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CytoScan™ Assay Automated Workflow

USER GUIDE

for 25 or 49 Samples

for use with:
Applied Biosystems™ CytoScan™ Arrays
Applied Biosystems™ CytoScan™ Reagent Kit
Applied Biosystems™ NIMBUS™ Target Preparation Instrument

Catalog Numbers 901808, 901833, and 901858

Document Part Number 703141

Publication Number MAN0025400

Revision B.0

ThermoFisher SCIENTIFIC

Affymetrix Pte Ltd |
7 Gul Circle #2M-01 |
Keppel Logistics Building |
Singapore 629563

Products manufactured at this site:

CytoScan™ Arrays

Thermo Fisher Scientific Baltics UAB |
V.A. Graiciuno 8, LT-02241 |
Vilnius, Lithuania

Products manufactured at this site:

CytoScan™ Reagent Kit

Revision history: MAN0025400 B.0 (English)

Revision	Date	Description
B.0	18 October 2023	• Updated product information. • Updated *Related documentation*.
A.0	16 November 2021	• Added recommendations for replacing the Bio-Rad™ HSS9641 (green) 96-well PCR plates with the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode. • Initial release in Thermo Fisher Scientific document control system. • Supersedes legacy Affymetrix publication number 703141. • Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty.

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

Contents

1 Introduction

About the CytoScan™ Assay Automated Workflow

The Applied Biosystems™ CytoScan™ Assay Automated Workflow is optimized for post-PCR processing from 25 or 49 samples at a time using the Applied Biosystems™ NIMBUS™ Target Preparation Instrument to obtain whole genome copy number and SNP information from Applied Biosystems™ CytoScan™ Arrays. The automated post-PCR steps include all stages, starting with PCR purification and finishing with Hybridization Master Mix preparation. This protocol is not intended for genome-wide association studies.

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

Cytogenetics research studies are performed to identify structural changes in DNA, such as copy number changes (CNV), loss of heterozygosity (LOH), and mosaicism. Individuals typically have 2 copies of the genome in each of their cells: 1 inherited from the mother and 1 inherited from the father. Chromosomal abnormalities, such as the following, are common in several disease states:

- Deletions—When 1 or both copies of a particular chromosome region are lost.
- Gains—When a chromosome or chromosomal region is duplicated or multiplied.
- Uniparental Disomies (UPDs)—When 2 copies of a chromosome or chromosomal region are present, but both have been inherited from a single parent.

Traditional cytogenetics techniques, such as karyotyping and fluorescent *in situ* hybridization (FISH), have been used to study chromosomal abnormalities for decades. However, karyotyping detects abnormalities only at low resolutions (larger than ~5 Mb), and FISH is a more focused and targeted approach without the benefit of genome-wide analysis. Further, these techniques are limited to providing only copy number information so that UPDs cannot be identified.

Together, the CytoScan™ Assay Automated Workflow, CytoScan™ Arrays, along with the Applied Biosystems™ GeneChip™ Command Console™ and Chromosome Analysis Suite software, enable you to perform high-resolution genome-wide DNA copy number analysis. The Thermo Fisher Scientific solution for cytogenetics research also provides genotyping information with over 750,000 SNPs, enabling detection of loss of heterozygosity (LOH), which can be used to detect UPDs. The combined high resolution DNA copy number data and the ability to detect gains, losses, mosaicism, and LOH on a single array makes the CytoScan™ solution a great tool for next generation cytogenetics research studies.

Workflows

Recommended 3-day workflow

A 3-day workflow is recommended for the CytoScan™ Assay Automated Workflow, where 1 operator processes 25 or 49 samples, including controls. In the 3-day workflow, you hybridize the samples onto arrays at the end of day 2. Day 1 and day 2 are extended work days (9 hours). Day 3 takes about half a day to wash the arrays and load the scanner.

