KaryoStat™ Assay 8, 16, and 24 Samples

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

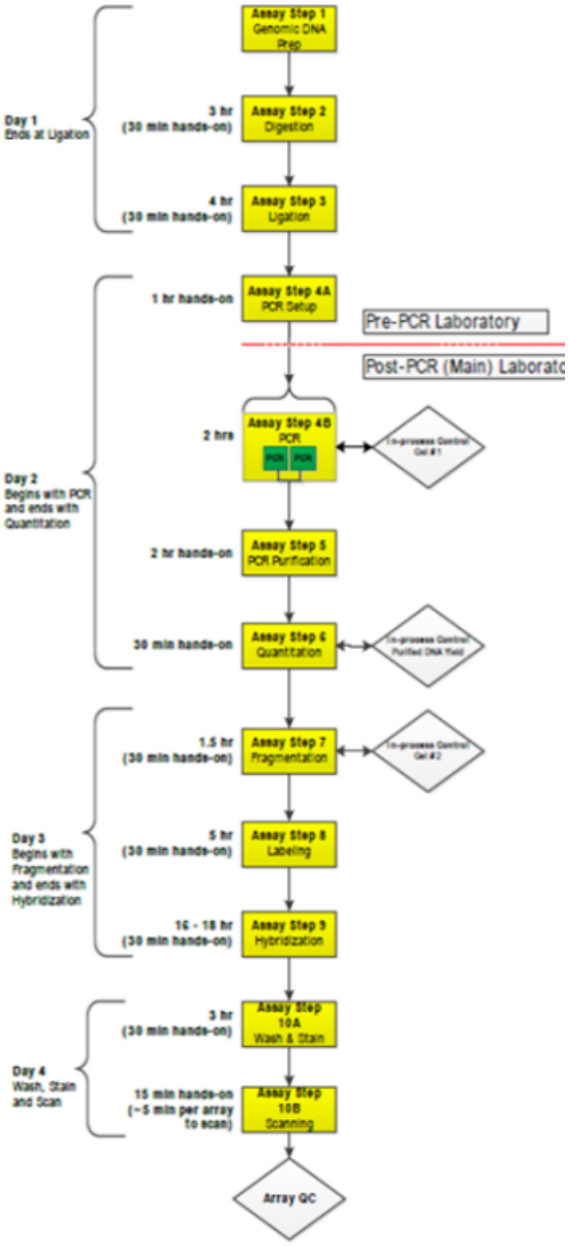
Catalog Number 905403

Pub. No. MAN0017123 **Rev.** A.0

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

Workflow overview

The KaryoStat™ Assay is optimized for processing 8 to 24 samples at a time to aid in the identification of chromosomal abnormalities and submicroscopic aberrations. The KaryoStat™ Assay supports processing of as little as 8 samples, at least one of which is recommended to be a negative control. The scheme below shows the 3.5-day protocol. Alternatively, the assay can be completed in 2.5 days. For details please refer to the *KaryoStat™ Assay User Guide*, Cat. No. 905403.



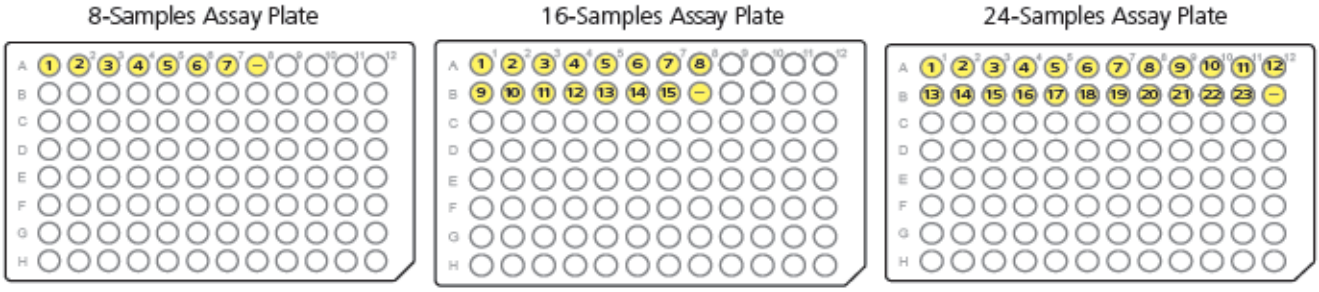
Prepare gDNA plate

Dilute stock gDNA to working concentration

1. Place gDNA plate in upper half of cooling block on ice.
2. Place gDNA at 15–30°C (RT) until thawed (≤30 min); place in cooling block on ice. Use within 1 hour.
3. Vortex gDNA samples for 3 seconds.
4. Centrifuge 650 × *g* for 1 minute; place in cooling block.
5. Use double-strand-specific quantitation method to determine sample concentration.
6. Dilute each sample to 50 ng/µL with Low EDTA TE Buffer in separate wells of 96-well plate.
7. Tightly seal plate.
8. Centrifuge 650 × *g* for 1 minute.
9. Place on cooling block.

Aliquot prepared gDNA into assay plate

1. Mark 96-well plate.
2. Place plate on lower half of cooling block.
3. Transfer 5 µL of first sample from gDNA stock plate to well A1 of DIG-LIG plate; transfer all samples in same way.



4. Add gDNA to wells marked 1 through 8 in plate diagram. For negative control, add 5 µL of Low EDTA TE Buffer instead of gDNA to a well and mark well clearly with "–".
5. Tightly seal DIG-LIG plate with new seal; centrifuge 650 × *g* for 1 minute and place on cooling block on ice.

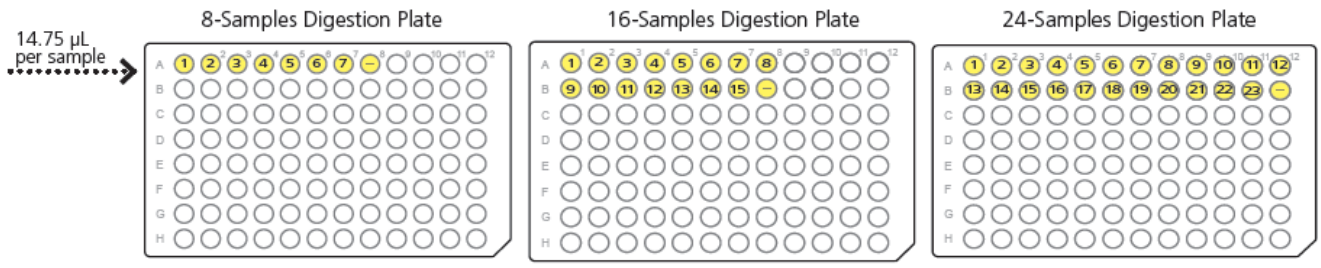
Stage 1 – Digestion

1. Thaw Nsp I Buffer and 100X BSA ≤30 minutes at room temperature (RT). Vortex and spin down; place on ice. Use within 1 hour.
2. Leave Nsp I at –15 to –20°C until ready to use.
3. Prepare Digestion Master Mix. Dispense within 10 minutes.

