

CytoScan™ Assay Automated Workflow on the Applied Biosystems™ NIMBUS™ Instrument—49 Samples

Catalog Numbers 901808, 901833, and 901858

Pub. No. MAN0017987 Rev. B.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *CytoScan™ Assay Automated Workflow on Applied Biosystems™ NIMBUS™ User Guide* (Pub. no. MAN0025400). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: This quick reference document is applicable to both Windows™ 7 and Windows™ 10 users.

Important notes

IMPORTANT! The NIMBUS™ Instrument software method for running the CytoScan™ Assay Manual Workflow is available for Windows™ 10 and Windows™ 7 operating systems. The Windows™ 10 and Windows™ 7 methods have different software version numbers, but the workflow and user interface information are identical.

IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are acceptable alternatives.

Note: The consumables kit does not include the 96-well Clear Flat Bottom UV-Transparent Plate (Corning™ Part. No. 3635). Purchase the Corning™ UV-Transparent Microplate from Fisher Scientific™ (Cat. No. 07-200-623).

Note: The CytoScan™ Assay Automated Workflow for 49 samples requires 2 CytoScan™ Reagent Kits (24 reactions per kit, Cat. No. 901808).

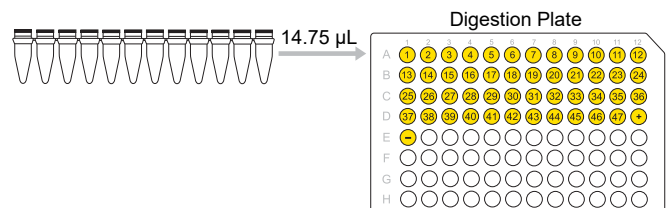
Stage 1—Restriction enzyme digestion

1. Add gDNA to wells marked 1 through 47 in the plate diagram.
2. Thaw the Genomic DNA tube, Nsp I Buffer (2 tubes), and 100X BSA (2 tubes) at room temperature. Vortex, centrifuge, then place on ice.
3. Leave the Nsp I 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 the Low EDTA TE Buffer as negative control to the well marked “–”.

6. Prepare the Digestion Master Mix.

Reagent	49 Samples MM (with 20% overage)	✓	Lot number
Chilled Nuclease-Free Water	665.2 µL		
Nsp I Buffer	115.2 µL		
100X BSA	11.6 µL		
Nsp I	57.6 µL		
Total volume	849.6 µL	–	–

7. Vortex the Digestion Master Mix at high speed 3 times, 1 second each time, then centrifuge.
8. Aliquot the Digestion Master Mix equally to strip tubes.
9. Use a multichannel pipette to add 14.75 µL to the samples.



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

- Ensure that the lid of the thermal cycler is preheated.
- Seal the plate with an adhesive film.
- Vortex the plate at high speed in 5 sector format, 1 second per sector.
- Centrifuge at 2,000 rpm for 1 minute.
- Load the plate onto the thermal cycler, then run the **CytoScan Digest** thermal cycler program.

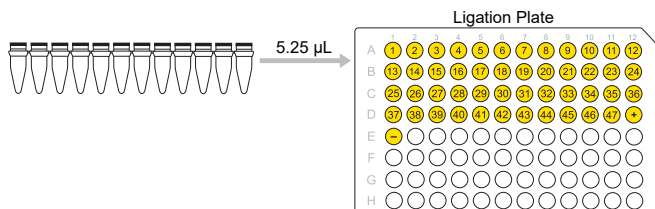
Temperature	Time
37°C	2 hours
65°C	20 minutes
4°C	Hold

Stage 2—Ligation

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

Reagent	49 samples MM (with 20% overage)	✓	Lot number
DNA Ligase Buffer	150.0 μL		
Adaptor, Nsp I	45.0 μL		
DNA Ligase	120.0 μL		
Total volume	315.0 μL	–	–

- Vortex the Ligation Master Mix at high speed 3 times, 1 second each time, then centrifuge.
- Aliquot the Ligation Master Mix equally to strip tubes.
- Use a multichannel pipette to add 5.25 μL to the samples.



Samples	Volume per sample
Digested Sample	19.75 μL
Ligation Master Mix	5.25 μL
Total volume	25.00 μL

- Seal the plate with an adhesive film.
- Vortex the plate at high speed in 5-sector format, 1 second per sector.
- Centrifuge at 2,000 rpm for 1 minute.
- Load the plate onto the thermal cycler, then run the **CytoScan Ligate** thermal cycler protocol.

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

- Proceed to Stage 3A—PCR reaction setup.

Stage 3A—PCR reaction setup

- Ensure that the ligation plate is sealed properly. Centrifuge at 2,000 rpm for 1 minute.
- Dilute the ligated samples.

Reagents	Volume
Ligated DNA	25 μL
Chilled Nuclease-Free Water	75 μL
Total volume	100 μL

