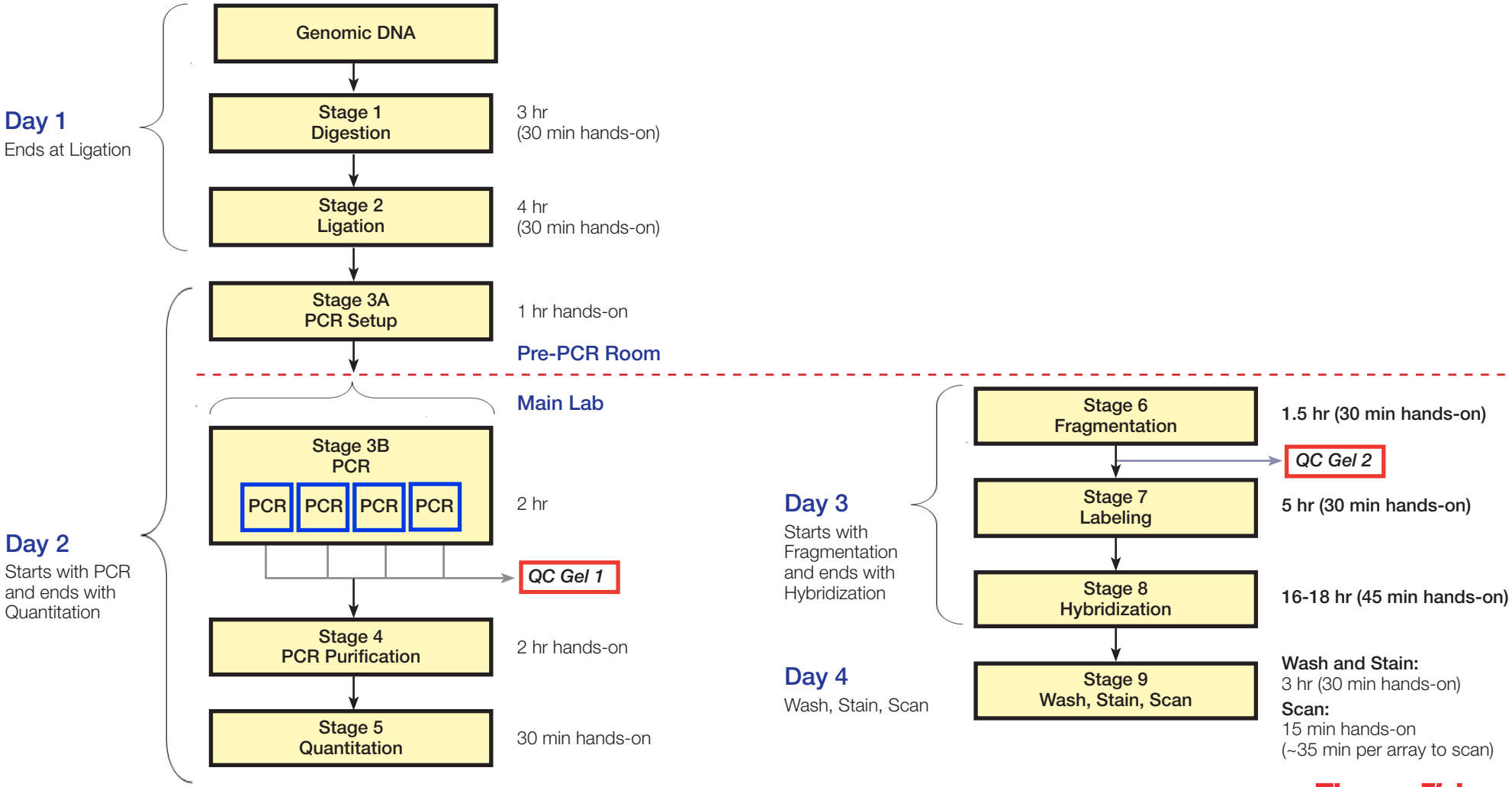
CytoScan™ Assay Manual Workflow (8 Samples)

Catalog Numbers 901835, 901834, 901859, 901860

Pub. No. 703039 **Rev.** 6

Workflow Overview

The CytoScan™ Assay manual workflow is optimized for processing 8 to 24 samples at a time to obtain whole genome copy number and SNP information from CytoScan™ Arrays. The CytoScan™ Assay manual workflow supports processing of as little as eight samples, two of which are a positive and negative control. This protocol is for research use only. Not for use in diagnostic procedures.