Reagent	Per sample	8 samples MM (with 20% overage)	16 samples MM (with 20% overage)	24 samples MM (with 20% overage)	Lot number
Water, Nuclease free	11.55 µL	110.9 µL	221.8 µL	332.6 µL	
Nsp I Buffer	2.00 µL	19.2 µL	38.4 µL	57.6 µL	
100X BSA	0.20 µL	1.9 µL	3.8 µL	5.8 µL	
Nsp I	1.00 µL	9.6 µL	19.2 µL	28.8 µL	
Total Volume	14.75 µL	141.6 µL	283.2 µL	424.8 µL	—

4. Vortex Master Mix before adding enzyme.
5. Vortex Digestion Master Mix at high speed 3 times, 1 second each time; spin down.
6. Add Digestion Master Mix to samples.

Samples	Volume/sample
gDNA (50 ng/µL)	5.00 µL (250 ng)
Digestion Master Mix	14.75 µL
Total Volume	19.75 µL



7. Ensure lid of thermal cycler is preheated.
8. Seal plate with adhesive film.

9. Vortex plate at high speed in 5 sector format, 1 second per sector.
10. Spin down at 650 × *g* for 1 minute.
11. Load plate onto thermal cycler and run CytoScan™ Optima Digest program.
12. Proceed to "Stage 2 – Ligation" on page 3.

Temp	Time
37°C	2 hour
65°C	20 minutes
4°C	5 minutes
4°C	∞ (Process plate within 2.5 hour)

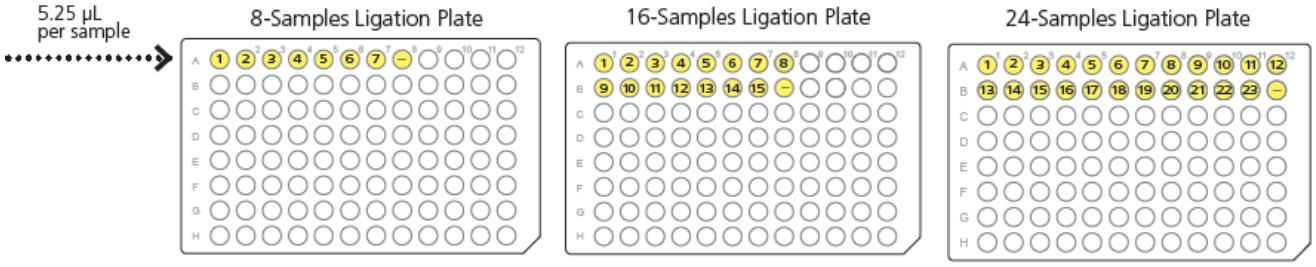
Stage 2 – Ligation

1. Thaw DNA Ligase Bfr and Adaptor, Nsp I at RT ≤30 minutes). Vortex to ensure any precipitate is resuspended and DNA Ligase Bfr is clear. Place on Ice. Use within 1 hour.
2. Leave DNA Ligase at –15 to –20°C until ready to use.
3. Prepare Ligation Master Mix. Dispense within 10 minutes.

Reagent	Per sample	8 samples MM (with 25% overage)	16 samples MM (with 25% overage)	24 samples MM (with 25% overage)	Lot number
DNA Ligase Bfr	2.50 µL	25.0 µL	50.0 µL	75.0 µL	
Adaptor, Nsp I	0.75 µL	7.5 µL	15.0 µL	22.5 µL	
DNA Ligase	2.00 µL	20.0 µL	40.0 µL	60.0 µL	
Total Volume	5.25 µL	52.5 µL	105.0 µL	157.5 µL	—

4. Vortex Ligation Master Mix before adding enzyme.
5. Vortex Ligation Master Mix at high speed 3 times, 1 second each time; spin down.
6. Add Ligation Master Mix to samples.

Samples	Volume/sample
Nsp I Digested Sample	19.75 µL
Ligation Master Mix	5.25 µL
Total Volume	25.00 µL



7. Ensure lid of thermal cycler is preheated.
8. Seal plate with adhesive film.
9. Vortex plate at high speed in 5 sector format, 1 second per sector.
10. Spin down at 650 × *g* for 1 minute.
11. Load plate onto thermal cycler and run CytoScan™ Optima Ligate program.

Temp	Time
16°C	3 hour
70°C	20 minutes
4°C	5 minutes
4°C	∞

Note: You can hold plate in thermal cycler at 4°C for up to 16-20 hours.

12. Proceed to "Stage 3A – PCR" on page 3.

STOPPING POINT Optional stopping point. You can freeze plate at –15 to –25°C for up to 1 week.

Stage 3A – PCR

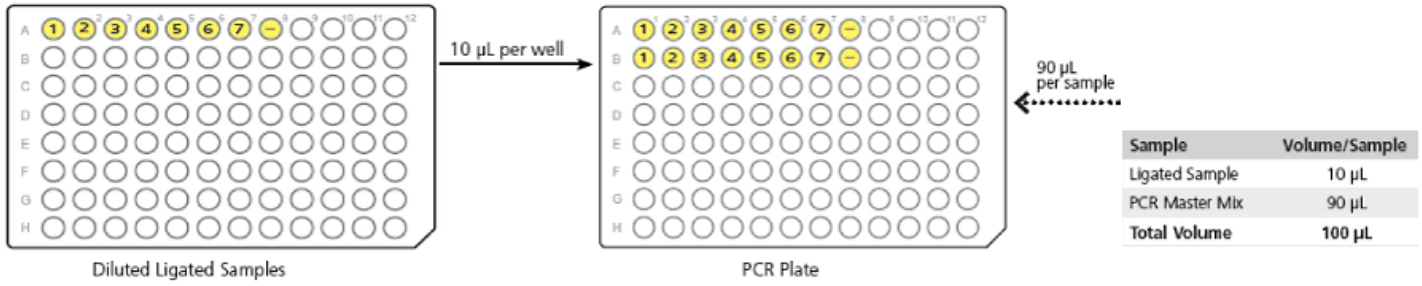
1. Thaw CytoScan™ Taq Buffer, dNTP and PCR Primer (≤30 minutes). Place on ice and use within 1 hour.
2. Ensure ligation plate is sealed properly.
3. Spin down at 650 × *g* for 1 minute.