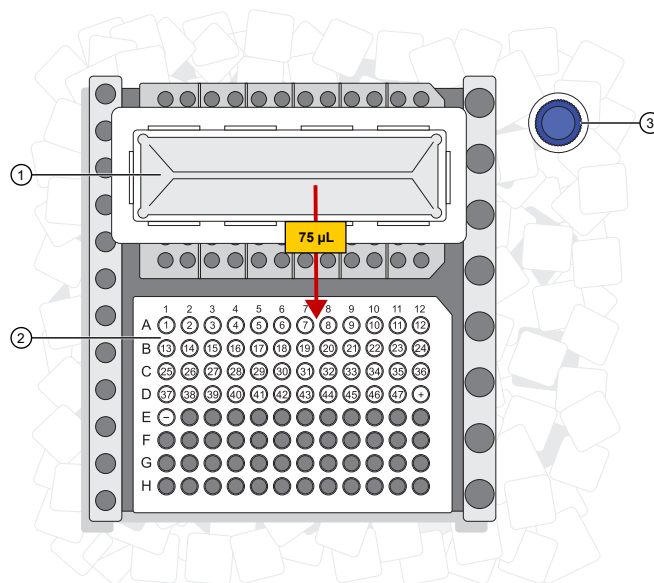
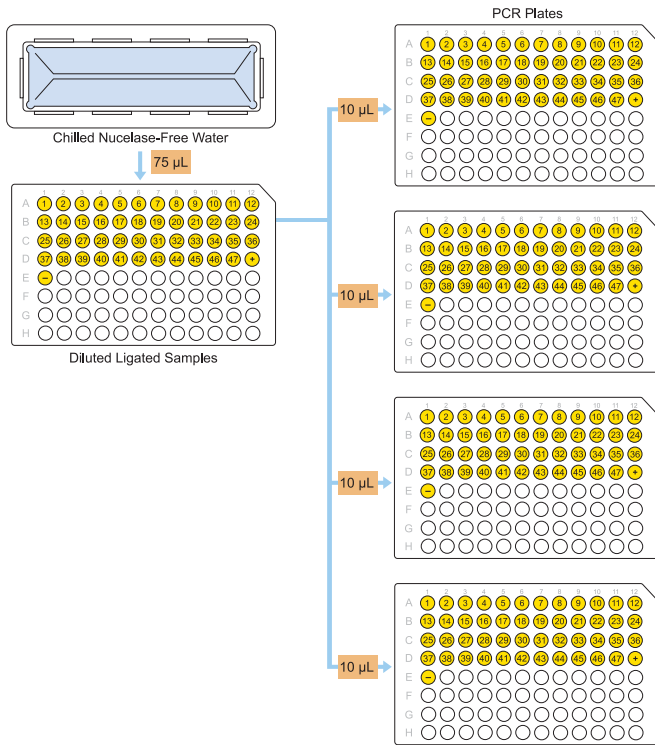


Figure 1 Dilute the ligated samples.

- Chilled Nuclease-Free Water in the reservoir.
- Ligated samples.
- Nuclease-Free Water.

- Seal the plate with an adhesive film.
- Vortex at high speed in 5 sector format, 1 second per sector. *Repeat* vortexing, then centrifuge at 2,000 rpm for 1 minute.
- Transfer four 10- μ L aliquots of each sample to a PCR plate.



- Thaw the 10X TITANIUM™ Taq PCR Buffer, dNTP Mixture, PCR Primer, and diluted ligated samples at room temperature. After thawing, immediately place on ice. Vortex, then centrifuge all reagents.
- Keep the GC-Melt Reagent and Nuclease-Free Water on ice.
- Leave the 50X TITANIUM™ Taq DNA Polymerase at -20°C until ready to use.
- Prepare the PCR Master Mix in a 50-mL centrifuge tube. Assemble the master mix on ice.
- Vortex the PCR Master Mix at high speed 3 times, 1 second each.
- Transfer the PCR Master Mix to a reservoir.

- Use a multichannel pipette to add 90 μ L to the samples.

Reagent	49 Samples MM (with 15% overage)	✓	Lot number
Chilled Nuclease-Free Water	8721.6 μ L		
10X TITANIUM™ Taq PCR Buffer	2,208.0 μ L		
GC-Melt Reagent	4,416.0 μ L		
dNTP Mixture (2.5 mM each)	3,091.2 μ L		
PCR Primer	993.6 μ L		
50X TITANIUM™ Taq DNA Polymerase	441.6 μ L		
Total volume	19,872.0 μL	—	—

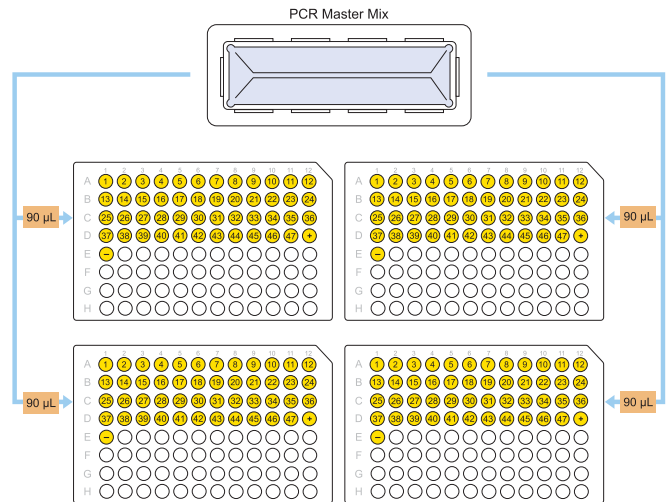


Table 1 PCR Plates.

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

- Seal the PCR plate, vortex at high speed in 5 sector format, 1 second per sector. *Repeat* vortexing, then centrifuge at 2,000 rpm for 1 minute.
- Keep the plate on ice and move to the Post-PCR Room/Area.

Stage 3B—PCR

1. Ensure that the thermal cycler lid is preheated.
2. Load the plates onto the thermal cyclers, then run the **CytoScan PCR** thermal cycler protocol.


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


Volume: 100 µL, specify *maximum* mode.