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Stage 1: Digestion (8 samples)

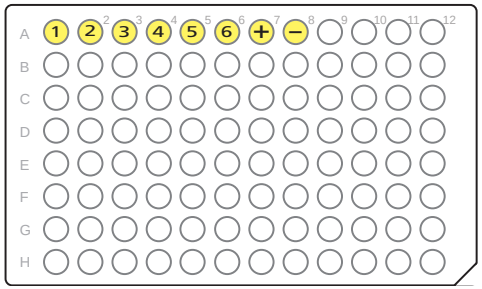
1. Add gDNA to wells marked 1 through 6 in the plate diagram.
2. Thaw Nsp I Buffer and 100X BSA at room temperature. Vortex and spin down, then place on ice.
3. Leave Nsp I enzyme at –20°C until ready to use.
4. Add 5 µL of the Genomic DNA, supplied in the kit as positive control, to the well marked "+".
5. Add 5 µL of Low EDTA TE as negative control to the well marked "–".
6. Prepare the Digestion Master Mix.

Reagent	Per sample	8 Samples MM (with 20% overage)	✓	Lot number
Chilled water, nuclease-free	11.55 µL	110.9 µL		
Nsp I Buffer	2.00 µL	19.2 µL		
100X BSA	0.20 µL	1.9 µL		
Nsp I	1.00 µL	9.6 µL		
Total volume	**14.75 µL**	**141.6 µL**	—	—

7. Vortex the Digestion Master Mix at high speed 3 times, 1 sec each time, and spin down.
8. Add Digestion Master Mix to the samples.

Samples	Volume/Sample
gDNA (50 ng/mL)	5.00 µL (250 ng)
Digestion Master Mix	14.75 µL
Total volume	**19.75 µL**

Digestion Plate



9. Ensure that the lid of the thermal cycler is preheated.
10. Seal the plate with an adhesive film.
11. Vortex the plate at high speed in 5 sector format, 1 sec per sector.
12. Spin down at 2000 rpm for 1 min.
13. Load the plate onto the thermal cycler and run the **CytoScan Digest** protocol.
14. Proceed to *Stage 2: Ligation*.

Temp	Time
37°C	2 hr
65°C	20 min
4°C	Hold

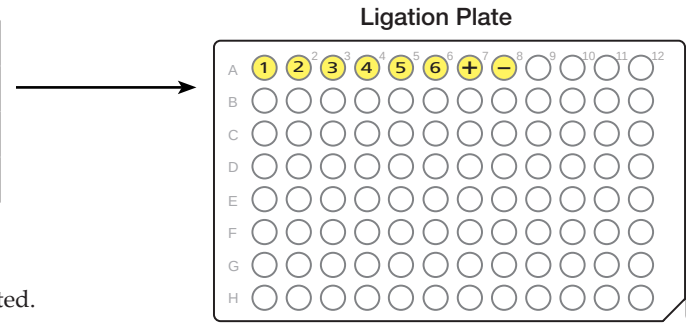
Stage 2: Ligation (8 samples)

1. Thaw the DNA Ligase Buffer and Adaptor, Nsp I at room temperature. Vortex to ensure any precipitate is resuspended and the buffer is clear. Place on ice.
2. Leave DNA Ligase at –20°C until ready to use.
3. Prepare the Ligation Master Mix.

Reagent	Per sample	8 Samples MM (with 25% overage)	✓	Lot number
● DNA Ligase Buffer	2.50 µL	25.0 µL		
● Adaptor, Nsp I	0.75 µL	7.5 µL		
● DNA Ligase	2.00 µL	20.0 µL		
Total volume	**5.25 µL**	**52.5 µL**	—	—

4. Vortex the Ligation Master Mix at high speed 3 times, 1 sec each time, and spin down.
5. Add Ligation Master Mix to the samples.

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



6. Ensure that the lid of the thermal cycler is preheated.
7. Seal the plate with an adhesive film.
8. Vortex the plate at high speed in 5 sector format, 1 sec per sector.
9. Spin down at 2000 rpm for 1 min.
10. Load the plate onto the thermal cycler and run the **CytoScan Ligate** protocol.