4. Thaw ligated samples at RT (≤30 mininutes). Immediately place on ice and dilute samples. Process within 1 hour.

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

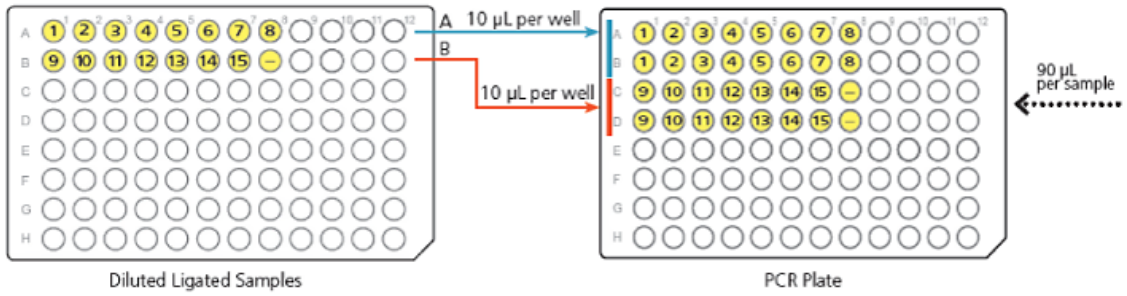
5. Seal plate with adhesive film.
6. Vortex at high speed in 5 sector format, 1 second per sector. Spin down at 650 × *g* for 1 minute.
7. Transfer two 10-µL aliquots of each sample to PCR plate.

8-Samples

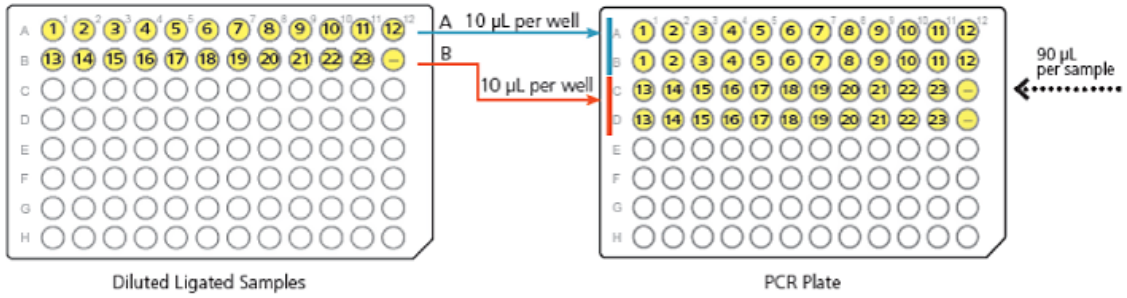


Sample	Volume/Sample
Ligated Sample	10 µL
PCR Master Mix	90 µL
Total Volume	**100 µL**

16-Samples



24-Samples



8. Vortex and spin down all reagents.
9. Keep 5M Betaine and Nuclease-free Water on ice. If 5M Betaine is frozen, thaw and vortex until fully dissolved.
10. Leave CytoScan™ Taq DNA Polymerase at –15 to –20°C until ready to use.

Reagent	Per reaction	8 samples MM (with 15% overage)	16 samples MM (with 15% overage)	24 samples MM (with 15% overage)	Lot number
Water, Nuclease free (chilled)	50.3 µL	965.8 µL	1,931.5 µL	2,897.3 µL	
CytoScan™ Taq Buffer	10.0 µL	192.0 µL	384.0 µL	576.0 µL	
5M Betaine	20.0 µL	384.0 µL	768.0 µL	1,152.0 µL	
dNTP	3.5 µL	67.2 µL	134.4 µL	201.6 µL	
PCR Primer	4.2 µL	80.6 µL	161.3 µL	241.9 µL	
CytoScan™ Taq DNA Polymerase	2.0 µL	38.4 µL	76.8 µL	115.2 µL	
Total Volume	90.0 µL	1,728 µL	3,456 µL	5,184 µL	—

11. Prepare PCR Master Mix in 15-mL centrifuge tube. Assemble PCR Master Mix on ice. Dispense within 20 min.
12. Vortex PCR Master Mix before adding enzyme.
13. Vortex PCR Master Mix at high speed 3 times, 1 second each.

14. Add PCR Master Mix to the samples.

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

15. Seal PCR plate, vortex TWICE at high speed in 5 sector format, 1 second per sector.

    Spin down at 650 × *g* for 1 minute.
16. Keep plate on ice and bring to Post-PCR Room/Area.
17. Ensure thermal cycler lid is preheated.
18. Load plate onto thermal cycler and run CytoScan™ Optima PCR program.

Temp	Time	Cycles
95°C	1 minute	1X
95°C	30 seconds	30
60°C	45 seconds	
68°C	60 seconds	
68°C	7 minutes	1X
4°C	5 minutes	—
4°C	Hold (can be held overnight)	

    Volume: 100 µL

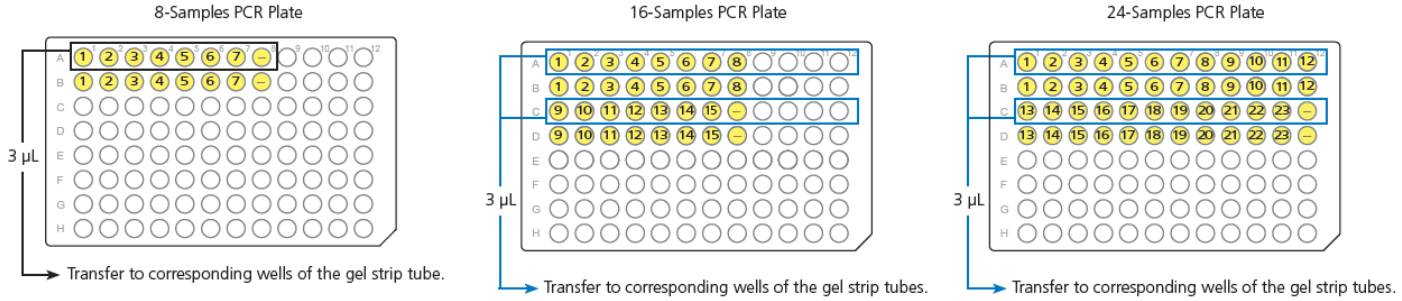
    Specify Maximum mode

    Note: You can hold the plate in the thermal cycler for 16-20 hours (overnight).
19. Proceed to "Stage 4 – PCR product purification" on page 5.

STOPPING POINT Optional: If not processing immediately, the plate maybe stored at –20°C for up to 1 week.