3. Hold at 4°C.

Stage 3C—PCR product check

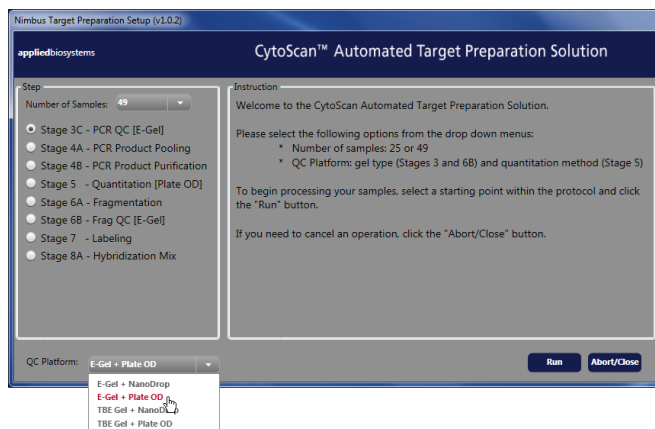
PCR products can be checked using E-Gel™ Agarose Gels or TBE gels.

1. Power on the NIMBUS™ Instrument and Multi TEC Controls.
2. To start the CytoScan™ Automated Target Preparation Solution, on the desktop, double-click the CytoScan™ **Start** icon .

If the software is already open, click the green arrow  in the toolbar.

PCR product check using E-Gel™ Agarose Gels

1. Dilute the TrackIt™ Cyan/Orange Loading Buffer by adding 50 µL of the loading buffer to 49.95-mL Nuclease-Free Water (total volume = 50 mL). Mix well, then store at room temperature.
2. On the **Welcome** screen, select **49 samples, Stage 3C**, then the **QC Platform** (E-Gel™ + quantitation method, either Plate OD or NanoDrop).



3. Click **Run**, then click **Yes** to confirm.

4. Set up labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen. Unseal one of the PCR plates, then discard the seal. Place the plate on the NIMBUS™ Instrument deck, then click **Next**.
5. Place a plate collar on the labware as shown in the **Place Plate Collar** screen, then click **Next**.
6. Place the tubes on NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
7. Start the method by clicking **Run** in the **Run Method** screen, then click **Yes** to confirm.
8. When the method is completed, remove the PCR plate from the NIMBUS™ Instrument deck. Seal the plate tightly with a new seal. If not proceeding to the purification step, store it at –20°C.
9. Remove the gel QC plate from the deck, then seal the plate tightly with an adhesive seal. Vortex the gel QC plate, then centrifuge briefly in a plate centrifuge.
10. After the method is finished, remove labware from the NIMBUS™ Instrument deck as shown in the **Clean Up** screen. Click **Finish**.
11. Power on the power for the E-Base™ device (red light).
12. Push the Power/Prg button to ensure that the program is set to EG mode (not EP).
13. Remove 1 or more combs from the E-Gel™ Agarose Gels, then wipe away any buffer that comes out of the gel or is on the surface.
14. Insert the E-Gel™ 48 Agarose Gels, 2% into the slot (12-well E-Gel™ Agarose Gels product can also be used if running a smaller number of samples).
15. Briefly vortex, then centrifuge the diluted samples before loading onto the E-Gel™ Agarose Gels.
16. Load all 20 µL of the diluted PCR product from the PCR QC gel plate onto the 48-well E-Gel™ 48 Agarose Gels, 2%.
17. Dilute the PCR marker (1:6 dilution, 4 µL in 20 µL of Nuclease-Free Water), then load all 15 µL into each marker wells. Fill empty wells with 20-µL water.
18. Set the run time to 21 minutes.
19. Push the Power/Prg button again (it changes from red to green).
20. The system automatically shuts off when the run time is reached (when the dye is near the end of the lane). The gel is then ready for imaging.

21. Start Stage 4A—PCR product pooling while the gel is running.

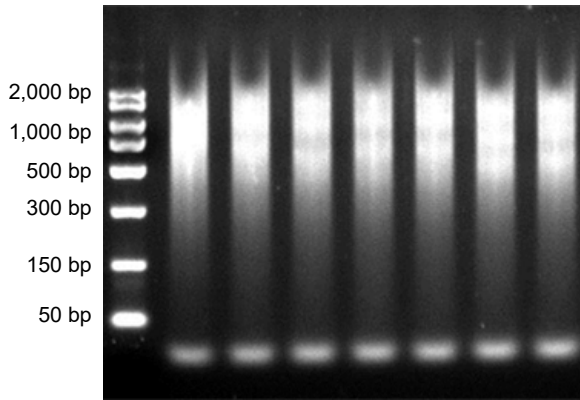


Figure 2 Gel image of the PCR product (from genomic DNA control) on E-Gel™ 48 Agarose Gels, 2%

PCR product check using TBE gels

1. On the **Welcome** screen, select **49 samples, Stage 3C**, then the **QC Platform** (TBE Gel + quantification method, either Plate OD or NanoDrop).
2. Click **Run**, then click **Yes** to confirm.
3. Fill the reservoir on the NIMBUS™ Instrument deck as shown in the **Fill Reservoir** screen, then click **Next**.
4. Set up labware on NIMBUS™ Instrument deck as shown in the **Place Labware** screen.
5. Unseal one of the PCR plates, then discard the seal. Place the plate on NIMBUS™ Instrument deck, then click **Next**.
6. Place a plate collar on the labware as shown in the **Place Plate Collar** screen, then click **Next**.
7. Place the tubes on NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
8. In the **Run Method** screen, click **Run** start the method, then click **Yes** to confirm.
9. After the method is finished, remove the PCR plate from NIMBUS™ Instrument deck. Seal the plate tightly with a new seal. If not proceeding to the purification step, store it at -20°C .
10. Remove the gel QC plate from the deck, then seal the plate tightly with an adhesive seal.
11. After the method is finished, remove labware from NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.
12. Vortex the gel QC plate, then centrifuge briefly in a plate centrifuge.
13. Load 8 μL of the sample mix from the gel strip tubes onto a 2% agarose gel. Load 5 μL of 25 bp DNA Ladder in the lanes before and after the samples. Run the gel at 5 V/cm for 45 minutes.