Temp	Time
16°C	3 hr
70°C	20 min
4°C	Hold

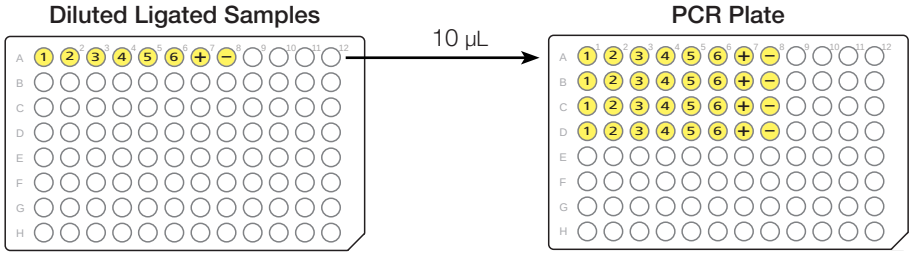
11. Proceed to the PCR setup.

Stage 3A: PCR (8 samples)

1. Ensure that the ligation plate is sealed properly.
2. Spin down at 2000 rpm for 1 min.
3. Dilute the ligated samples.

Samples	Volume/Sample
Ligated Samples	25 µL
Chilled water, nuclease-free	75 µL
Total volume	**100 µL**

4. Seal the plate with an adhesive film.
5. Vortex at high speed in 5 sector format; spin down at 2000 rpm for 1 min.



6. Transfer four 10 µL aliquots of each sample to the PCR plate.
7. Thaw 10X TITANIUM™ *Taq* PCR Buffer, dNTP Mixture, PCR Primer, and diluted ligated samples at room temperature. After thawing, immediately place on ice. Vortex and spin down all reagents.
8. Keep the GC-Melt Reagent and nuclease-free water on ice.
9. Leave the 50X TITANIUM™ *Taq* DNA Polymerase at –20°C until ready to use.
10. Prepare the PCR Master Mix in a 15 mL centrifuge tube. Assemble the master mix on ice.

Reagent	Per Reaction	8 Samples MM (with 15% overage)	✓	Lot number
Chilled water, nuclease-free	39.5 µL	1453.6 µL		
10X TITANIUM™ *Taq* PCR Buffer	10.0 µL	368.0 µL		
GC-Melt Reagent	20.0 µL	736.0 µL		
dNTP Mixture	14.0 µL	515.2 µL		
PCR Primer	4.5 µL	165.6 µL		
50X TITANIUM™ *Taq* DNA Polymerase	2.0 µL	73.6 µL		
Total volume	**90.0 mL**	**3312.0 µL**	—	—

11. Vortex the PCR Master Mix at high speed 3 times, 1 sec each.
12. Add PCR Master Mix to the samples.

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

13. Seal the PCR plate, vortex at high speed in 5 sector format, 1 sec per sector.

    REPEAT vortexing one more time. Spin down at 2000 rpm for 1 min.

Pre-PCR Area

Post-PCR Area

14. Keep the plate on ice and bring to the Post-PCR Room/Area.
15. Ensure that the thermal cycler lid is preheated.
16. Load the plate onto the thermal cycler and run the **CytoScan PCR** protocol.
17. Hold at 4°C.

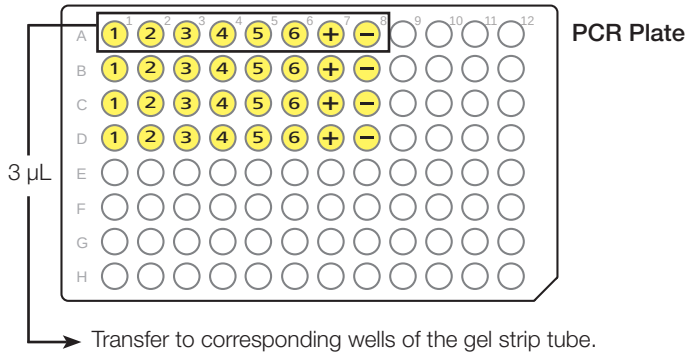
CytoScan PCR – ABI 9700

Temp	Time	Cycles
94°C	3 min	—
94°C	30 sec	30
60°C	45 sec	
68°C	15 sec	
68°C	7 min	—
4°C	Hold	—

