spin down strip tubes. Place on a cold block.
9. Press **Pause** when cycler reaches 37 °C, remove the *1st PCR* Plate and place a cold block on ice for 1 min.
10. Spin plate and return plate to cold block.
11. Add 25 µL CM Mix to each reaction. Pipet up and down 3X.
12. Seal, vortex, and spin down.
13. Place *1st PCR* Plate back on thermal cycler, press **Resume**.
14. Set a timer for 25 min to make the PCR mix described in the next section: **Prepare and Add the 1st PCR Master Mix**.

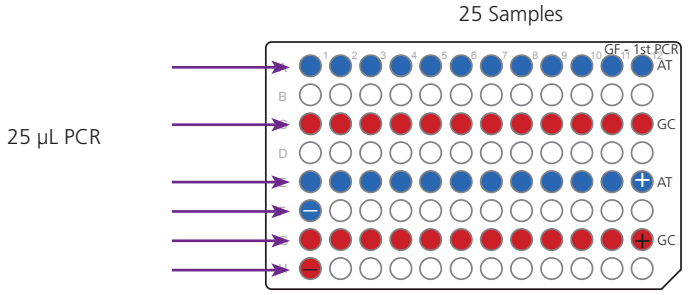


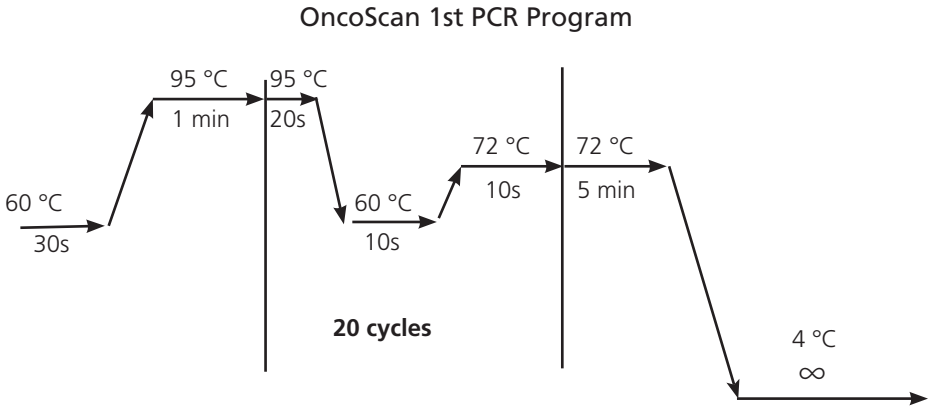
Prepare and Add the 1st PCR Master Mix

1. Label a 12-strip tube, place in a cold block.
2. Label a 1.5 mL Eppendorf tube with the letters *PCR* and place on ice.
3. Remove the Taq from the –20 °C (keep cold). Vortex, spin and return to ice.
4. Vortex, spin down the thawed PCR Mix. Return to ice.
5. Add reagents according to the table below to the *PCR* tube. Pipet up and down 3X.

Reagent	1 Reaction	50 Reactions (~25% overage)
PCR Mix	24.4 µL	1528 µL
Taq Polymerase	0.56 µL	34.9 µL
Total	**25.0 µL**	**1562 µL**
Volume per Tube for Tube Strip		120.0 µL 12-Tube Strip

6. Vortex, spin down and return the *PCR* tube to ice.
7. Aliquot 60 µL of the PCR Master Mix to each of the 12 tubes in the strip.
8. Spin down strip tube. Place strip tube in cold block.
9. When *OncoScan GAP Fill* program ends, remove the *1st PCR* Plate, place on a cold block for 1 min.
10. Vortex, spin down the *1st PCR* Plate.
11. Start the *OncoScan 1st PCR* program, **Pause** the program when thermal cycler reaches 60 °C.
12. Add 25 µL PCR Master Mix to each reaction. Pipet up and down 3X.
13. Seal, vortex, and spin the *1st PCR* Plate.
14. Place on thermal cycler, press **Resume**. At the end of *OncoScan 1st PCR* program, take the plate directly to the Post-Amp Lab.