14. Start Stage 4A—PCR product pooling while the gel is running.

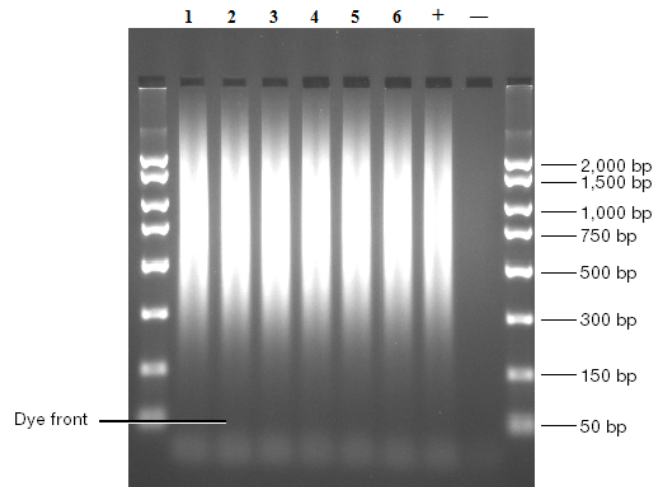
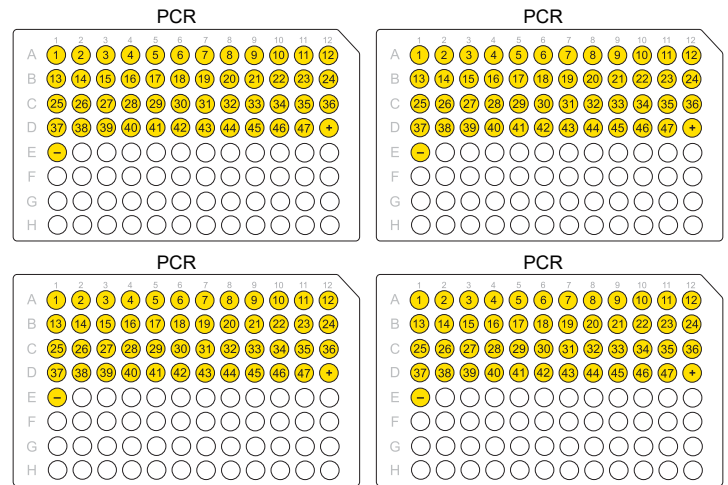




Figure 3 Gel image of PCR products run on a 2% TBE gel at 5 V/cm for 45 minutes

Most product should be between 150 bp and 2,000 bp.

Stage 4A—PCR product pooling


The PCR plate layout must match the format that is shown in the following image. Matching the format allows the NIMBUS™ Instrument to pool the PCR reactions for each sample.




1. Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.
2. Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.
If the software is already open, click the green arrow  in the toolbar.
3. From the **QC Platform** list, select **49 samples, Stage 4A**, and the gel type and quantification method.
4. Click **Run**, then click **Yes** to confirm.
5. Set up labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen.

- Place the 2 PCR plates on the PCR tube storage racks, unseal the plates, then discard the seals. Place the plates on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen.
- Place a plate collar on the labware as shown in the **Place Plate Collar** screen, then click **Next**.
- In the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.
- After the method is finished, remove labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

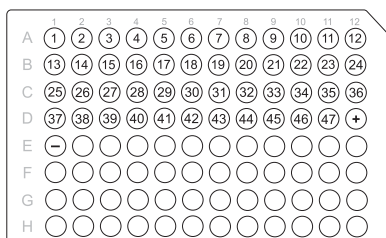
Stage 4B—PCR product purification

- Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.
- Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.

If the software is already open, click the green arrow  in the toolbar.

- From the **QC Platform** list, select **49 samples, Stage 4B**, and the gel type and quantification method.
- Click **Run**, then click **Yes** to confirm.
Note: Ensure that 45 mL of absolute ethanol has been added to the Purification Wash Buffer before use.
- Transfer the entire content of each reagent bottle into its respective reservoir position as shown in the **Fill Reservoir** screen, then click **Next**.
- Place the labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen. Place a 2.2-mL ABgene™ plate under the tip isolator and a rack of 300-µL tips on top, then click **Next**.
- Place a plate collar on the labware as shown in the **Place Plate Collar** screen.
- On the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.

After the purification method is completed, the NIMBUS™ Instrument transfers 47 µL of eluted sample to the appropriate wells of a new 96-well plate.





- Visually examine the purified sample plate to determine whether the wells contain the same volume. If a well has lower volume:
 - Measure the volume using a pipette.

- If the volume is less than 47 µL, bring the volume to 47 µL by adding Elution Buffer.
- Tightly seal the plate, then vortex at high speed for 1 second in all corners and in the center, then centrifuge at 2,000 rpm for 1 minute.
 - Remove labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Stage 5—Quantitation

Perform quantitation using a SpectraMax® Plus 384 Microplate Spectrophotometer or a NanoDrop™ Spectrophotometer.

- Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.
- Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.
If the software is already open, click the green arrow  in the toolbar.