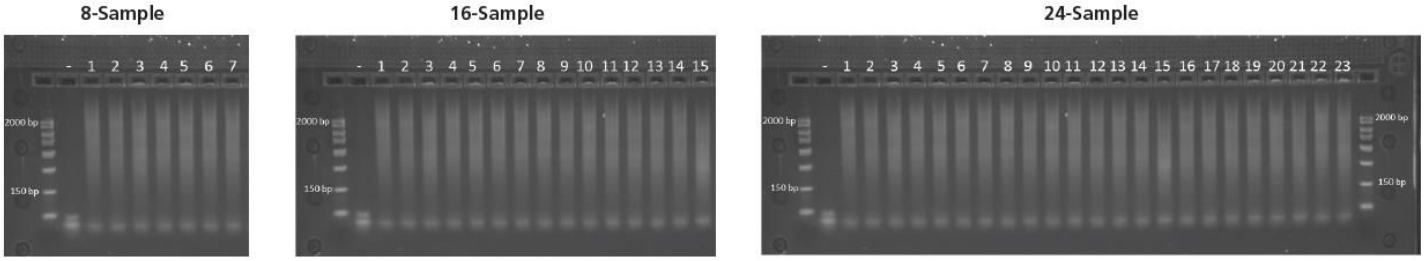
Stage 3B – PCR product check

1. Transfer 3 µL of each PCR product from one row to corresponding wells of gel strip tube.
2. Add 17 µL of diluted loading buffer.
3. Seal gel strip tubes.



4. Vortex and spin down.
5. Load entire sample onto a 2% agarose gel.
6. Follow manufacturer's instructions to run gel that meets requirements.

   Example of PCR products run on a 2% agarose gel. Majority of product must be 150 to 2,200 bp.

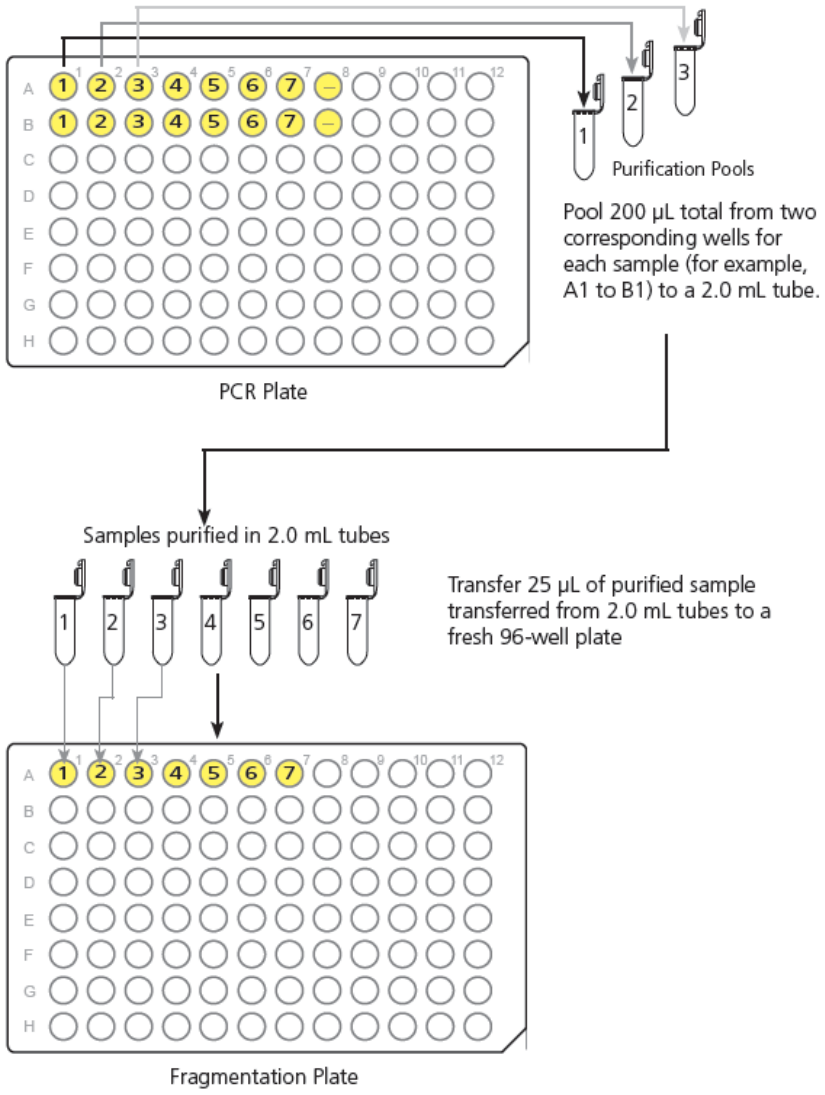


Stage 4 – PCR product purification

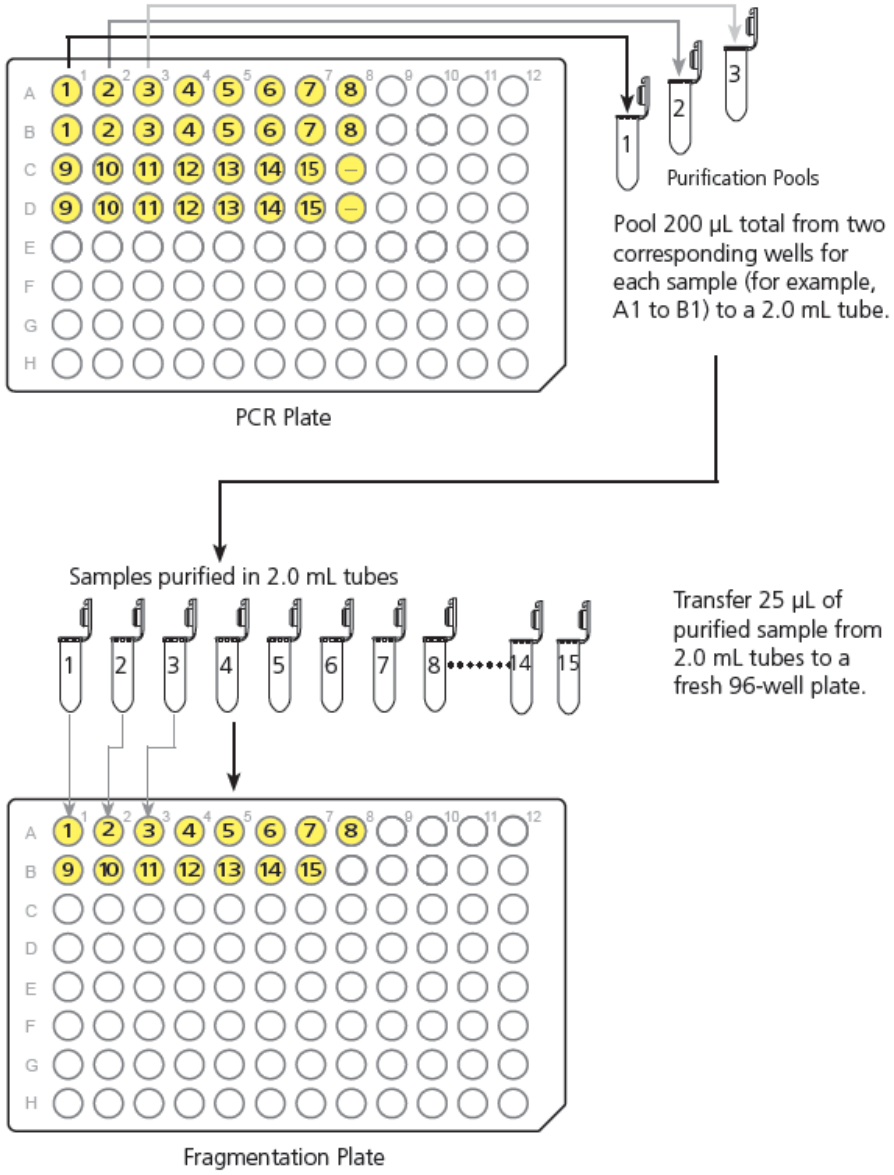
1. Add 45 mL of absolute ethanol to Purification Wash Buffer prior to use. Cap bottle tightly and mix by inverting Purification Wash Buffer bottle 10 times.
2. Pool both PCR products for each sample by transferring all PCR reactions to appropriately marked 2.0 mL tube.
3. Examine PCR plate to that ensure all volume from each well is transferred.
4. Thoroughly mix Purification Beads stock by inverting bottle up and down 10 times until mixture is homogeneous. Examine bottom of bottle to ensure that solution appears homogeneous.
5. Add 360 µL of Purification Beads to each pooled sample using single-channel P1000 pipette (>8 samples, use multichannel pipette).