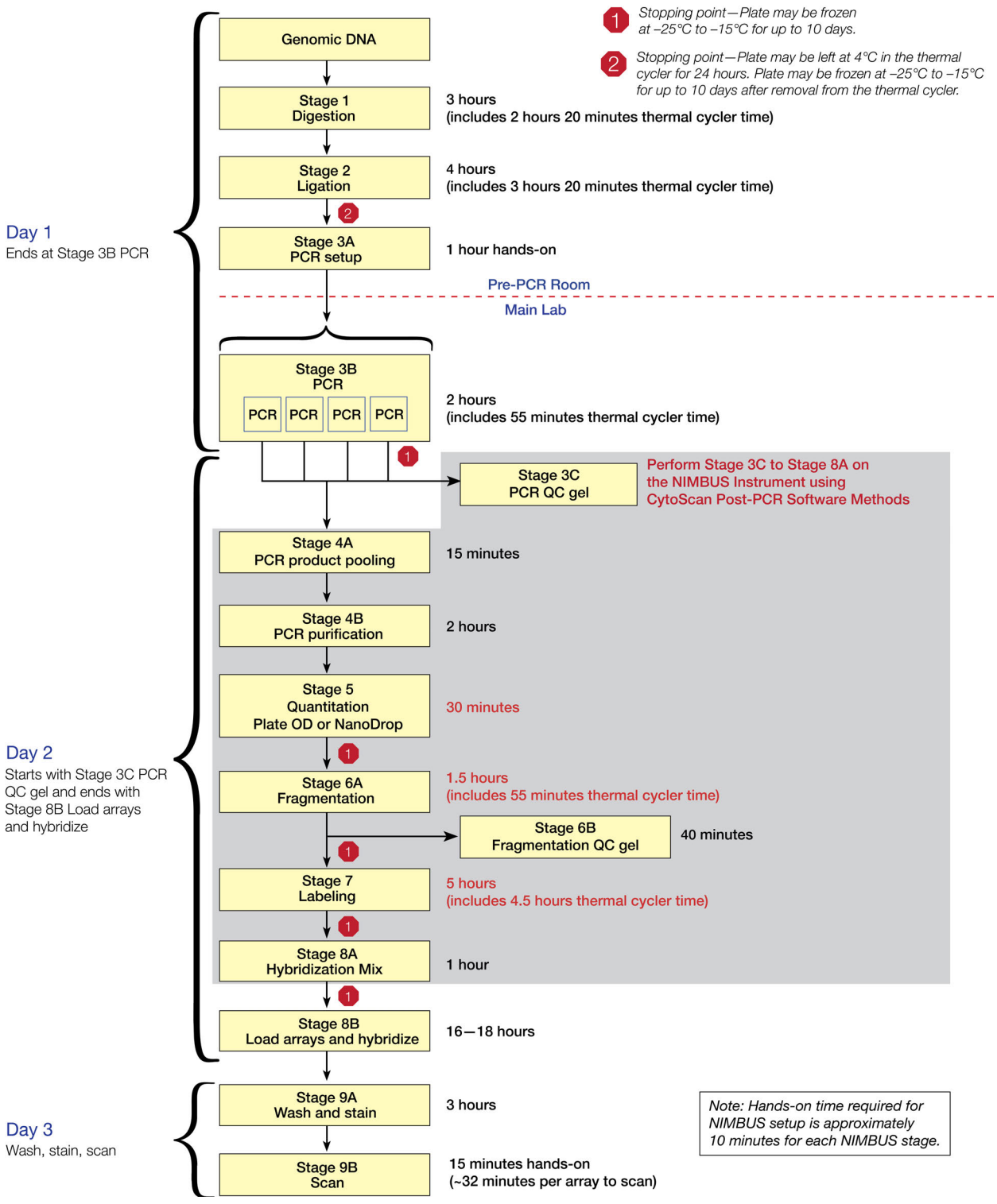


Figure 1 Recommended 3-Day workflow.

Optional 4-day workflow

An optional 4-day workflow can be used for the CytoScan™ Assay Automated Workflow, where 1 operator processes 25 or 49 samples including controls. Day 1 to day 3 are 8-hour work days. The process on day 4 takes about half a day to wash the arrays and load the scanner.

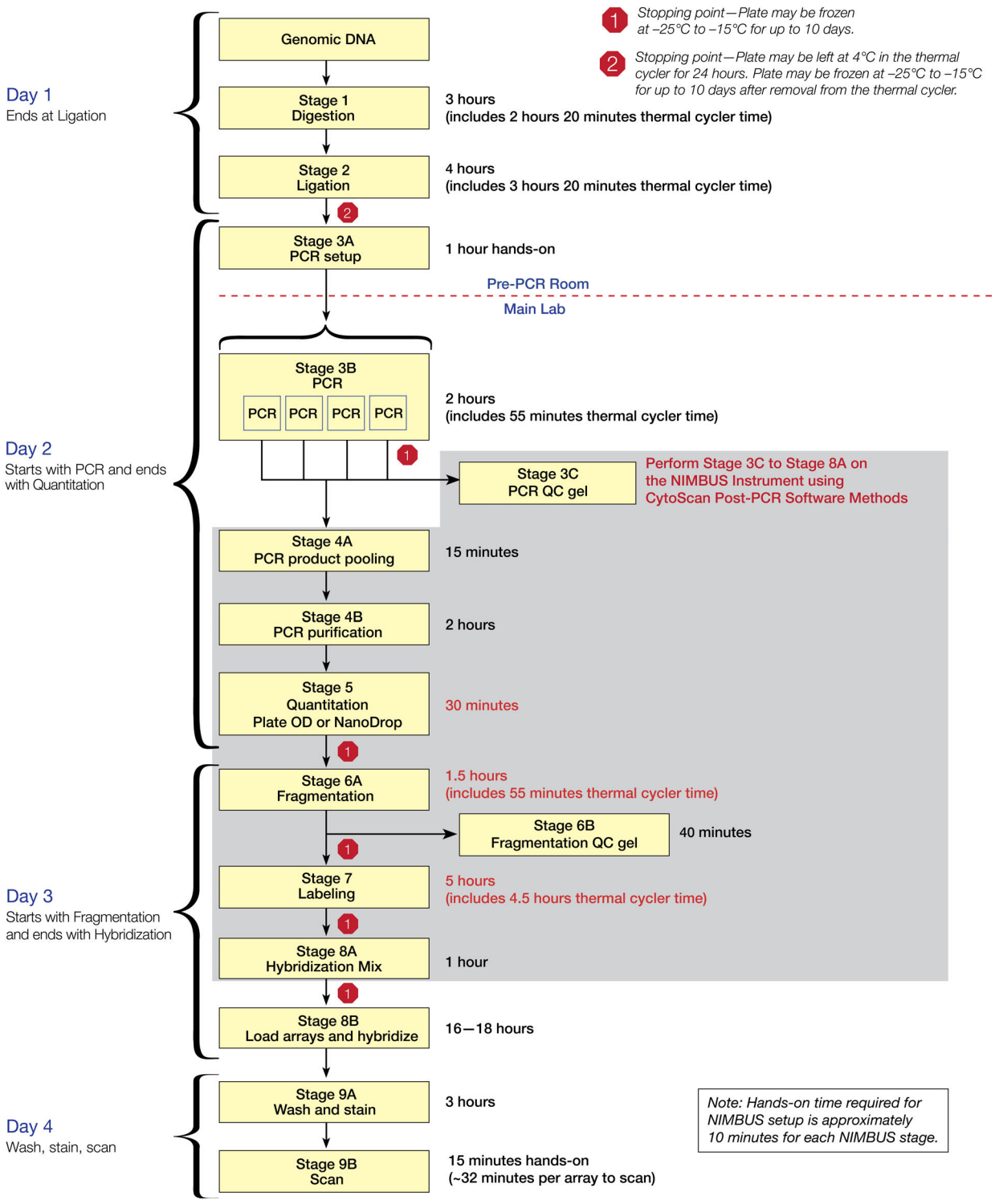


Figure 2 Optional 4-day workflow.

2 Set up the CytoScan™ Assay Automated Workflow

This chapter contains information describing the equipment, materials, and set-up procedures required for running the CytoScan™ Assay Automated Workflow on the NIMBUS™ Target Preparation Instrument.