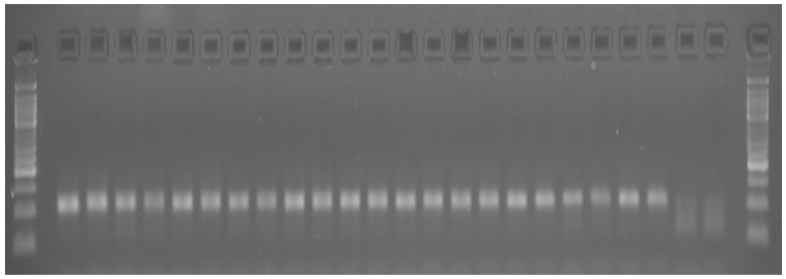




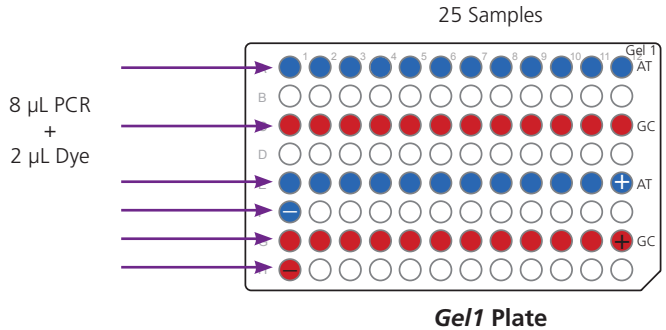
Stage 3 — First QC Gel and 2nd PCR (Post-Amp Lab)

Prepare the QC Gel 1 Plate

1. Label one fresh PCR plate *Gel1*.
2. Prepare diluted gel loading dyes and diluted buffers as instructed on the right.
3. Aliquot 8 µL of *1st PCR* Plate product to wells 1-7.
4. Add 2 µL of 1:10 gel loading dye. Pipet up and down 3X.
5. Seal *1st PCR* Plate and keep in a cold block on ice.
6. Seal, vortex, and spin down.



7. Load 10 µL of each reaction onto a 3% agarose gel.
8. Add 5.0 µL of the diluted 50 bp ladder to the lanes marked as "M".
9. Run the gel at 150 V for 15 min.
10. Examine the gel in a gel imager to ensure PCR products are approximately 120 bp.



***Gel1* Plate**

Reagents for Gel Loading

1. Glycerol-EDTA Buffer (50% Glycerol + 50 mM EDTA).

Reagent	Initial Concentration	Final Concentration	Volume
100% Glycerol	100%	50%	1000.0 µL
0.5 M EDTA	500 mM	50 mM	200.0 µL
Nuclease-Free Water	N/A	N/A	800.0 µL
Total Volume			**2000.0 µL**

2. 1:10 Dilution of 6X Gel Loading Dye (store at 4 °C for long-term storage).

Reagent	Volume
Glycerol-EDTA Buffer (50% Glycerol + 50 mM EDTA)	900.0 µL
NEB 6X Loading Dye	100.0 µL
Total Volume	**1000.0 µL**

3. Dilution of the 50 bp ladder (1 mL of the diluted ladder can be used in ~200 lanes, Store at –20 °C for long-term storage).

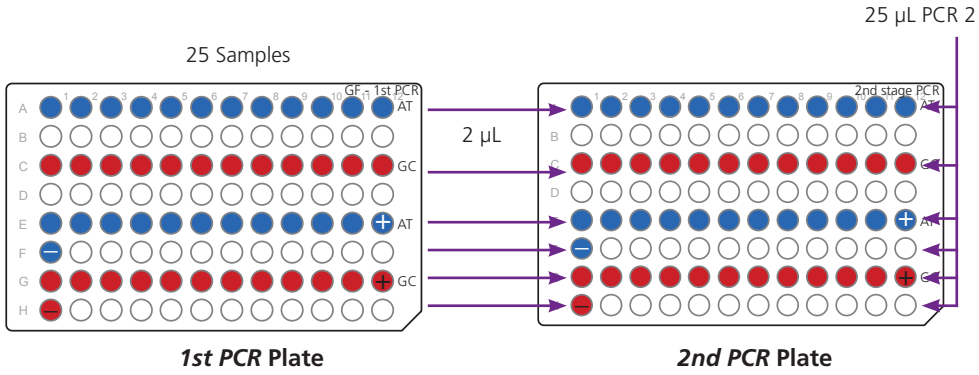
Reagent	Volume
Glycerol-EDTA Buffer (50 % Glycerol + 50 mM EDTA)	830.0 µL
NEB 6X Loading Dye	100.0 µL
NEB 50 bp Ladder	70.0 µL
Total Volume	**1000.0 µL**