6. Securely cap each tube and mix well by inverting 10 times.
7. Incubate at room temperature for 10 minutes.
8. Centrifuge tubes—with hinges facing out—for 3 minutes at 16,000 × *g*.
9. Place tubes on magnetic stand so that cap hinge is over magnet.
10. Leave tubes in stand, pipet off supernatant without disturbing bead pellet. Discard supernatant.
11. With P1000 pipette add 800 µL Purification Wash Buffer to each tube.
12. Cap tubes, load into foam adapter, vortex at maximum setting 2 minutes.
13. Centrifuge tubes 3 minutes at 16,000 × *g* with hinges facing out.
14. Place tubes back on magnetic stand.
15. Leave tubes in stand, pipet off supernatant without disturbing bead pellet. Discard supernatant.
16. Spin tubes 30 seconds at 16,000 × *g* with hinges facing out; place them back on magnetic stand.
17. With a P20 pipette remove any drops of Purification Wash Buffer from bottom of each tube.
18. Allow any remaining Purification Wash Buffer to evaporate by taking tubes OFF magenetic stand and leaving them UNCAPPED at room temperature for 7 minutes.
19. With a P100 pipette add 27 µL of Elution Buffer to each tube, dispense directly on beads.
20. Cap tubes, load into foam adapter, vortex at maximum speed 10 minutes to resuspend beads.
21. If beads are not fully resuspended, flick tubes to dislodge pellet and vortex additional 2 minutes.
22. Centrifuge tubes 3 minutes at 16,000 × *g* with hinges facing out.
23. Place tubes on magnetic stand 10 minutes until all beads are pulled to side.
24. Transfer 25 µL of eluted sample to appropriate well of fresh 96-well plate.
25. Proceed to "Stage 5 – Quantitation" on page 8.

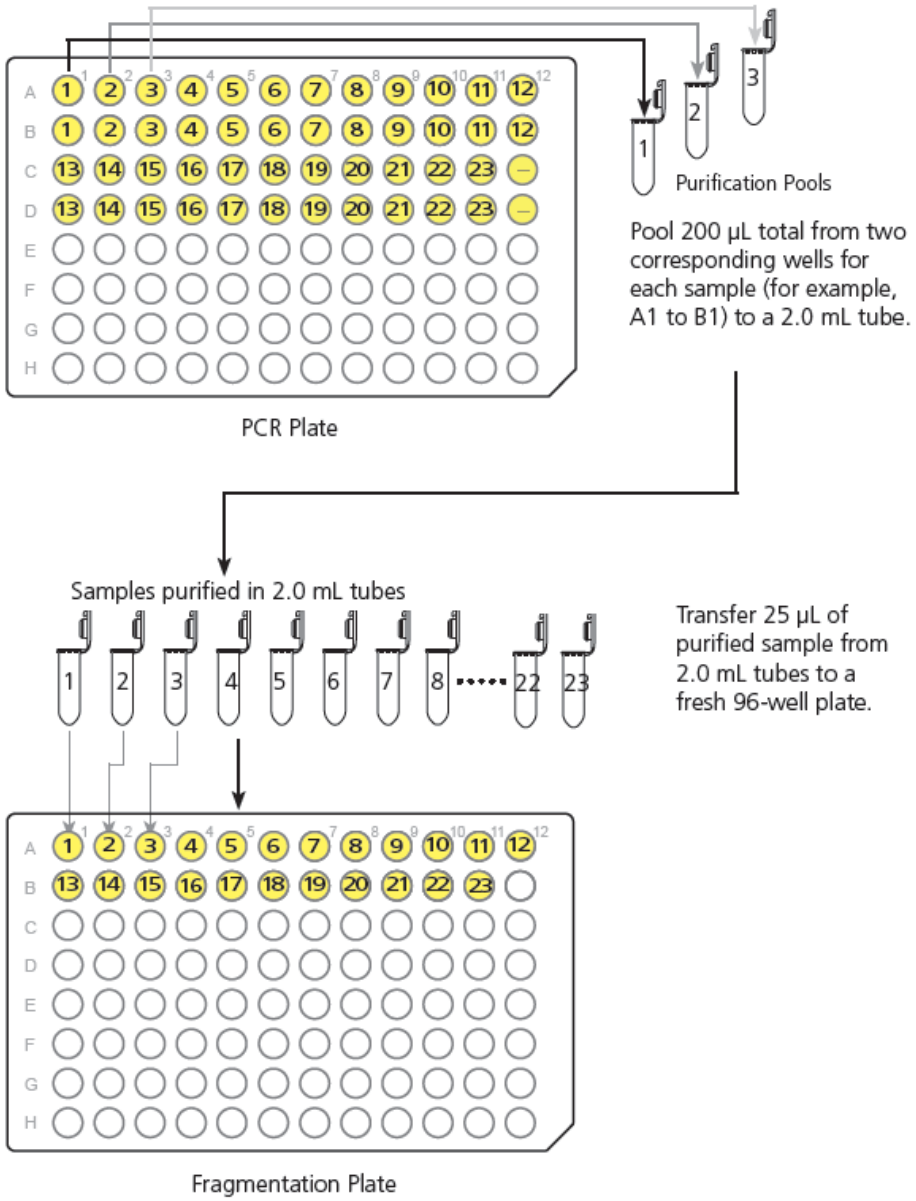
8-Samples



16-Samples


A 1 2 3 4 5 6 7 8 9 10 11 12
B 1 2 3 4 5 6 7 8
C 9 10 11 12 13 14 15 –
D 9 10 11 12 13 14 15 –
E
F
G
H
PCR Plate
1
2
3
Purification Pools
Pool 200 µL total from two corresponding wells for each sample (for example, A1 to B1) to a 2.0 mL tube.
Samples purified in 2.0 mL tubes
1 2 3 4 5 6 7 8 14 15
Transfer 25 µL of purified sample from 2.0 mL tubes to a fresh 96-well plate.
A 1 2 3 4 5 6 7 8 9 10 11 12
B 9 10 11 12 13 14 15
C
D
E
F
G
H
Fragmentation Plate