To ensure operator safety and assay performance, operators must be familiar with the content before starting target preparation described in Chapter 5, “CytoScan™ Assay Automated Workflow for 25 samples” and Chapter 6, “CytoScan™ Assay Automated Workflow for 49 samples”.

Software required

Item	Source
GeneChip™ Data Collection Software	Version 1.0 or later
GeneChip™ Command Console™	Version 6.1 or later
GeneChip™ Chromosome Analysis Suite (ChAS) Software Requires 64-bit workstation, Windows™ operating system, 8-GB RAM.	901394
CytoScan™ post-PCR software methods for the NIMBUS™ Instrument • Windows™ 7 operating system • Windows™ 10 operating system	 • 600732 • 611397

Equipment, reagents, consumables, and arrays required

Pre-PCR Clean Area equipment required

When performing the pre-PCR stages of the CytoScan™ Assay Automated Workflow, take great care to avoid sample contamination with PCR products. If the assay is to be run in a single room, we strongly recommend that the pre-PCR stages be performed in a laminar flow or PCR cabinet.

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

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Laminar cabinet. Recommended if protocol is to be performed in 1 room only: • Laminar flow cabinet, 6-foot • PCR cabinet	• ESCO™, SVE-6A, or equivalent • C.B.S. Scientific™, P-048-02, or equivalent
Benchtop cooler, –20°C	Agilent™, 401349
Biocooler aluminum block, 96-well Required if processing more than 8 samples. (1 for 9–16 samples, 2 for 17–24 samples)	BioSmith, 81001
Double Cooling Block for 0.2 mL tubes	Diversified Biotech™, CHAM-1020
Freezer, –20°C; deep freeze; manual defrost; 17 cu ft	—
Corning™ Rectangular Ice Pan, Maxi 9L	Fisher Scientific™ 07-210-093
Mini microcentrifuge (for tubes and strip tubes)	**MLS**
96-well tube storage racks	**MLS**
Single-channel P20, 2–20 µL	Rainin™ L-20, **MLS**
Single-channel P200, 20–200 µL	Rainin™ L-200, **MLS**
Single-channel P1000, 100–1,000 µL	Rainin™ L-1000, **MLS**
Pipette, 12-channel, 2–20 µL	Rainin™ L12-20, **MLS**
Pipette, 12-channel, 20–200 µL	Rainin™ L12-200, **MLS**
Plate centrifuge, multipurpose	Eppendorf™ 5804 or 5810, **MLS**
Vortexer	**MLS**
Thermal cycler: capable of holding 200 µL volume and 96-well plate; heat block capable of holding temperature of 4–99.9°C; temperature accuracy of ±0.25°C (at 35–99.9°C); average heating and cooling rate of 2.6°C per second; thermal uniformity of ±0.5°C.	See Table 2 on page 41.

Note: Electronic multichannel pipettes are not recommended.

Post-PCR Area equipment required

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

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration.	00-0401
Applied Biosystems™ GeneChip™ Fluidics Station 450	00-0079
Tubing, silicone peristaltic for GeneChip™ Fluidics Station 450	400110
Applied Biosystems™ GeneChip™ Hybridization Oven 645	00-0331
Applied Biosystems™ GeneChip™ Scanner 3000 7G System	00-0213
(Optional) Applied Biosystems™ GeneChip™ System 3000Dx v2, running AMDS v1.1 or higher	00-0334
Thermal cycler, capable of holding 200 µL volume and 96-well plate; heat block, capable of holding temperature of 4–99.9°C, temperature accuracy of ±0.25°C (at 35–99.99°C), average heating and cooling rate of 2.6°C per second, thermal uniformity of ±0.5°C.	See Table 2 on page 41.
Invitrogen™ Mother E-Base™ Device	EBM03
Invitrogen™ Daughter E-Base™ Device	EBD03
Freezer, –20°C; deep freeze; manual defrost; 17 cu ft	**MLS**
Refrigerator, 2–8°C	**MLS**
Benchtop Cooler, –20°C	Agilent™, 401349
Corning™ Rectangular Ice Pan, Maxi 9L	Fisher Scientific™ 07-210-093
Scientific Industries™ Vortex-Genie 2 (for plates and microtubes)[1]	Fisher Scientific™ 50-728-002 (120 V/60 Hz) 50-728-004 (230 V/50 Hz)
Mini microcentrifuge (for tubes and strip tubes)	**MLS**
Eppendorf™ Benchtop Centrifuge, multipurpose plate centrifuge	Eppendorf™ 5804R or 5810R, **MLS**
Bel-Art™ SP Scienceware® Cryo-Safe™ Mini Quick-Freeze Microcentrifuge Tube Cooler, –15°C[1]	Fisher Scientific™, 03-410-497
Pipet-Aid™ Pipette Controller	**MLS**
Single-channel P20, 2–20 µL	Rainin™ L-20, **MLS**

(continued)

Item	Source
Single-channel P200, 20–200 µL	Rainin™ L-200, **MLS**
Single-channel P1000, 100–1,000 µL	Rainin™ L-1000, **MLS**
Gel imager	**MLS**
Electrophoresis gel box	**MLS**
Thermo Scientific™ Owl™ EC-105 Compact Power Supply	105ECA-115
Spectrophotometer, one of the following: • SpectraMax® Plus 384 Microplate Reader • NanoDrop™ Spectrophotometer	 • Molecular Devices™ PLUS 384 • NanoDrop™ ND-1000

[1] Equivalent item from other manufacturer is acceptable.

Note: Electronic multichannel pipettes are not recommended.

Reagents and arrays required

CytoScan™ Reagent Kit

Always use the 24-reaction Applied Biosystems™ CytoScan™ Reagent Kit (Cat. No. 901808) for this assay, whether running 25 or 49 samples.

- 25 sample method (23 customer samples, 1 positive control, 1 negative control) uses one 24-reaction CytoScan™ Reagent Kit.
- 49 sample method (47 customer samples, 1 positive control, 1 negative control) uses two 24-reaction CytoScan™ Reagent Kits.

The reagent kit is sufficient for 1-time use only on the NIMBUS™ Instrument.