Quantitation using the SpectraMax® Plus 384 Microplate Spectrophotometer

- From the **QC Platform** list, select **49 samples, Stage 5**, and the gel type and quantitation method.
- Click **Run**, then click **Yes** to confirm.
- Fill the reservoir as shown in the **Fill Reservoir** screen, then click **Next**.
- Set up labware on NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
- Place a plate collar on the labware as shown in the **Place Plate Collar** screen.
- On the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.
- After the method is finished, tightly seal the plate of purified samples with a new seal, then store at -20°C.
- Seal the UV plate, using a laboratory tissue on the vortex adaptor surface, vortex, then centrifuge at 2,000 rpm for 1 minute.
- Remove labware from NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Quantitate the diluted PCR product

Apply the convention that 1 absorbance unit at 260 nm equals 50 µg/mL (equivalent to 0.05 µg/µL) for double-stranded PCR products. This convention assumes a path length of 1 cm. See the spectrophotometer handbook for further information.

1. Measure the OD of each sample at 260, 280, and 320 nm.
OD₂₈₀ and OD₃₂₀ are used as controls.
2. Determine the OD₂₆₀ measurement for the water blank and average.
3. Determine the concentration of each PCR product.
 - a. Calculate 1 OD reading for every sample:
OD = (sample OD) – (average water blank OD).
 - b. Calculate the undiluted concentration for each sample in µg/µL:
Undiluted sample concentration = OD x 0.05 µg/µL x 100.


Quantitation using the NanoDrop™ Spectrophotometer


1. From the **QC Platform** list, select **49 samples, Stage 5**, and the gel type + NanoDrop.
2. Click **Run**, then click **Yes** to confirm.
3. Fill the reservoir as shown in the **Fill Reservoir** screen, then click **Next**.
4. Set up labware on NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
5. Place a plate collar on the labware as shown in the **Place Plate Collar** screen, then click **Next**.
6. On the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.
7. After the method is finished, tightly seal the plate of purified samples with a new seal, then store at –20°C.
8. Seal the UV plate, using a laboratory tissue on the vortex adaptor surface, vortex, then centrifuge at 2,000 rpm for 1 minute.
9. Remove labware from NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Quantitate the PCR product

1. Blank the NanoDrop™ Spectrophotometer with water.
2. Take 2 µL of the diluted sample and:
 - a. Measure the OD of each sample at 260, 280 and 320 nm.
OD₂₈₀ and OD₃₂₀ are used as controls.
 - b. Calculate the undiluted concentration for each sample as follows.
Undiluted sample concentration in µg/µL = (NanoDrop™ concentration in ng/µL × 10) ÷ (1,000).

Stage 6A—Fragmentation

1. Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.
2. Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.

If the software is already open, click the green arrow  in the toolbar.

Note: Confirm that the cooling block and Thermoshake plate adapter on the NIMBUS™ Instrument deck are cooling down to 4°C. The cooling block takes ~20 minutes to reach 4°C.

3. Turn down the plate centrifuge to 4°C before starting the fragmentation step.
4. Power on the thermal cycler to preheat the lid.
5. If frozen, remove the plate of purified, quantitated samples from –20°C storage, then thaw.
Seal the plate tightly, vortex, then centrifuge. Place the plate on the lower half of the cooling block on ice, then chill for 10 minutes before use.

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

6. Thaw the Fragmentation Buffer at room temperature. When thawed, immediately place on ice.
7. Prepare the Fragmentation Buffer.
 - Vortex 3 times, 1 second each time.
 - Centrifuge for 3 seconds.
 - Place on ice.
8. Remove the Fragmentation Reagent from the freezer, then immediately place it in the cooler that is chilled to –20°C.
 - Vortex the Fragmentation Reagent at high speed 1 time for 1 second.
 - Immediately centrifuge for 3 seconds to bring down any reagent that can be clinging to the top of the tube.
 - Immediately place in the –20°C cooler.
9. From the **QC Platform** list, select **49 samples, Stage 6A**, and the gel type and quantification method.

10. Click **Run**, then click **Yes** to confirm.
11. Wait until the block temperature reaches 4°C, then click **Next**.
12. Wait until the shaker temperature reaches 4°C, then click **Next**.
13. Set up labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
14. Place a plate collar on the labware as shown in the **Place Plate Collar** screen, then click **Next**.
15. Place tubes on the NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
16. On the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.
17. After the method is complete, immediately place the fragmentation sample plate on a cooling block. Tightly seal the plate with a new seal.


IMPORTANT! Always carry the sample plate to the centrifuge or the thermal cycler on the cooling block in the ice box.


18. Vortex the plate at high speed in 5 sector format, 1 second per sector.
19. Bring the sample plate to the centrifuge on the cooling block in the ice box. Centrifuge at 2,000 rpm for 1 minute in a prechilled centrifuge.
20. Ensure that the thermal cycler block is preheated. Carry the sample plate on the cooling block in the ice box, then immediately load the Fragmentation Plate onto the thermal cycler.
21. Run the **CytoScan Fragment** thermal cycler protocol.

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	Hold
22. After the method is complete, remove labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Stage 6B—Fragmentation QC

Perform fragmentation QC using E-Gel™ Agarose Gels or TBE gels.

1. Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.
2. Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.

If the software is already open, click the green arrow  in the toolbar.

Note: Confirm that the block is cooling. It takes ~20 minutes for the block to reach 4°C.