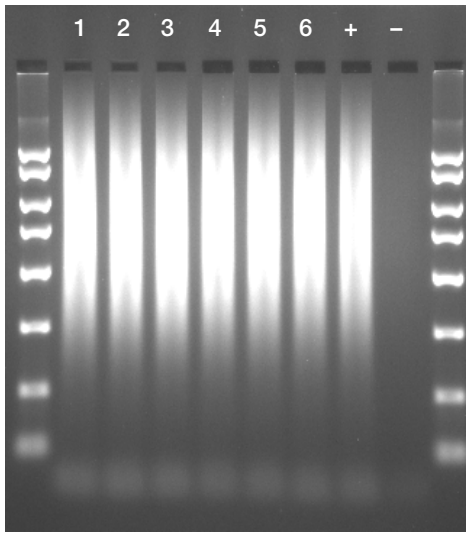
Stage 3B: PCR product check (8 samples)

Follow this protocol if you are using Agarose gels.

1. Aliquot 5 µL of nuclease-free water and 2 µL of loading dye into 8 wells of a new gel strip tube.
2. Transfer 3 µL of each PCR product from one row to the corresponding wells of the gel strip tube.
3. Seal the gel strip tube.



4. Vortex and spin down.
5. Load 8 µL of the sample mix from the gel strip tube onto a 2% agarose gel. Load 5 µL of 50-2000 bp Ladder in the lanes before and after the samples. Run the gel at 5V/cm for 45 min.



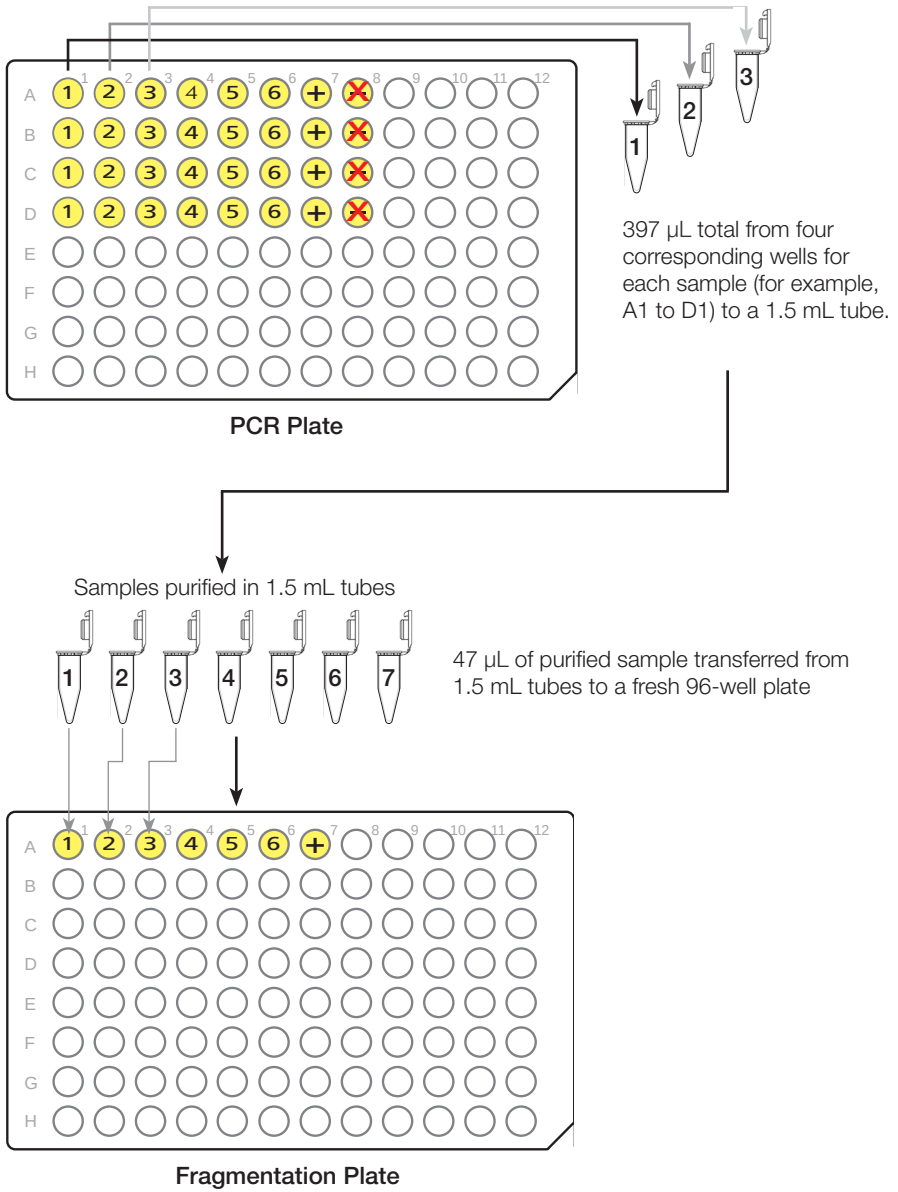
Example of PCR products run on a 2% TBE gel at 5 V/cm for 45 min.
Majority of product should be between 150 and 2000 bp.