24-Samples



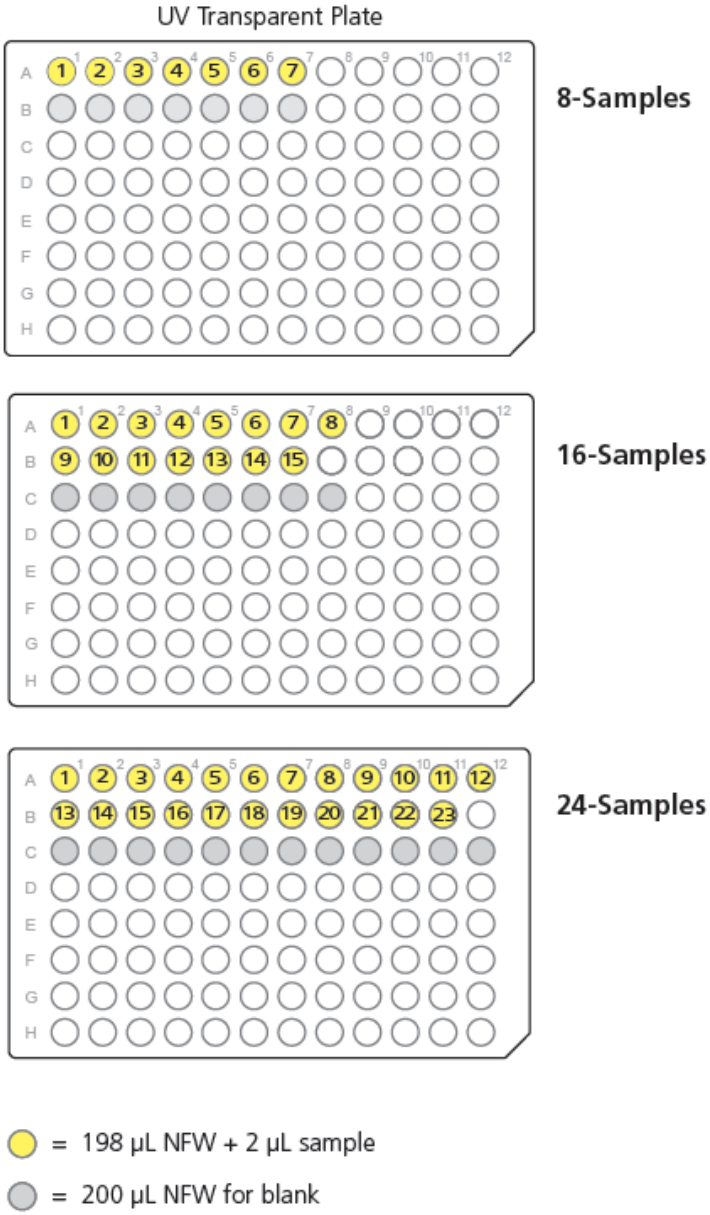
Stage 5 – Quantitation

Prepare the quantitation plate

1. Add 198 µL Nuclease-free Water into UV plate.
2. Add 200 µL of Nuclease-free Water into each well of empty row to use as blank.
3. Add 2 µL of each purified sample to corresponding well of UV plate.
4. Seal plate, vortex, spin down.

Plate spectrophotometer

1. Use OD_{280} and OD_{320} as controls.
2. Measure the OD of each PCR product at 260, 280, and 320 nm.



3. Determine the OD_{260} measurement for the water blank and calculate average.
4. Calculate one OD_{260} reading for every sample:

   OD_{260} = (sample OD_{260}) – (average water blank OD_{260})
5. Calculate the undiluted concentration for each sample in µg/µL:

   OD_{260} × 0.05 µg/µL × 100

Assess OD readings

- Average purification yield for ≥7 samples must be ≥3.0 µg/µL. Do not further process samples with yields <2.5 µg/µL.
- The OD_{260}/OD_{280} ratio should be between 1.7 and 2.1.
- The OD_{320} measurement must be very close to zero (≤0.1).

Note: Stopping Point: Optional: The plate maybe stored at –15 to –25°C for up to 1 week.

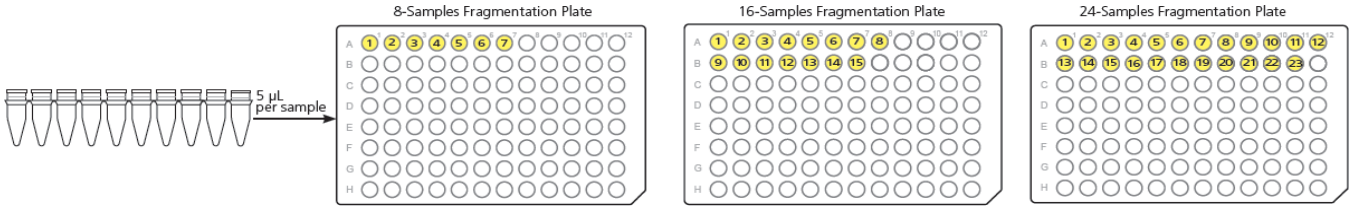
Stage 6A – Fragmentation

1. Chill the plate centrifuge to 4°C before proceeding.
2. Turn on the thermal cycler to preheat the lid.
3. Remove the plate of purified, quantitated samples from –15 to –20°C storage and thaw at room temperature (≤30 minutes). Seal tightly and spin down plate at 650 × *g* for 1 minute. Place the plate on lower half of cooling block on ice and chill for 10 minutes before use. Process within 1 hour.
4. Thaw the Fragmentation Buffer at room temperature (≤30 min). Vortex and spin down; place on ice. Use within 1 hour.

5. Leave the Fragmentation Reagent at –15 to –20°C until ready to use.
6. Keep all reagents, including water, on ice. Perform all additions on ice.
7. Prepare the Fragmentation Master Mix. Dispense within 10 minutes.

Reagent	2.5 U/µL	✓	Lot number
Water, Nuclease free	215 µL		
Fragmentation Buffer	275 µL		
Fragmentation Reagent	10.0 µL		
Total volume	500.0 µL	–	–

8. Vortex the Fragmentation Master Mix before adding enzyme.
9. Vortex the Fragmentation Master Mix at high speed 3 times, 1 second each time, pulse spin 3 seconds, place in cooling block.
10. Add Fragmentation Master Mix equally to strip tubes.
11. With a multichannel pipette, add 5 µL Fragmentation Master Mix to each sample.