Fragmentation QC using E-Gel™ Agarose Gels

1. When the **CytoScan Fragment** thermal cycler protocol is finished, remove the sample plate from the thermal cycler.
2. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute. Place on a cooling block on ice.
3. On the **Welcome** screen, select **49 samples, Stage 6B**, then the **QC Platform** (E-Gel + quantification method, either Plate OD or NanoDrop).
4. Click **Run**, then click **Yes** to confirm.
5. Fill the reservoir as shown in the **Fill Reservoir** screen, then click **Next**.
6. Wait until the shaker temperature reaches 4°C, then click **Next**.
7. Set up labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
8. Place a plate collar on labware as shown in the **Place Labware** screen, then click **Next**.
9. Place tubes on the NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
10. On the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.
11. After the method is finished, remove the Fragmentation Plate from the NIMBUS™ Instrument deck. Seal the plate tightly with a new seal. If proceeding to Stage 7—Labeling next, hold the plate on ice until then. If not proceeding to Stage 7—Labeling, store the plate at –20°C.
12. Seal, then store the Fragmentation QC Sample Dilution Plate at –20°C for further analysis using the Agilent™ 2100 Bioanalyzer™.
13. Tightly seal the Fragmentation QC Sample Plate with a new seal, vortex, then centrifuge.
14. Remove the labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Run the E-Gel™ Agarose Gel

1. Power on the E-Base™ device (red light).
2. Push the Power/Prg button to ensure that the program is set to EG mode (not EP).
3. Remove the comb or combs from the E-Gel™ Agarose Gel, then wipe away any buffer that comes out of the gel or is on the surface.
4. Insert the E-Gel™ 48 Agarose Gels, 4% into the slot. A12-well E-Gel™ Agarose Gel can also be used if running a smaller number of samples.
5. Load 20 µL of the fragmented product from the QC sample plate onto the E-Gel™ 48 Agarose Gels, 4%.
6. Dilute the 25 bp DNA Marker (1:6 dilution, 4 µL in 20 µL of Nuclease-Free Water), then load 15 µL into each marker well, if needed. Fill empty wells with 20-µL water.
7. Set the run time to 19 minutes.
8. Push the Power/Prg button again (it changes from red to green).

The system automatically powers off when the run time is reached (when the dye is near the end of the lane, as shown in the following example). The gel is then ready for imaging.

9. Verify that the fragmented product distribution is between 25 bp to 125 bp, then, proceed to Stage 7—Labeling.

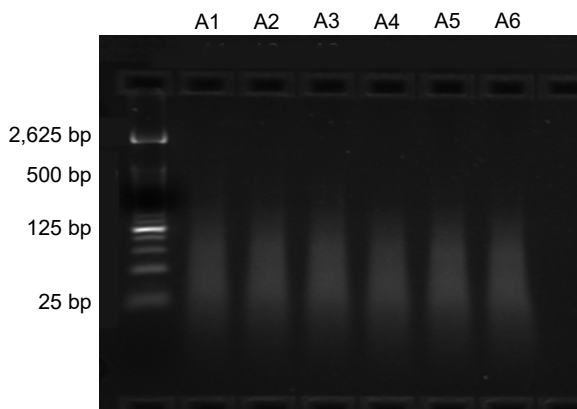


Figure 4 Gel image of fragmented product (from Genomic DNA) on E-Gel™ 48 Agarose Gels, 4%.

Fragmentation QC using TBE gels

1. When the **CytoScan Fragment** thermal cycler protocol is finished, remove the sample plate from the thermal cycler.
2. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute. Place on a cooling block on ice.
3. On the **Welcome** screen, select **49 samples, Stage 6B**, then the **QC Platform** (TBE + quantification method, either Plate OD or NanoDrop).
4. Click **Run**, then click **Yes** to confirm.
5. Fill the reservoir as shown in the **Fill Reservoir** screen, then click **Next**.
6. Wait until the shaker temperature reaches 4°C, then click **Next**.
7. Set up labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
8. Place a plate collar on the labware that is shown in the **Place Plate Collar** screen, then click **Next**.
9. Place tubes on the NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
10. On the **Run Method** screen, click **Run** to start the method, then click **Yes** to confirm.
11. Seal, then store the Fragmentation QC Sample Dilution Plate at -20°C for further analysis using the Agilent™ 2100 Bioanalyzer™.

12. Tightly seal the Fragmentation QC Sample Plate with a new seal, vortex, then centrifuge.

13. Remove labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Run the TBE gel

1. Load 8 µL of the samples from the QC Sample Plate onto the gel.
2. Load 2 µL of the 25 bp DNA Ladder to the first and last lanes.
3. Run the samples on a 4% TBE gel at 5 V/cm for 45 minutes or until the dye front reaches at least 75% of distance down the gel.

- Inspect the gel, then compare it against the example that is shown in the following figure. Most fragment distribution should be between 25 bp to 125 bp.

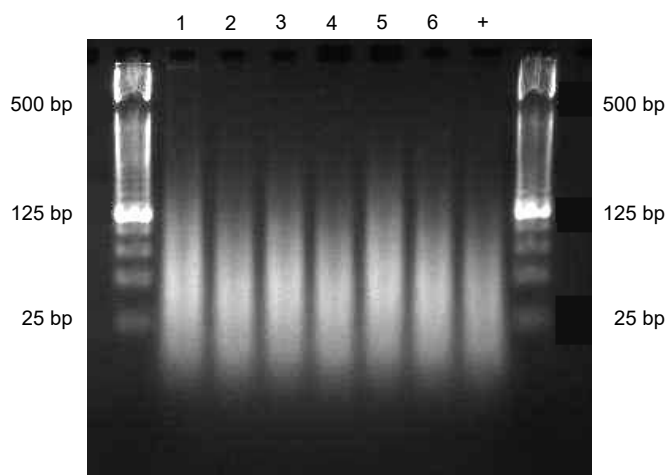



Figure 5 Example of fragmented samples run on a 4% TBE gel at 5 V/cm for 45 minutes.

Average fragment distribution is between 25 bp to 125 bp.