We recommend freezing the negative control and not proceeding with hybridization; use the negative control for troubleshooting purpose only. We recommend including the positive control along with other samples for hybridization.

Reagent and cap color	Part No.	Storage
CytoScan™ Module 1: Pre-Lab Restriction and Ligation	**904004**	–25°C to –15°C
Nsp I	901718	
Nsp I Buffer	901719	
100X BSA	901720	
Adaptor, Nsp I	902702	
DNA Ligase Buffer	901722	
DNA Ligase	901723	
Genomic DNA (REF DNA 103 (50 ng/µL))	900421	
PCR Primer	902674	

(continued)

Reagent and cap color	Part No.	Storage
CytoScan™ Module 2: Pre-Lab TE Buffer and Water	**904001**	2°C to 8°C
Low EDTA TE Buffer	902979	
Water, Nuclease-Free	902976	
CytoScan™ Module 3: Post-Lab Fragmentation, Labeling, and Hybridization	**904002**	
Fragmentation Reagent	902428	
Fragmentation Buffer	903001	
TdT Enzyme	902675	
TdT Buffer	902676	
DNA Labeling Reagent	902677	–25°C to –15°C
Oligo Control Reagent	902678	
Hyb Buffer Part 1	901725	
Hyb Buffer Part 2	901726	
Hyb Buffer Part 3	901727	
Hyb Buffer Part 4	901728	
CytoScan™ Module 4: Post-Lab Stain, Holding Buffer, Beads, and Water	**904005**	
Stain Buffer 1	901751	
Stain Buffer 2	901752	2°C to 8°C
Array Holding Buffer	901733	
Purification Beads	901807	
Water, Nuclease-Free	901781	
CytoScan™ Module 5: Post-Lab Elution Buffer and Purification Wash Buffer	**903000**	
Elution Buffer	901738	15°C to 30°C
Purification Wash Buffer	901372	
Wash A	901680	15°C to 30°C
Wash B	901681	

CytoScan™ Amplification Kit

The Applied Biosystems™ CytoScan™ Amplification Kit has been designed to be used at the PCR amplification step of the CytoScan™ Accel Assay and is manufactured by Takara™ Bio USA, Inc (Clontech™). It is equivalent to the Clontech™ Titanium™ DNA Amplification Kit (Cat. No. 639240, 300 reactions, or Cat. No. 639243, 400 reactions).

The CytoScan™ Amplification Kit contains enough reagents to prepare 400 PCR reactions, equivalent to 96–100 samples processed with the CytoScan™ Accel Assay.

The CytoScan™ Amplification Kit (Cat. No. 902975) is included in the CytoScan™ HD Accel Plus 24 Kit (Cat. No. 952465) and the CytoScan™ HD Accel Plus 96 Kit (Cat. No. 952466).

Table 1 Components of the CytoScan™ Amplification Kit.

Component	Clontech™ Part No. for identification	Storage
50X TITANIUM™ *Taq* DNA Polymerase Mix	S4851	–25°C to –15°C
10X TITANIUM™ *Taq* PCR Buffer	S4852	
dNTP Mixture	S4853	
GC-Melt Reagent	S4854	

Other reagents required

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

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Novex™ Hi-Density TBE Sample Buffer (5X)	LC6678
Absolute Ethanol	Sigma-Aldrich™, 459844
Bleach (6.15% Sodium Hypochlorite)	**MLS**
Invitrogen™ Quant-iT™ PicoGreen™ dsDNA Assay Kit	P7589
Applied Biosystems™ 25 bp DNA Ladder	931343
Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer	10482028
Invitrogen™ E-Gel™ 96 High Range DNA Marker	12352019
Water, Nuclease-free, Molecular Biology Grade, Ultrapure	10-977-015, **MLS**
Invitrogen™ E-Gel™ *48* Agarose Gel, 1%	G800801
2% Gel for PCR analysis: • Invitrogen™ E-Gel™ *48* Agarose Gels, 2%, or • TBE Gel, 2%, BMA Reliant™ precast (depending on method selection)	G800802 or Lonza™ Bioscience, 54939
4% Gel for PCR analysis: • Invitrogen™ E-Gel™ *48* Agarose Gels, 4%, or • TBE Gel, 4%, BMA Reliant™ precast (depending on method selection)	G800804 or Lonza™ Bioscience, 54929
1X TBE Buffer for electrophoresis (only if using TBE gel method)	**MLS**

CytoScan™ 750K reagent and array kits

Item	Source
Applied Biosystems™ NIMBUS™ CytoScan™ Training Kit	902169
Applied Biosystems™ CytoScan™ Reagent Kit, 24 reactions per kit	901808
Applied Biosystems™ CytoScan™ 750K Array Kit, 6 pack	901858
Applied Biosystems™ CytoScan™ 750K Array Kit and Reagent Kit Bundle, 24 arrays and reactions	901859
Applied Biosystems™ CytoScan™ 750K Training Kit	901860
Applied Biosystems™ CytoScan™ 750K Kit Plus 24	905924
Applied Biosystems™ CytoScan™ 750K Plus 96	905996

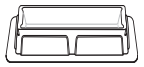
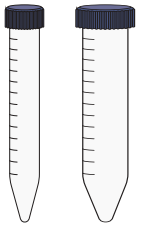
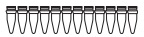
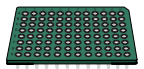
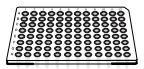
CytoScan™ HD reagents and array kits required

Item	Source
Applied Biosystems™ NIMBUS™ CytoScan™ Training Kit	902169
Applied Biosystems™ CytoScan™ Reagent Kit, 24 reactions per kit	901808
Applied Biosystems™ CytoScan™ HD Array Kit, 6 pack	901833
Applied Biosystems™ Applied Biosystems™ CytoScan™ HD Training Kit	901834
Applied Biosystems™ CytoScan™ HD Array Kit and Reagent Kit Bundle, 24 arrays and reactions	901835
Applied Biosystems™ CytoScan™ HD Kit Plus 24	905824
Applied Biosystems™ CytoScan™ HD Kit Plus 96	905896
CytoScan™ HD FAS Onsite Training	000802

Consumables required

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

Catalog numbers that appear as links open the web pages for those products.

Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
96-well unskirted PCR plates for Stage 1 Digestion and Stage 2 Ligation stages only.	Bio-Rad™ MLP-9601		✔		
Reagent Reservoir, 25 mL	Diversified Biotech™, RESE-3000		✔		
Tube, centrifuge, 15 mL and 50 mL	**MLS**		✔		
Strip tubes, 8-well or 12-well, 0.2 mL	**MLS**		✔		
Adhesive film for 96-well plates	Applied Biosystems™ MicroAmp™ Clear Adhesive Film, 4306311		✔	✔	
Plates, 96-well half-skirt For stages not related to the NIMBUS™ Instrument (digestion, ligation, and PCR setup stages) order these plates separately. **Note:** The user interface on the method uses the term “half-skirt” which is equivalent in meaning to “semi-skirt”.	Included in the CytoScan™ Consumables Kit for NIMBUS™, 902168. Purchase separately, one of the following: Bio-Rad™ Plates, 96-well, semi-skirted, HSS9641 (green) or HSS9601 (clear)	 	✔	✔	

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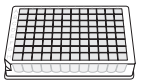
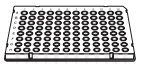
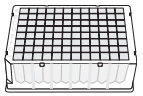
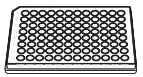
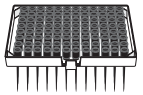
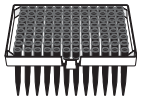
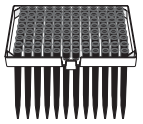
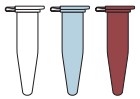
Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
Plates, 96-well half-skirt For stages not related to the NIMBUS™ Instrument (digestion, ligation, and PCR setup stages) order these plates separately. **Note:** The user interface on the method uses the term “half-skirt” which is equivalent in meaning to “semi-skirt”.	Included in the CytoScan™ Consumables Kit for NIMBUS™ v2, 952449. Purchase separately: MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode, 4483354 or 4483352 This plate is an alternative to the Bio-Rad™ Plates, 96-well, semi-skirted, HSS9641 (green) or HSS9601 (clear).		✔	✔	
2-mL skirted tube 2.0-mL Screw Tube, Skirted Base, Graduated, Writing Area, Clear	Included in the CytoScan™ Consumables Kit for NIMBUS™, 902168, or v2 952449. Purchase separately: Thomas Scientific™649020			✔	
1.2-mL square-well plate, round bottom Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate	Included in the CytoScan™ Consumables Kit for NIMBUS™, 902168, or v2 952449. Purchase separately: Thermo Scientific™, AB1127			✔	
4-row reservoir	Included in the CytoScan™ Consumables Kit for NIMBUS™, 902168, or v2 952449. Purchase separately: Thomas Scientific™, 1149Q43			✔	

(continued)

Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
96-well full-skirt plate Axygen™ 96-well PCR Microplate	Included in the CytoScan™ Consumables Kit for NIMBUS™, 902168, or v2 952449. Purchase separately: Fisher Scientific™ 14-222-326			✔	
2.2-mL storage plate Abgene™ 96 Well 2.2 mL Polypropylene Deepwell Storage Plate	Included in the CytoScan™ Consumables Kit for NIMBUS™, 902168, or v2 952449. Purchase separately: Fisher Scientific™ AB0932			✔	
96-well UV plate 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).			✔	
CO-RE™ II Filter Tips, 50 µL, conductive, in frames (case of 5,760)	Hamilton™ Company, 235948			✔	
CO-RE™ II Filter Tips, 300 µL, conductive, in frames (case of 5,760)	Hamilton™ Company, 235903			✔	
CO-RE™ II Filter Tips, 1,000 µL, conductive, in frames (case of 3,840)	Hamilton™ Company, 235905			✔	
Pipette tips, 20-µL filter tips	Rainin™ GP-L10F,**MLS**	—	✔	✔	✔
Pipette tips, 200-µL filter tips	Rainin™ GP-L200F, **MLS**	—	✔	✔	✔
Pipette tips, 1,000-µL filter tips	Rainin™ GP-L1000F, **MLS**	—	✔	✔	✔
10-mL serological pipette	**MLS**	—	✔	✔	

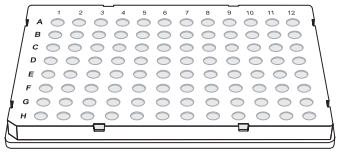
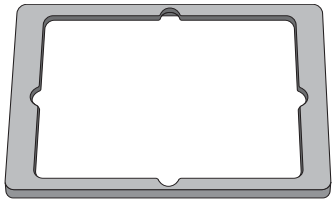
(continued)

Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
25-mL serological pipette	**MLS**	—	✔	✔	
Tough-Spots™ labels, 1/2-inch	Diversified Biotech™, Spot 2200	—			✔
USA Scientific™ Inc Tough-Spots™, 3/8 inch diameter, white, on sheets	Fisher Scientific™ NC9504463	—			✔
Eppendorf™ Safe-Lock™ Tubes, microcentrifuge tube, 1.5 mL,natural	Eppendorf™, 022363204				✔
Eppendorf™ Safe-Lock™ Tubes, microcentrifuge tube, 1.5 mL, blue	Eppendorf™, 022363247				✔
Eppendorf™ Safe-Lock™ Tubes, microcentrifuge tube, 1.5 mL, amber	Eppendorf™, 022363221				✔