Samples	Volume/sample
Purified PCR Product	23 µL
Fragmentation Master Mix	5 µL
Total volume	28 µL

12. Seal the sample plate with an adhesive film.
13. Vortex at high speed in 5 sector format, 1 second per sector.
14. Spin down at 650 × *g* for 1 minute in a prechilled centrifuge.
15. Ensure that the thermal cycler block is preheated.
16. Load the plate onto the thermal cycler and run the CytoScan™ Optima Fragment program.

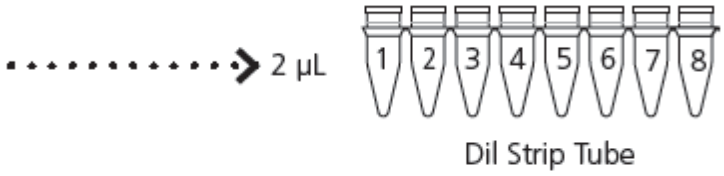
Temp	Time
37°C	35 minutes
95°C	15 minutes
4°C	5 mininutes
4°C	∞ (Process plate within 2.5 hours)

17. Proceed immediately to "Stage 6B – Fragmentation QC gel" on page 10.

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

Stage 6B – Fragmentation QC gel

1. Prepare the loading buffer dilution.
2. Add 14 µL Nuclease-free Water into strip tubes labeled "Dil".



3. Transfer 2 µL of each fragmented sample to corresponding Dil strip tube. Seal strip, vortex, spin down.
4. Label strip tubes "Gel Analysis."
5. Add 8 µL diluted fragmented product to 12 µL diluted loading buffer.
6. Load 20 µL samples onto 4% agarose gel.

   Fill empty wells with 20 µL Nuclease-free Water.

Example of fragmented samples run on 4% agarose gel. Average fragment distribution must be between 25 and 125 bp.

7. If quality control results pass, proceed to "Stage 7 – Labeling" on page 11.

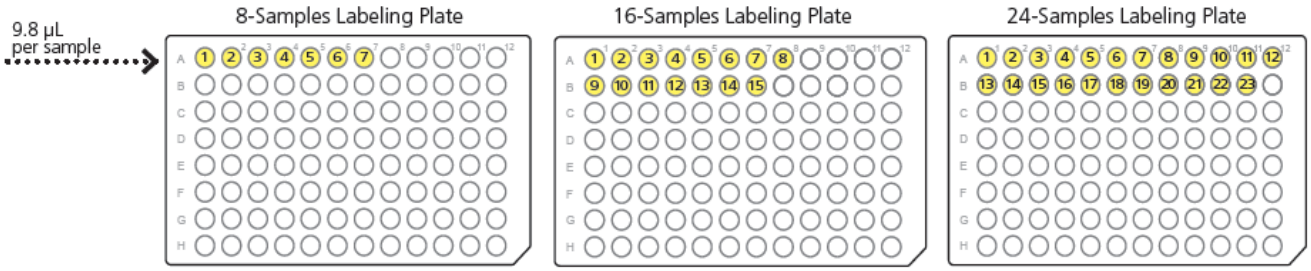
Stage 7 – Labeling

1. Thaw TdT Buffer and DNA Labeling Reagent at RT (≤30 min); place on ice. Use within 1 hour.
2. Leave TdT Enzyme at –15 to –20°C until ready to use.
3. Prepare Labeling Master Mix. Dispense within 10 minutes.
4. Vortext Labeling Master Mix before adding enzyme.

Reagent	Per sample	8 samples MM (with 20% overage)	16 samples MM (with 20% overage)	24 samples MM (with 20% overage)	Lot number
TdT Buffer	7 µL	67.2 µL	134.4 µL	201.6 µL	
DNA Labeling Reagent	1 µL	9.6 µL	19.2 µL	28.8 µL	
TdT Enzyme	1.8 µL	16.8 µL	33.6 µL	50.4 µL	
Total Volume	9.8 µL	93.6 µL	187.2 µL	280.8 µL	—

5. Vortex Labeling Master Mix and spin down. Add 9.8 µL of Labeling Master Mix to each sample.

Samples	Volume/sample
Fragmented DNA (less 2.0 µL for gel analysis)	26 µL
Labeling Master Mix	9.8 µL
Total Volume	35.8 µL



6. Tightly seal plate, vortex at high speed in 5 sector format, 1 second per sector.
7. Spin down at 650 × *g* 1 minute.
8. Load plate onto thermal cycler and run CytoScan™ Optima Label program.

Temp	Time
37°C	4 hour
95°C	15 minutes
4°C	5 minutes
4°C	∞

9. Proceed to "Stage 8 – Hybridization" on page 11.

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

Stage 8 – Hybridization

Create test request and register array

1. Unpack the arrays and allow to equilibrate to room temperature prior to use.
2. Preheat the hybridization oven for at least 1 hour at 50°C with the rotation turned on.
3. Create a Batch Registration file using AGCC.
4. Prepare the Hybridization Master Mix in a 15 mL conical tube on ice.

Reagent	Per sample	8 samples MM (with 20% overage)	16 samples MM (with 20% overage)	24 samples MM (with 20% overage)	✓	Lot number
Hyb Buffer Part 1	82.5 µL	792 µL	1,584 µL	2,376.0 µL		
Hyb Buffer Part 2	7.5 µL	72 µL	144.0 µL	216.0 µL		
Hyb Buffer Part 3	3.5 µL	33.6 µL	67.2 µL	100.8 µL		
Hyb Buffer Part 4	0.5 µL	4.8 µL	9.6 µL	14.4 µL		
Oligo Control Reagent	1.0 µL	9.6 µL	19.2 µL	28.8 µL		
Total Volume	95 µL	912 µL	1,824 µL	2,736 µL	—	—

5. Mix well by vortexing the master mix at high speed 3 times, 3 seconds each; then pour it into a reservoir on the cold block. Refer to.

6. Add 95 µL of Hybridization Master Mix to each sample.
7. Tightly seal the plate, vortex TWICE at high speed in 5 sector format, and spin down at 2,000 rpm for 1 minute.