6. While the gel is running, begin *Stage 4: PCR product purification*.

Stage 4: PCR product purification (8 samples)

1. Pool all four PCR products for each sample by transferring all PCR reactions to the appropriately marked 1.5 mL tube.
2. Examine the PCR plate to ensure all of the volume from each well has been transferred.
3. Thoroughly mix the Purification beads by inverting the bottle up and down until the mixture is homogeneous.
4. Add 720 µL of Purification Beads to each pooled sample using a single-channel P1000 pipette.
5. Securely cap each tube and mix well by inverting 10 times.
6. Incubate at room temperature for 10 min.
7. Centrifuge the tubes—with hinges facing out—for 3 min at maximum speed (16,100 rcf).
8. Place the tubes on a magnetic stand (for example, MagnaRack™).
9. Leaving the tubes in the stand, pipet off the supernatant without disturbing the bead pellet. Discard the supernatant.

   Note: Be sure to add **45 mL of absolute ethanol** to the Purification Wash Buffer prior to use.
10. Using a P1000 pipet, add 1.0 mL Purification Wash Buffer to each tube.
11. Cap the tubes, load into the foam adapter, and vortex at maximum setting for 2 min.
12. Centrifuge the tubes for 3 min at 16,100 rcf with hinges facing out.
13. Place the tubes back on the magnetic stand.
14. Leaving tubes in the stand, pipet off the supernatant without disturbing the bead pellet. Discard the supernatant.
15. Spin the tubes for 30 sec at 16,100 rcf with hinges facing out, then place them back on the magnetic stand.
16. Using a P20 pipet, remove any drops of Purification Wash Buffer from the bottom of each tube.
17. Allow any remaining Purification Wash Buffer to evaporate by taking the tubes OFF the magenetic stand and leaving them UNCAPPED at room temperature for 10 min.
18. Using a P100 pipet, add 52 µL of Elution Buffer to each tube, directly dispensing on the beads.
19. Cap the tubes, load into the foam adapter, and vortex at maximum power for 10 min to resuspend the beads.
20. If the beads are not fully resuspended, flick the tubes to dislodge the pellet and vortex an additional 2 min.
21. Centrifuge the tubes for 3 min at maximum speed 16,100 rcf with hinges facing out.
22. Place the tubes on the magnetic stand for 10 min until all beads are pulled to the side.
23. Transfer 47 µL of eluted sample to the appropriate well of a fresh 96-well plate.
24. Seal the plate tightly. Vortex at high speed for 1 sec each in all corners and in the center. Spin down at 2000 rpm for 1 min.



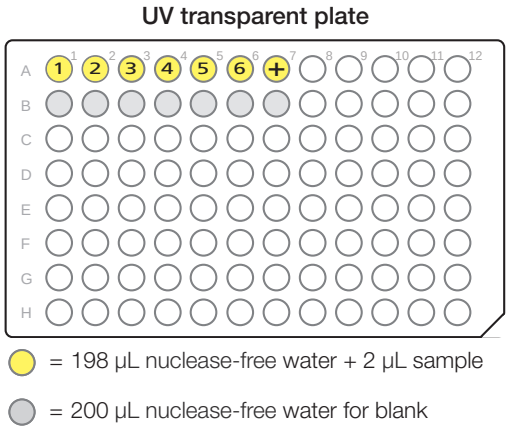
Stage 5: Quantitation (8 samples)

Prepare the Quantitation Plate

1. Aliquot 198 µL of nuclease-free water into a UV plate.
2. Add 2 µL of each purified sample.
3. Seal the plate, vortex, and spin down.