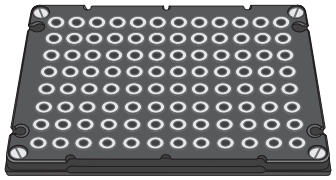
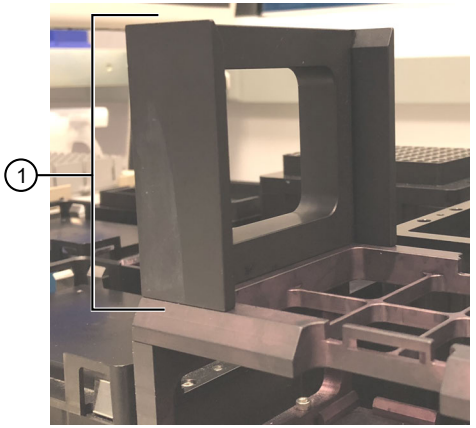
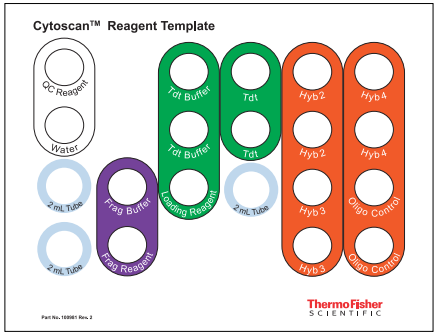
Accessories required

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

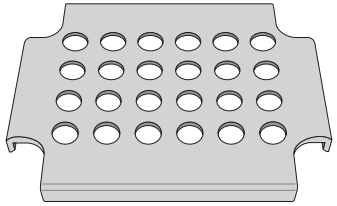
Catalog numbers that appear as links open the web pages for those products.

Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
(Optional) MicroAmp™ Adhesive Film Applicator	4333183	—	✔	✔	✔
Double 96-well Block Double Cooling Chamber for 0.2 mL tubes. Dim.: 6.125"W x 6.75"L x 1”H	Fisher Scientific™, NC9762282 Alternate source: Diversified Biotech™ CHAM-1020		✔	✔	✔
PCR Tube Racks Used as holder for 96 half-skirt plate.	Included in the CytoScan™ Automation Starter Kit for NIMBUS™, Cat. No. 902167. Alternate source: Bio-Rad™, TRC 9601			✔	
Plate Collar	Included as part of the NIMBUS™ Instrument configuration, 68319-01			✔	

(continued)

Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
96S Super Magnet Plate	Included in the CytoScan™ Automation Starter Kit for NIMBUS™, Cat. No. 902167. Purchase separately: ALPAQUA 96S Super Magnet, A001322			✔	
Tip loading tool	Included as part of the NIMBUS™ Instrument configuration.	 1 Tip loading tool attached to deck.		✔	
CytoScan™ Reagent Template	Included in the CytoScan™ Automation Starter Kit for NIMBUS™, Cat. No. 902167.			✔	

(continued)

Labware	Source	Image	Where used		
			Pre-PCR	NIMBUS	Array processing
Tube Collar	Included as part of the NIMBUS™ Instrument configuration, 93717-01			✔	
Corning™ Rectangular Ice Pan, Maxi 9L	Fisher Scientific™ 07-210-093	—	✔	✔	✔
Laboratory tissue	**MLS**	—	✔	✔	✔
Markers, permanent, fine point	**MLS**	—	✔	✔	✔

Pipette tips required for the NIMBUS™ Instrument

The following tables provide the CO-RE™ II Filter Tips usage by stage, by run option, and by 25 and 49 samples for 1 full run of the CytoScan™ Assay Automated Workflow on the NIMBUS™ Target Preparation Instrument.

CO-RE™ II Filter Tips usage by stage for the E-Gel + Nanodrop run option

Stage	E-Gel + Nanodrop run option 25 samples			E-Gel + Nanodrop run option 49 samples		
	50 µL	300 µL	1,000 µL	50 µL	300 µL	1,000 µL
Stage 3C—PCR product check	32	1	0	56	1	0
Stage 4A—PCR product pooling	0	25	0	0	49	0
Stage 4B—PCR product purification	0	121	38	0	182	62
Stage 5—Quantitation	0	25	7	0	49	7
Stage 6A—Fragmentation	36	7	2	60	7	2
Stage 6B—Fragmentation QC	32	8	0	56	8	0
Stage 7—Labeling	25	9	2	49	15	2
Stage 8A—Hybridization Mix	0	34	20	0	58	24
Total tips	**125**	**230**	**69**	**218**	**369**	**97**

CO-RE™ II Filter Tips usage by stage for the E-Gel + OD run option

Stage	E-Gel + OD run option 25 samples			E-Gel + OD run option 49 samples		
	50 µL	300 µL	1,000 µL	50 µL	300 µL	1,000 µL
Stage 3C—PCR product check	32	1	0	56	1	0
Stage 4A—PCR product pooling	0	25	0	0	49	0
Stage 4B—PCR product purification	0	121	38	0	182	62
Stage 5—Quantitation	25	32	0	49	56	0
Stage 6A—Fragmentation	36	7	2	60	7	2
Stage 6B—Fragmentation QC	32	8	0	56	8	0
Stage 7—Labeling	25	9	2	49	15	2
Stage 8A—Hybridization Mix	0	34	20	0	58	24
Total tips	**150**	**237**	**62**	**270**	**376**	**90**

CO-RE™ II Filter Tips usage by stage for the TBE Gel + Nanodrop run option

Stage	TBE Gel + Nanodrop run option 25 samples			TBE Gel + Nanodrop run option 49 samples		
	50 µL	300 µL	1,000 µL	50 µL	300 µL	1,000 µL
Stage 3C—PCR product check	38	2	0	62	2	0
Stage 4A—PCR product pooling	0	25	0	0	49	0
Stage 4B—PCR product purification	0	121	38	0	182	62
Stage 5—Quantitation	25	32	0	49	56	0
Stage 6A—Fragmentation	36	7	2	60	7	2
Stage 6B—Fragmentation QC	38	8	0	62	8	0
Stage 7—Labeling	25	9	2	49	15	2
Stage 8A—Hybridization Mix	0	34	20	0	58	0
Total tips	**162**	**238**	**62**	**282**	**377**	**90**

CO-RE™ II Filter Tips usage by stage for the TBE Gel + OD run option

Stage	TBE Gel + OD run option 25 samples			TBE Gel + OD run option 49 samples		
	50 µL	300 µL	1,000 µL	50 µL	300 µL	1,000 µL
Stage 3C—PCR product check	32	1	0	56	1	0
Stage 4A—PCR product pooling	0	25	0	0	49	0
Stage 4B—PCR product purification	0	121	38	0	182	62
Stage 5—Quantitation	25	32	0	49	56	0
Stage 6A—Fragmentation	36	7	2	60	7	2
Stage 6B—Fragmentation QC	32	8	0	56	8	0
Stage 7—Labeling	25	9	2	49	15	2
Stage 8A—Hybridization Mix	0	34	20	0	58	24
Total tips	**150**	**237**	**62**	**270**	**376**	**90**

Consumable kits required for the NIMBUS™ Target Preparation Instrument

CytoScan™ Consumables Kit for NIMBUS™

The CytoScan™ Consumables Kit for NIMBUS™ (Cat. No. 902168) contains labware sufficient for 4 x 25 or 2 x 49 reaction runs.