Prepare and Add the 2nd PCR Master Mix

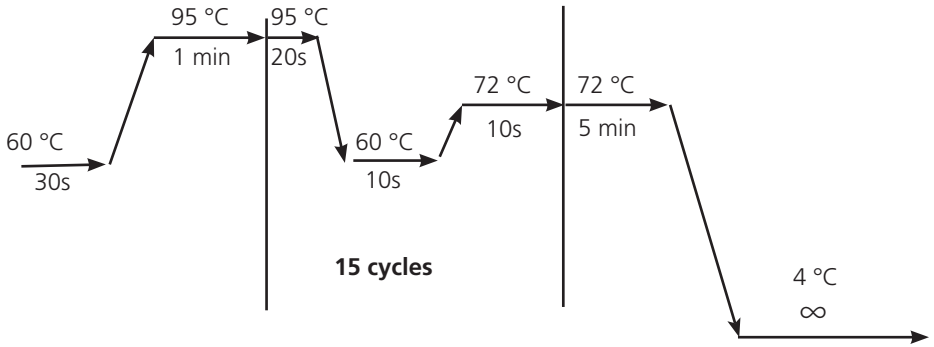
1. Start the *OncoScan 2nd PCR* program and **Pause** cycler reaches 60 °C.
2. Thaw the PCR Mix at room temperature.
3. Label a new 96-well half-skirt PCR plate as *2nd PCR* and keep on cold block.
4. Label row A as "**AT**" in **BLUE** and row C as "**GC**" in **RED**.
5. Label a 12-tube strip and a 1.5 mL Eppendorf tube as *PCR 2*.
6. Place strip tube in a cold block and the *PCR 2* tube on ice.
7. Remove the Taq from the –20 °C (keep cold).
8. Vortex and spin the Taq. Return to ice. Vortex and spin down the thawed PCR Mix and return to ice.
9. Add the PCR Mix and Taq Enzyme according to the table below to the *PCR 2* tube. Pipet up and down 3X.

Reagent	1 Reaction	50 Reactions (~25% overage)
PCR Mix	24.4 µL	1528 µL
Taq Polymerase	0.56 µL	34.9 µL
Total	**25.0 µL**	**1562 µL**
Volume per Tube for Tube Strip		120.0 µL 12-Tube Strip

10. Vortex and spin down the *PCR 2* tube. Keep on ice.
11. Aliquot 60 µL of the PCR Master Mix to each of the 12 tubes in the strip.
12. Spin down strip and return to cold block.
13. Add 25 µL PCR 2 Master Mix to the wells in the new *2nd PCR* Plate.
14. Add 2 µL from each well of the *1st PCR* Plate to the wells in the *2nd PCR* Plate. Pipet mix 3X.
15. Vortex, spin, and place the *2nd PCR* Plate on the thermal cycler.
16. Run the *OncoScan 2nd PCR* program.



OncoScan 2nd PCR Program



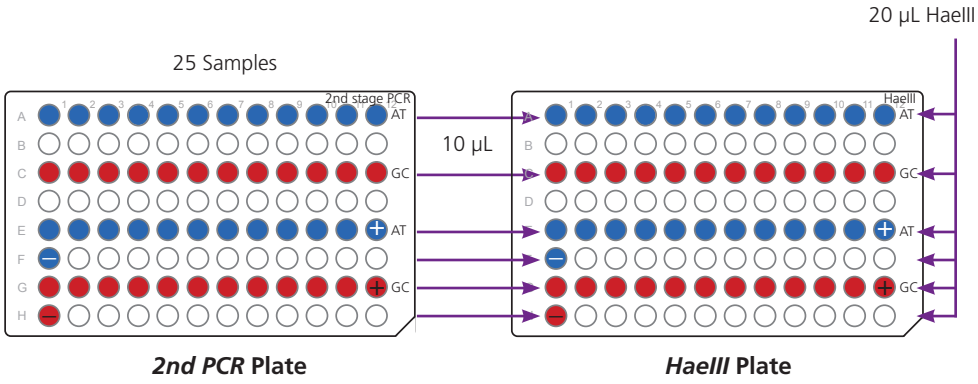
Stage 4 — HaeIII Digest and Second QC Gel (Post-Amp Lab)