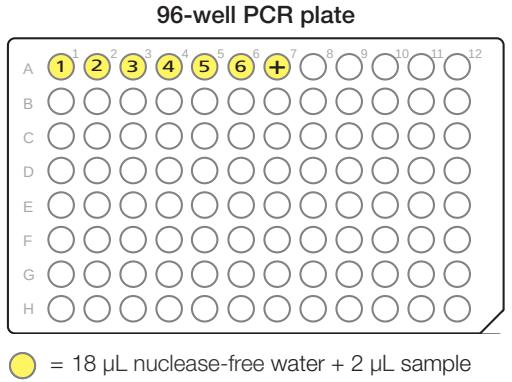
Plate spectrophotometer

1. Measure the OD of each PCR product at 260, 280 and 320 nm.
2. Determine the OD_{260} measurement for the water blank and average.
3. Calculate one OD reading for every sample:
   OD = (sample OD) – (average water blank OD)
4. Calculate the undiluted concentration for each sample in µg/µL: OD X 0.05 µg/µL X 100.



Nanodrop

1. Aliquot 18 µL of nuclease-free water to the corresponding wells of a 96-well plate.
2. Using a P20 pipette, transfer 2 µL of each purified sample to the corresponding well of a 96-well plate.
3. Seal the plate, vortex, and spin down.
4. Blank the NanoDrop using nuclease-free water.
5. Take 2 µL of diluted sample and measure the OD of each PCR product at 260, 280 and 320 nm.
6. Calculate the undiluted concentration of each sample in µg/µL: (concentration in ng/µL X 10) ÷ 1000.



Assess OD readings

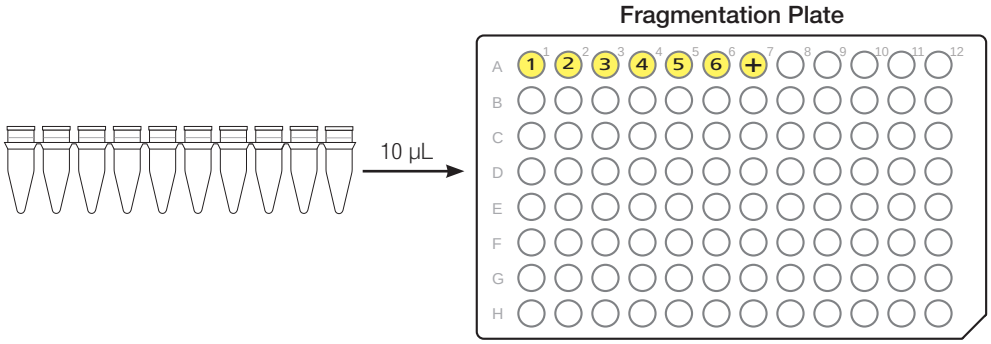
- The average purification yield for 7 or more samples should be ≥3.0 µg/µL. We do not recommend further processing of samples with yields <2.5 µg/µL If the yields are outside of the range indicated here, please consult the troubleshooting section of the *CytoScan™ Assay User Guide* (Pub. No. 703038).
- The OD_{260}/OD_{280} ratio should be between 1.8 and 2.0.
- The OD_{320} measurement should be very close to zero (< 0.1).

Stage 6A: Fragmentation (8 samples)

1. Turn down the plate centrifuge to 4°C before proceeding into the fragmentation step.
2. Turn on the thermal cycler to pre-heat the lid.
3. Remove the plate of purified, quantitated samples from –20°C storage and thaw. Seal the plate tightly, then vortex and spin down. Place the plate on the lower half of the cooling block on ice and chill for 10 min prior to use.
4. Leave the Fragmentation Reagent (2.5 U/µL) at –20°C until ready to use.
5. Keep all reagents, including water, on ice. Perform all additions on ice.
6. Always prepare Fragmentation Master Mix according to the table below even when processing less than 24 samples.

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

7. Vortex the master mix at high speed 3 times, 1 sec each time.
8. Aliquot the Fragmentation Master Mix equally to strip tubes.
9. Using a multi-channel pipet, add 10 µL of Fragmentation Master Mix to each sample.



Samples	Volume/Sample
Purified PCR Product	45 µL
Fragmentation Master Mix	10 µL
Total volume	**55 µL**

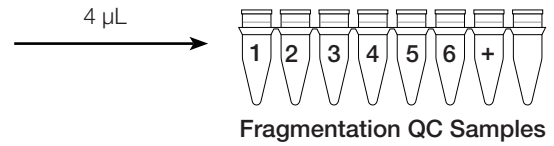
10. Seal the sample plate with an adhesive film.
11. Vortex at high speed in 5 sector format, 1 sec per sector.
12. Spin down at 2000 rpm for 1 min. in a **pre-chilled centrifuge**.
13. Ensure the thermal cycler block is preheated.
14. Load the plate onto the thermal cycler and run the **CytoScan Fragment** protocol. →
15. Proceed immediately to *Stage 6B: Fragmentation QC gel*.