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).

Component	Part No.	Usage per run		Number per kit
		25 samples	49 samples	
Plates, 96-well, semi-skirted	203009	5	7	20
Tube, screw cap, no ribs, 2 mL	202006	6	6	24
1.2 mL Square Well Round Bottom Storage Plate	202154	1	1	4
Reservoir 4 ROW PYR PP 73 mL	203010	6	6	25
96-well Full Skirt PCR Plate, clear	203023	7	7	28
2.2 mL Square Well Storage Plate	202920	3	3	12

CytoScan™ Consumables Kit for NIMBUS™ v2

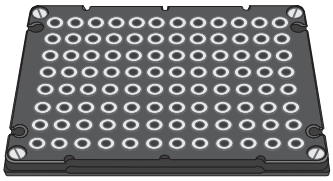
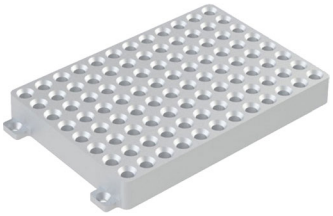
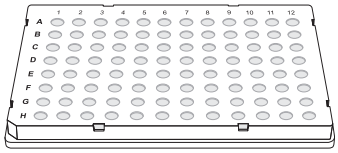
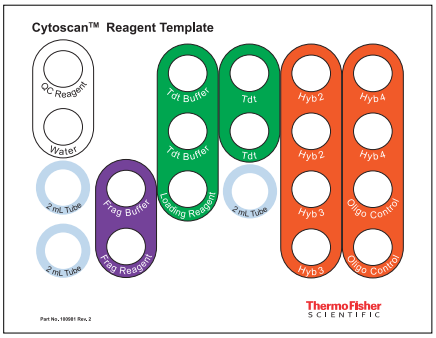
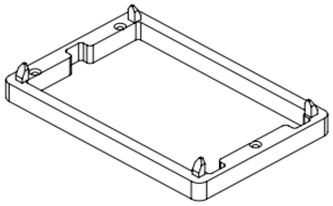
The CytoScan™ Consumables Kit for NIMBUS™ v2, (Cat. No. 952449) contains labware sufficient for 4 x 25 or 2 x 49 reaction runs.

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).

Component	Part No.	Usage per run		Number per kit
		25 samples	49 samples	
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	203244	5	7	20
Tube, screw cap, no ribs, 2 mL	202006	6	6	24
1.2 mL Square Well Round Bottom Storage Plate	202154	1	1	4
Reservoir 4 ROW PYR PP 73 mL	203010	6	6	25
96-well Full Skirt PCR Plate, clear	203023	7	7	28
2.2 mL Square Well Storage Plate	202920	3	3	12

CytoScan™ Automation Starter Kit for NIMBUS™

The CytoScan™ Automation Starter Kit for NIMBUS™, Cat. No. 902167, contains the following components.

Quantity	Item	Image	Part No.
1	96S Super Magnet Plate		900929
1	Micro Plate 96 PCR Adapter		100987
4	PCR Tube Racks		100988
2	CytoScan™ Reagent Template		100981
1	Plate Retainer		111705

Set up the NIMBUS™ Instrument prerun

Equipment

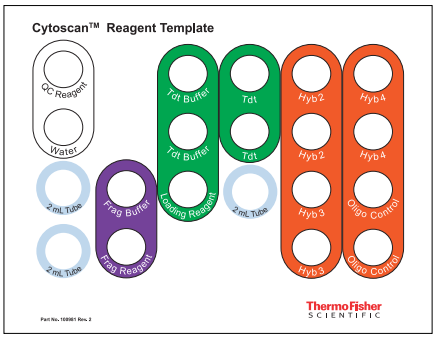
The Applied Biosystems™ NIMBUS™ Target Preparation Instrument (Cat. No. 00-0401) is required to run the CytoScan™ Assay Automated Workflow. This workstation includes the accessories, software, and deck configuration listed in the following figure.

Figure 3 CytoScan™ deck configuration for the NIMBUS™ Instrument.

① Variomag™ Thermoshake with adapter and plate collar
② CPAC
③ Precision DWP Pedestal
④ DWP Pedestal
⑤ DWP Pedestal
⑥ Tip Adapter
⑦ Precision DWP Pedestal
⑧ DWP Pedestal
⑨ FTR Pedestal
⑩ FTR Pedestal
⑪ Tip isolator

Cooling block template

The CytoScan™ Reagent Template fits precisely on top of the INHECO™ CPAC device. Using this template helps ensure the proper placement of reagent tubes onto the block for each method.



Place the reagent template onto the CPAC

1. Place the CytoScan™ reagent template directly on top of the CPAC with the 24 Tube Rack Adapter (Figure 4).

Figure 4 CPAC with 24 tube rack adaptor.