Prepare and Add the Hae III Digest

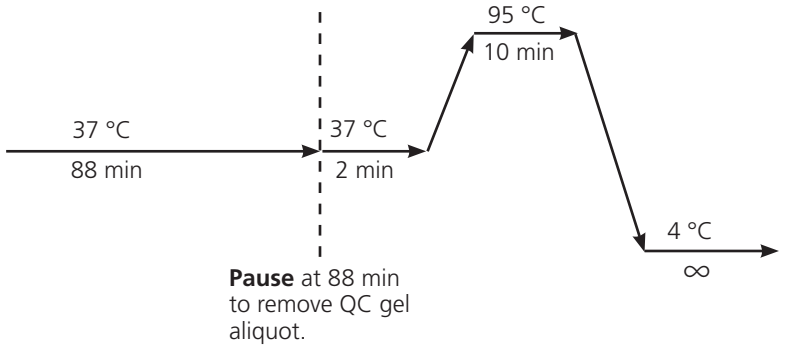
1. Thaw Buffer B at room temperature. Once thawed, vortex, spin and place on ice.
2. Label one fresh 96-well half-skirt PCR plate *HaeIII*, label row A as "**AT**" in **Blue** and row C as "**GC**" in **Red**.
3. Place *HaeIII* Plate in a cold block.
4. Upon completion of *OncoScan 2nd PCR* program, place the *2nd PCR* Plate on a cold block for 1 min. Vortex, spin down, and return to cold block.
5. Label a 12-tube strip and a 1.5 mL Eppendorf tube with *HaeIII* and place on cold block or ice.
6. Remove the Hae III and Exo enzymes from the –20 °C. Vortex and spin down. Place on ice.
7. Add the reagents according to the table to the *HaeIII* tube. Pipet up and down 3X.

Reagent	1 Reaction	50 Reactions (~20% overage)
Buffer B	19.10 µL	1146 µL
HAE III Enzyme	0.40 µL	24.0 µL
Exo Enzyme	0.50 µL	30.0 µL
Total	**20.0 µL**	**1200 µL**
Volume per Tube for Tube Strip		96.0 µL 12-Tube Strip

8. Vortex and spin the *HaeIII* tube. Keep on ice.
9. Aliquot 45 µL of the HaeIII Master Mix to a 12-tube strip. Spin down and place on a cold block.
10. Add 20 µL of HaeIII Master Mix to each well of the *HaeIII* Plate.
11. Add 10 µL of *2nd PCR* Plate wells to the *HaeIII* Plate. Pipet up and down 3X.
12. Seal, vortex, and spin down the *HaeIII* Plate.
13. Place the plate on a thermal cycler and run *OncoScan HaeIII* program.
14. Set timer for 85 min for Hae III QC Gel.