Temp	Time
37°C	35 min
95°C	15 min
4°C	Hold

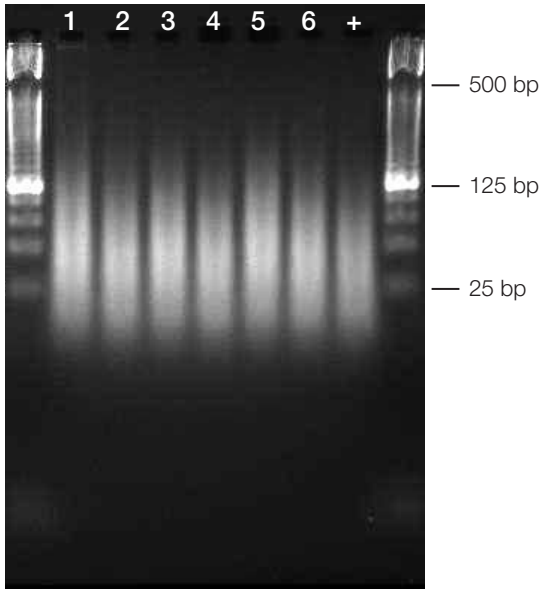
Stage 6B: Fragmentation QC gel (8 samples)

1. Transfer 4 µL of each fragmented sample into strip tubes and label as Fragmentation QC Samples.
2. Add 28 µL of nuclease-free water to each strip tube. Seal the strip, vortex, and spin down.

   Note: This procedure is optimized if you are using Agarose gels.



3. Take an 8 µL aliquot out and add to a strip tube, labeled "Gel Analysis". Add 12 µL of diluted loading dye to each tube of the strip. Seal the strip tubes tightly, vortex, and spin down.
4. Load 20 µL of the samples onto a 4% TBE gel. Load 2 µL of 25 bp DNA Ladder before and after the samples to the first and last lanes.



Example of fragmented samples run on a 4% TBE gel at 5 V/cm for 45 min.
Average fragment distribution is between 25 to 125 bp.

5. Run the gel at 5V/cm for 45 min.
6. Inspect the gel and compare against the figure shown above.
7. Store the remaining 24 µL aliquot of the Fragmentation QC samples at –20°C for further analysis on the Bioanalyzer.
8. If the QC results are good, proceed to *Stage 7: Labeling*.

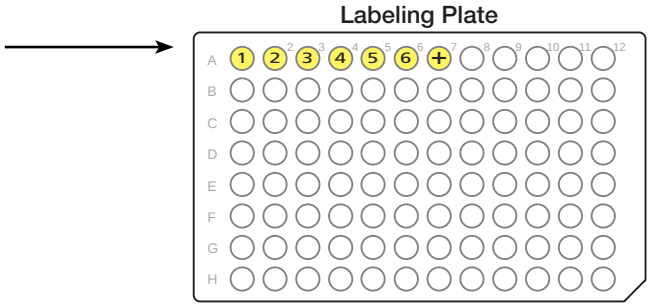
Stage 7: Labeling (8 samples)

1. Thaw the TdT Buffer and DNA Labeling Reagent at room temperature, then place on ice.
2. Leave the TdT Enzyme at –20°C until ready to use.
3. Prepare the Labeling Master Mix.

Reagent	Per sample	8 Samples MM (with 20% overage)	✓	Lot number
● TdT Buffer	14.0 µL	134.4 µL		
● DNA Labeling Reagent	2.0 µL	19.2 µL		
● TdT Enzyme	3.5 µL	33.6 µL		
Total volume	**19.5 µL**	**187.2 µL**	—	—

4. Vortex the Labeling Master Mix and spin down. Add 19.5 µL of Labeling Master Mix to each sample.

Samples	Volume/Sample
Fragmented DNA (less 4.0 µL for gel analysis)	51 µL
Labeling Mix	19.5 µL
Total volume	**70.5 µL**



5. Tightly seal the plate, and vortex at high speed in 5 sector format.
6. Spin down at 2000 rpm for 1 min.
7. Load the plate onto the thermal cycler and run the **CytoScan Label** protocol.