Stage 7—Labeling

- Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.

- Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.

If the software is already open, click the green arrow  in the toolbar.

Note: Confirm that the cooling block and Thermoshake plate adapter on the NIMBUS™ Instrument deck are cooling down to 4°C. The cooling block takes ~20 minutes to reach 4°C.

- Power on the thermal cycler to preheat the lid. Leave the block at room temperature.
- Thaw the TdT Buffer and DNA Labeling Reagent at room temperature. When thawed, immediately place on cooling block on ice.

IMPORTANT! Leave the TdT Enzyme at –20°C until ready to use.



- Prepare the TdT Buffer and DNA Labeling Reagent by vortexing each reagent at high speed 3 times, 1 second each time. Centrifuge each reagent for 3 seconds, then place in the cooling block.
- If the fragmented samples were frozen, allow them to thaw at room temperature. Immediately centrifuge the plate at 2,000 rpm for 1 minute and place on the cooling block on ice.
- Remove the TdT Enzyme from the freezer and immediately place in the cooler that is chilled to –20°C. Vortex the enzyme at high speed 1 time for 1 second. Centrifuge the enzyme for 3 seconds, then immediately place back in the –20°C cooler.

- On the **Welcome** screen, select **49 samples, Stage 7**.
- Click **Run**, then click **Yes** to confirm.
- Wait until the cooling block reaches 4°C, then click **Next**.
- Wait until the shaker temperature reaches 4°C, then click **Next**.
- Set up the labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
- Place a plate collar on labware as shown in the **Place Plate Collar** screen, then click **Next**.
- Place tubes on the NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
- On the **Run Method**, click **Run** to start the method, then click **Yes** to confirm.
- Tightly seal the plate, then vortex in 5-sector format, 1 second per sector.
- Centrifuge at 2,000 rpm for 1 minute.
- Load the plate onto the preheated thermal cycler block, then run the **CytoScan Label** thermal cycler protocol.

Temperature	Time
37°C	4 hours
95°C	15 minutes
4°C	Hold

- 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.
- Remove labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Stage 8A—Hybridization Mix

- Confirm that the NIMBUS™ Instrument and Multi TEC Control are powered on.
- Start the CytoScan™ Automated Target Preparation Solution by double-clicking the **Start** icon  on the workstation desktop.
If the software is already open, click the green arrow  in the toolbar.
Note: Confirm that the cooling block and Thermoshake plate adapter on the NIMBUS™ Instrument deck are cooling down to 4°C. The cooling block takes ~20 minutes to reach 4°C.
- Place a double-cooling block on ice.
- Prepare the samples.
 - If the labeled samples from the previous stage were frozen, allow them to thaw on the benchtop to room temperature, then centrifuge at 2,000 rpm for 1 minute.

- b. Immediately place the plate in the lower half of the cooling block on ice.
5. Thaw the following reagents at room temperature. When thawed, immediately place on cooling block on ice.
 - Hyb Buffer Part 1
 - Hyb Buffer Part 2
 - Hyb Buffer Part 3
 - Hyb Buffer Part 4
 - Oligo Control Reagent
6. Vortex each reagent at high speed 3 times, 1 second each time.
7. Briefly centrifuge for 3 seconds, then place in the cooling block.
8. On the **Welcome** screen, select **49 samples, Stage 8A**.
9. Click **Run**, then click **Yes** to confirm.
10. Wait until the cooling block reaches 4°C, then click **Next**.
11. Wait until the shaker temperature reaches 4°C, then click **Next**.
12. Fill the reservoir as shown in the **Fill Reservoir** screen, then click **Next**.
13. Set up labware on the NIMBUS™ Instrument deck as shown in the **Place Labware** screen, then click **Next**.
14. Place a plate collar on labware as shown in the **Place Plate Collar** screen, then click **Next**.
15. Place tubes on the NIMBUS™ Instrument deck as shown in the **Place Tubes** screen, then click **Next**.
16. On the **Run Method** screen, click **Run**, then click **Yes** to confirm.
17. After the method is finished, tightly seal the plate, then store at -20°C.
18. Remove labware from the NIMBUS™ Instrument deck as shown in the **Cleanup** screen, then click **Finish**.

Stage 8B—Hybridization

1. Unpack the arrays, then allow to equilibrate to room temperature before use.
2. Preheat the hybridization ovens for at least 1 hour at 50°C with the rotation on.
3. Create a Batch Registration file.
4. From the **Probe Array type set to** list, select the appropriate array type.
 - If using the CytoScan™ HD Array, select **CytoScanHD_Array**.
 - If using the CytoScan™ 750K Array, select **CytoScan750K_Array**.

5. Load the plate onto the thermal cycler, then run the **CytoScan Hybridization** thermal cycler protocol.

Temperature	Time
95°C	10 minutes
49°C	Hold

6. Allow the samples to incubate at 49°C for at least 1 minute before loading.
7. Leave the samples on the thermal cycler, then load 200 µL of sample onto each array using a single-channel P200 pipette. Hybridize only up to 8 arrays at a time.



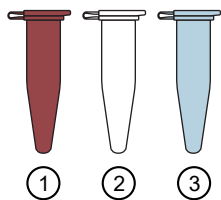
8. Clean any excess fluid from near the septa.
9. Apply Tough-Spots™ labels to the septa, then press firmly.
10. Immediately load the arrays into the hybridization oven.
11. Hybridize the arrays for 16–18 hrs at 50°C and 60 rpm.

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 that bubbles are not visible through the window.

Stage 9—Wash, stain, and scan

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



- ① 500 μ L Stain Buffer 1 into amber tubes.
- ② 500 μ L Stain Buffer 2 into clear tubes.
- ③ 800 μ L Array Holding Buffer into blue tubes.