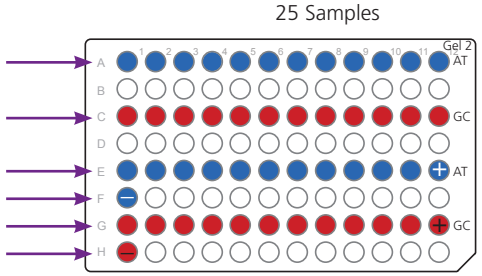
OncoScan HaeIII Program



Prepare and Run the Second Quality Control Gel

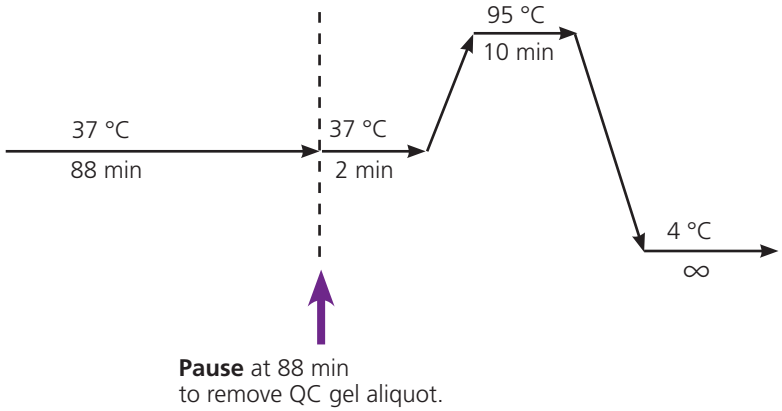
1. Label a 96-well PCR plate *Gel2*.
2. Add 4 µL of 1X TE to the gel plate.
3. Add 2 µL of 1/10th diluted Gel Loading Dye to each reaction. Pipet up and down 3X.
4. At 88 min at 37 °C during the *OncoScan HaeIII* program, **Pause** the cycler.
5. Remove the *HaeIII* Plate, and place it on a cold block for 1 minute.
6. Vortex, spin down, and place on cold block.
7. Remove 4 µL of HaeIII digest sample and add it to the HaeIII QC Gel plate.
8. Seal the *HaeIII* Plate and place it back on the thermal cycler. **Resume** the *OncoScan HaeIII* program.
9. Seal the *Gel2* Plate. Vortex and spin down.
10. Load 10 µL of each reaction and 3.5 µL of the diluted 50 bp ladder onto a 3% agarose gel.
11. Run the gel at 150 V for 15 min.
12. Examine the gel in a gel imager to ensure that you see a double band.

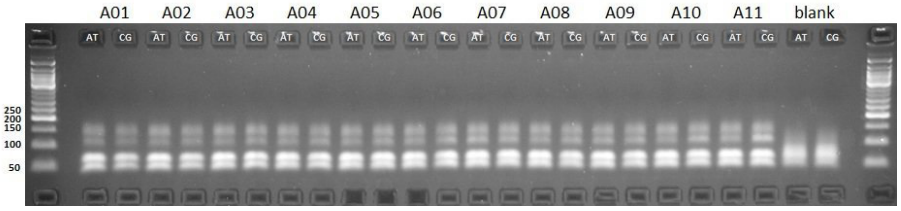
4 µL TE +
2 µL Dye +
4 µL HaeIII Product



***Gel 2* Plate**

OncoScan HaeIII Program



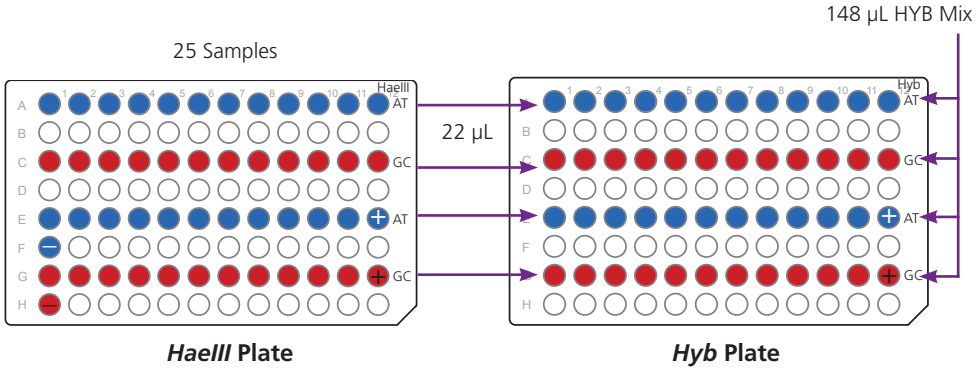


Stage 5 — Hybridization (Post-Amp Lab)

1. Unpack the arrays and equilibrate to room temperature.
2. Preheat the hybridization oven for an 1 hr at 49 °C with rotation.
3. Create a Batch Registration file (AGCC) or Test Requests (AMDS).
4. Prepare the Hybridization Master Mix in a 15 mL conical tube on ice.