Temp	Time
37°C	4 hr
95°C	15 min
4°C	Hold

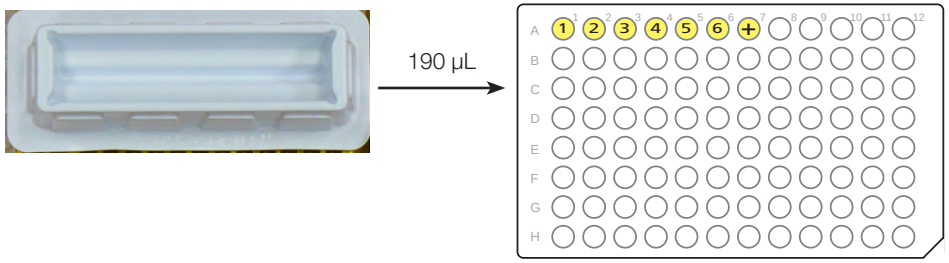
8. If not proceeding to hybridization, store the Labeling plate overnight at –20°C. Otherwise, you can also hold the Labeling Plate at 4°C overnight.

Stage 8: Hybridization (8 samples)

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

Reagent	Per sample	8 samples MM (with 20% overage)	✓	Lot number
Hyb Buffer Part 1	165.0 µL	1584.0 µL		
Hyb Buffer Part 2	15.0 µL	144.0 µL		
Hyb Buffer Part 3	7.0 µL	67.2 µL		
Hyb Buffer Part 4	1.0 µL	9.6 µL		
Oligo Control Reagent	2.0 µL	19.2 µL		
Total volume	**190.0 µL**	**1824.0 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.



6. Add 190 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 2000 rpm for 1 min.
8. Load the plate onto the thermal cycler and run the **CytoScan Hyb** protocol.

Temp	Time
95°C	10 min
49°C	Hold

9. Allow the samples to incubate at 49°C for at least 1 min before loading.
10. Leaving the samples on the thermal cycler, load 200 µL of sample onto each array using a single-channel P200 pipette. Only hybridize up to 4-6 arrays at a time.

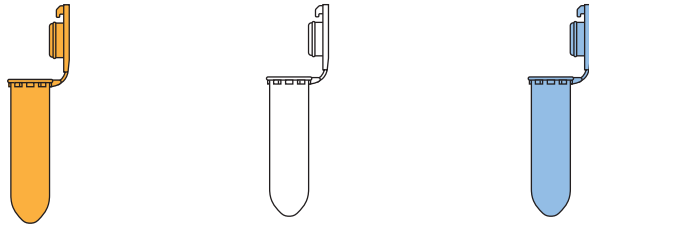
11. Clean any excess fluid from around the septa.
12. Apply Tough-Spots® to the septa and press firmly.
13. Immediately load the arrays into the hybridization oven, four at a time.
14. Hybridize the arrays 16 to 18 hrs at 50°C and 60 rpm.

IMPORTANT POINTS:

- Samples must remain on the thermal cycler 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.

Stage 9: Wash, stain, and scan (8 samples)

1. Aliquot the following reagents into separate 1.5 mL microfuge tubes for each array:
   a. 500 µL Stain Buffer 1 solution into amber tubes
   b. 500 µL Stain Buffer 2 into clear tubes
   c. 800 µL Array Holding Buffer into blue tubes



500 µL Stain Buffer 1 | **500 µL Stain Buffer 2** | **800 µL Array Holding Buffer**

Washing and staining arrays

1. Prime the Fluidics Station with the Wash buffers. Load the stain solutions and select the appropriate fluidics protocol.
   • For CytoScan™ HD Array: CytoScanHD_Array_450
   • For CytoScan™ 750K Array: CytoScan750K_Array_450
2. Start the fluidics protocol and leave the cartridge lever down in the **Eject** position.
3. Remove the Tough-Spots® from each array.
4. Load the arrays onto the Fluidics Station.

IMPORTANT POINTS:

- Aliquot Stain1 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 *GeneChip® Fluidics Station 450/250 User Guide*, Pub. No. 08-0092) or remove the bubbles manually.

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 *CytoScan™ Assay User Guide* (Pub. No. 703038).

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

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28 September 2017

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