Wash and stain arrays

1. Turn on the fluidics station.
2. Prime the fluidics station with the wash buffers. Load the stain solutions, then select the appropriate fluidics protocol.
 - a. If using the CytoScan™ HD Array, select **CytoScanHD_Array_450**.
 - b. If using the CytoScan™ 750K Array, select **CytoScan750K_Array_450**.
3. Start the fluidics protocol, leaving the cartridge lever down in the Eject position.
4. Remove the Tough-Spots™ labels from each array.
5. Load the arrays onto the fluidics station.

Before scanning

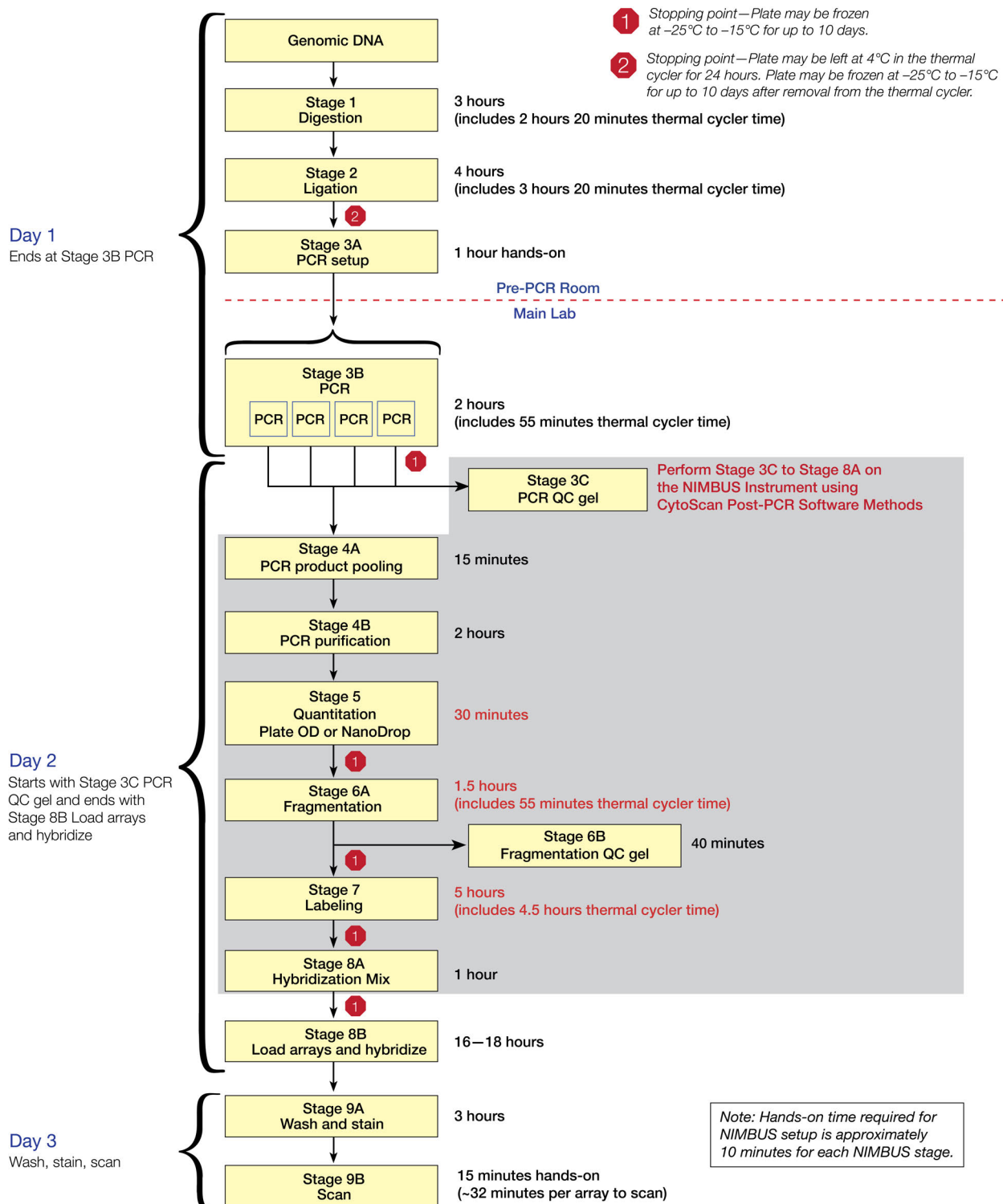
1. Ensure that no bubbles are visible through the window.
2. Cover the septa with Tough-Spots™ labels, then load onto the scanner.
3. Scan the arrays as described in the *CytoScan™ Assay Automated Workflow on Applied Biosystems™ NIMBUS™ User Guide* (Pub. No. MAN0025400).

Important points

- Aliquot Stain Buffer 1 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 User Guide*, Pub. No. 08-0295) or remove the bubbles manually.

Workflow overview

The CytoScan™ Assay Automated Workflow is optimized for sample processing on the NIMBUS™ Target Preparation Instrument to obtain whole-genome copy number and SNP allelic information from CytoScan™ Arrays. This document covers the processing of 49 samples at a time, 2 of which are positive and negative controls.



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Revision history: Pub. No. MAN0017987

Revision	Date	Description
B.0	16 November 2021	Added recommendations for replacing the Bio-Rad™ HSS9641 (green) PCR plate with the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode.
A.0	11 September 2018	Initial release in Thermo Fisher Scientific document control system. Supersedes legacy Affymetrix publication number 703144. Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty.

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