Reagent	For 1 Array	48 Arrays
Water	30.0 µL	1.80 mL
Hybridization Mix	118.0 µL	7.08 mL
Total	**148.0 µL**	**8.88 mL**

5. Vortex, spin down briefly, and place it on ice.
6. Once *OncoScan HaeIII* program is complete, remove the *HaeIII* Plate. Vortex, spin down, place on ice.
7. Label a fresh 96-well plate as *Hyb*. Label row A "**AT**" in **Blue** and row C "**GC**" in **Red**.
8. Place reagent reservoir on ice and pour Hyb Master Mix into reservoir.
9. Aliquot 148 µL of Hybridization Master Mix to the appropriate wells of the *Hyb* Plate.
10. Transfer 22 µL of each reaction from the *HaeIII* Plate to the *Hyb* Plate. Pipet up and down 3X.

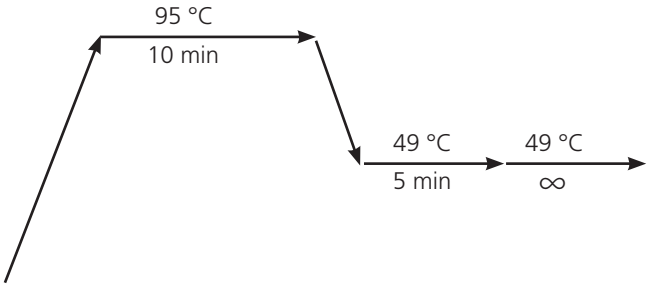


***HaeIII* Plate** ***Hyb* Plate**

148 µL HYB Mix +
22 µL HaeIII Product =
Total volume each well: 170 µL

11. Seal, vortex, and spin the *HYB* Plate.
12. Place the *Hyb* Plate on the thermal cycler.
13. Start the *OncoScan Hybridization* program.

OncoScan Hybridization Program



Load Arrays

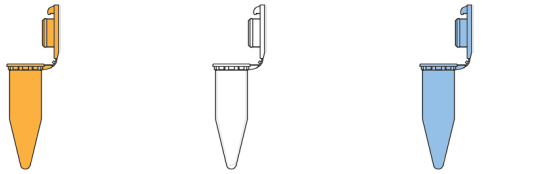
Allow the samples to incubate at 49 °C for at least 5 min before loading.

1. Leave the samples on the thermal cycler, load 160 µL of sample onto each array using a single-channel P200 pipette. Only hybridize up to 8 arrays at a time.

2. Clean any excess fluid from around the septa.
3. Apply Tough-Spots® to the septa and press firmly.
4. Immediately load the arrays into the hybridization oven, four at a time.
5. Hybridize the arrays 16 to 18 hrs at 49 °C and 60 rpm.
6. After 16 to 18 hrs of hybridization, move on to the Wash, Stain, and Scan portion of the assay.

Wash, Stain and Scan

1. Aliquot the following reagents into separate 1.5 mL micofuge tubes for each array:
   - ☐ 500 µL Stain Buffer 1 into amber tubes
   - ☐ 500 µL Stain Buffer 2 into clear tubes
   - ☐ 1000 µL Array Holding Buffer 1 into blue tubes



500 µL Stain Buffer 1 | 500 µL Stain Buffer 2 | 1000 µL Array Holding Buffer

2. Prime the Fluidics Station with the Wash buffers. Load the stain solutions and select the appropriate Fluidics Protocol: **OncoScan_450**
3. Start the Fluidics Protocol and leave the cartridge lever down in the **Eject** position.
4. Remove the Tough-Spots® from each array.
5. Load the arrays onto the Fluidics Station.

Before Scanning

1. Ensure no bubbles are visible through the window.
2. Cover the septa with Tough-Spots®, then load onto the scanner.
3. Scan the arrays as described in the *OncoScan™ FFPE Assay Kit User Manual* (P/N 703175).

Important Points

- Aliquot Stain 1 Buffer into amber tubes.
- Aliquot Array Holding Buffer into blue tubes.
- Stain Buffer 1 and Array Holding Buffer are light sensitive.
- If there is a delay after aliquoting into the tubes, store the tubes at 4 °C, protected from light.
- Remove the bubbles from the arrays on the Fluidics Station (see the *Affymetrix® GeneChip® Fluidics Station 450/250 User's Guide*, P/N 08-0092) or remove the bubbles manually.

For Research Use Only
Not for use in Diagnostic Procedures.

P/N 703189 Rev. 2