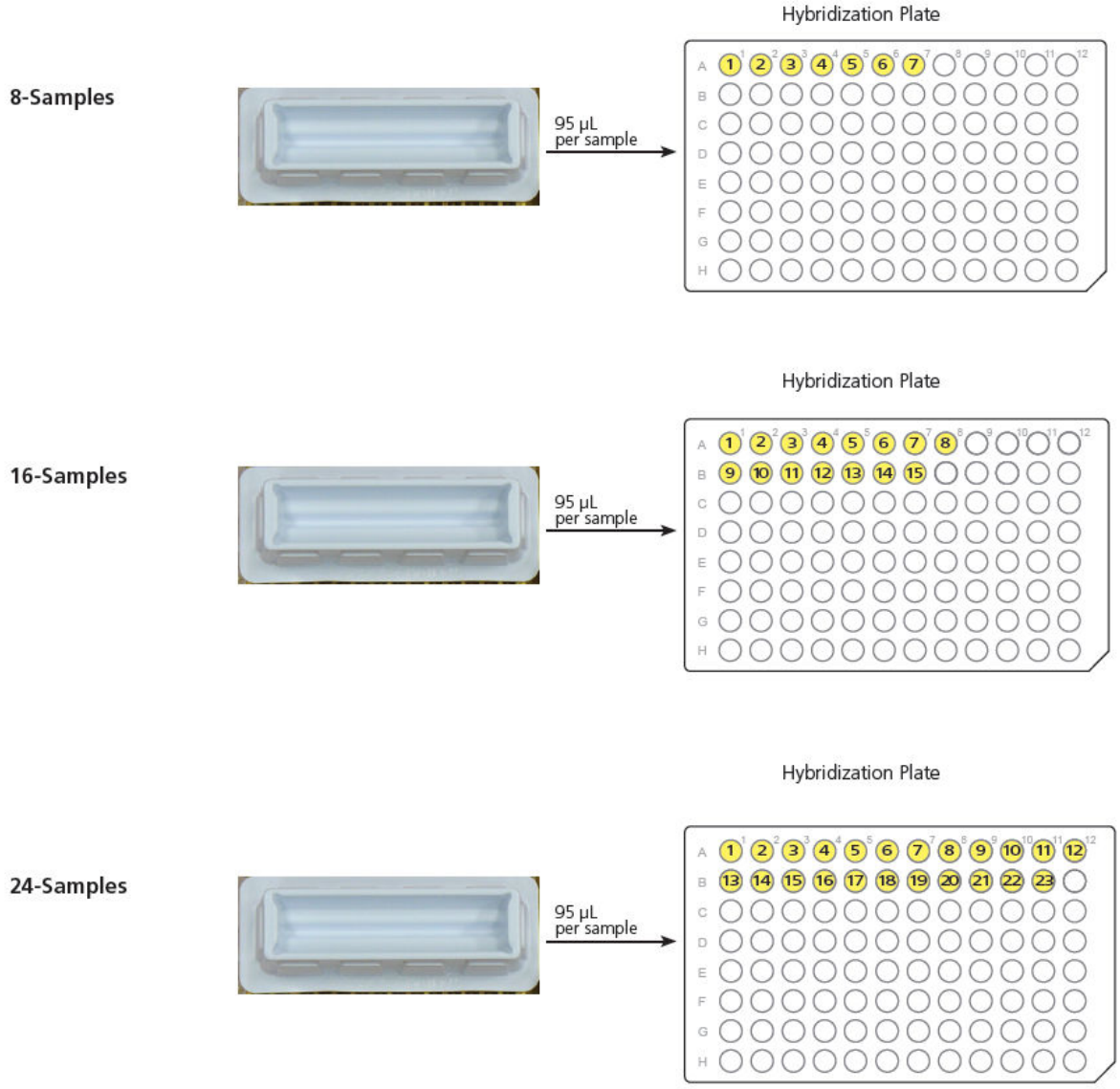
STOPPING POINT Optional: The plate can be held at –15 to –25°C for up to a week.

8. Load the plate onto the thermal cycler and run the CytoScan™ Optima Hyb program.

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

9. Allow the samples to incubate at 49°C for at least 3 minutes before loading.

10. Leaving the samples on the thermal cycler, load 100 µL of sample onto each array using a single-channel P200 pipette. Only hybridize up to 6 arrays at a time.



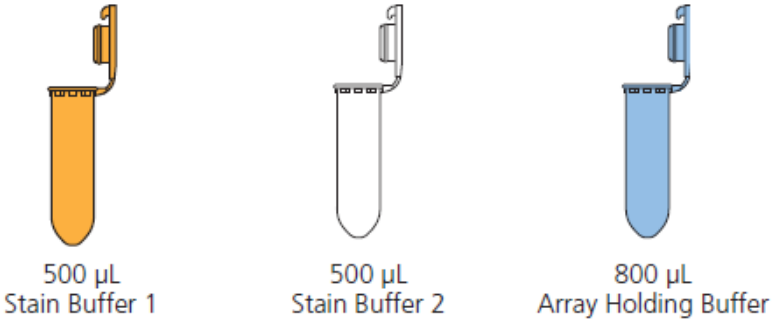
- Samples must remain on the thermal cycle while loading the arrays.
- To avoid damaging the septa, use a single-channel P200 pipette to load the arrays.
- If bubbles adhere to the array surface, tap the array lightly on the edge of a countertop, then gently shake the array a few times to ensure bubbles are not visible through the window.

11. Clean any excess fluid from around the septa.
12. Apply Tough-Spots™ to the septa and press firmly.
13. Immediately load the arrays into the hybridization oven, four to six at a time.
14. Hybridize the arrays 16 to 18 hours at 50°C and 60 rpm.

Stage 9 – Wash, stain, and scan

Aliquot the following reagents into separate 1.5 mL microfuge tubes for each array:

1. 500 µL Stain Buffer 1 solution into amber tubes
2. 500 µL Stain Buffer 2 into clear tubes
3. 800 µL Array Holding Buffer into blue tubes



Washing and staining arrays

1. Prime fluidics station with Wash A and Wash B. Load stain solutions and select correct fluidics protocol.
2. Start fluidics protocol and leave cartridge lever down in Eject position.
3. Remove adhesive label dots from each array.
4. Load arrays onto fluidics station.
5. Refer to the *KaryoStat™ Assay User Guide* (Cat. No. 905403) for detailed instructions on priming the fluidics stations and washing/staining the CytoScan™ Optima Arrays in AGCC.

Before scanning

1. Ensure no bubbles are visible through window. Clean array surface.
2. Cover septa with thin, 3/4-inch adhesive label dots; load onto scanner. Thicker dots can get caught in autoloader.
3. Scan arrays as described in *KaryoStat™ Assay User Guide* (Cat. No. 905403).

Important points

- Add Stain Buffer 1 to amber tubes.
- Add Array Holding Buffer to blue tubes.
- Stain Buffer 1 and Array Holding Buffer are light sensitive.
- If there is a delay after adding to tubes, store tubes at 4°C, protected from light.
- Remove bubbles from arrays on the fluidics station or remove bubbles manually (see *GeneChip™ Fluidics Station 450 User Guide for AGCC*, Cat. No. 08-0295).

Customer and technical support

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

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

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

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