2. Place reagent tubes as instructed before running a method.

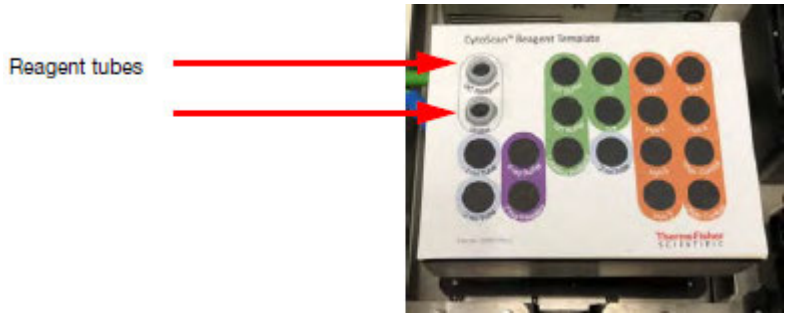


Figure 5 Example of tube in the 24 tube rack adaptor.

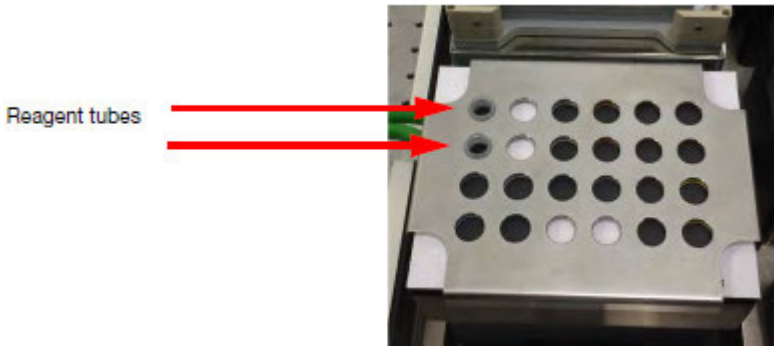


Figure 6 Tube collar placed over reagent templates with reagent tubes inserted.

3 Guidelines for use

Controls

Using positive and negative controls is recommended to evaluate the performance of each run. We recommend that you use the Genomic DNA control supplied in the CytoScan™ Reagent Kit as a positive control through the entire assay, up to hybridization on the arrays. Use the Low EDTA TE Buffer supplied in the CytoScan™ Reagent Kit as a negative control only through the PCR gel QC stage.

Equipment and calibration

Keep dedicated equipment in each of the areas used for this protocol, including pipettors, ice buckets, coolers, and so on. It is critical to use equipment that conforms to the guidelines and specifications detailed in this guide. To avoid contamination, do not move equipment back and forth from the Post-PCR Area to the Pre-PCR Clean Area.

Laboratory instrumentation plays an important role in the successful execution of this assay. To help maintain consistency across samples and operators, all equipment must be well maintained and routinely calibrated per manufacturer recommendations, including:

- All thermal cyclers
- NIMBUS™ Target Preparation Instrument
- GeneChip™ Hybridization Oven 645
- GeneChip™ Fluidics Station
- GeneChip™ Scanner 3000 7G System

- SpectraMax® Plus 384 Microplate Spectrophotometer or NanoDrop™ Spectrophotometer
- All single and multichannel pipettes

Pipetting

Automation of the CytoScan™ Assay involves a series of ordered stages. Because the output of 1 stage directly impacts the performance of the subsequent stage, use the following precautions to efficiently process samples:

- Always use pipettes that have been calibrated according to the manufacturer's specifications.
- It is essential that operators be proficient with the use of single- and multichannel pipettes.
- Always use filter tips for pipetting. This is essential to reduce sample contamination.

To become familiar with the use of multichannel pipettes, we strongly recommend that you practice several times before processing actual samples. Use water to get a feel for aspirating and dispensing solutions to multiple wells simultaneously. Take special care to observe complete evacuation of liquid from all pipette tips when using a multichannel pipette.

Reagent handling and storage

IMPORTANT! Always use the 24 reaction CytoScan™ Reagent Kit (Cat. No. 901808) for this protocol. You can freeze/thaw the reagents in the 24 reaction kit ≤5 times. The post-PCR reagents used on the NIMBUS™ Instrument are all single use only.

Proper storage and handling of reagents is essential for robust performance. Follow these guidelines to ensure best results:

- Use reagents from the recommended vendors only.
- Store all reagents at the recommended temperatures and conditions. Do not use reagents that have been improperly stored. Storage methods can profoundly impact activity.
- Upon receipt of the reagent kit, store the Nuclease-Free Water at 4°C and the Low EDTA TE Buffer at room temperature for convenience.
- Do not use expired reagents or reagents that have undergone more than the recommended number of freeze-thaw cycles.
- Seal all vials and bottle caps well after use to prevent evaporation.
- Do not store enzymes in a frost-free freezer.
- Store the reagents used for digestion, ligation, and PCR only in the Pre-PCR Clean Area.

When using reagents at the lab bench

- Properly chill essential equipment such as cooling blocks and reagent coolers before use.
- Unless otherwise indicated, keep all reagents (except enzymes) on ice, or in a cooling block that has been chilled to 4°C and placed on ice during use.
- Ensure that enzymes are kept at –20°C until needed. When removed from the freezer, immediately place in a bench top reagent cooler that has been chilled to –20°C.
- Keep all tubes and working solutions in chilled cooling blocks on ice.
- Since enzyme activity is a function of temperature, ensure that all temperature transitions to incubation temperatures are rapid and/or well-controlled to help maintain consistency across samples.

Prepare the work area for each stage

Many of the stages in the CytoScan™ Assay Automated Workflow must be performed rapidly at 4°C. A chilled adapter is used during robotic handling to carefully control enzyme activity and temperature transitions. Therefore, before starting each stage, hold reagents on ice (except for enzymes) until equipment and consumable setup is complete.

Automation

Place the sample plate on the deck

When you place the sample plate on the Thermoshake adapter, always ensure that the plate is flat on the adapter. After placing the plate on the adapter, press on all 4 corners to verify that the plate is sitting flat.

Filling the reservoir for the purification step

Ensure that correct reagent volumes are added to the reservoir compartments as shown in the on-screen instructions.

Transfer all contents of the Elution Buffer tube, then ensure that the buffer is evenly spread across the reservoir.

Laboratory workflow

- Maintain a single direction workflow. Do not reenter the Pre-PCR Clean Area after entering the Post-PCR Area until you have showered and changed into freshly laundered clothing.
- Never bring amplified products into the Pre-PCR Clean Area.
- Keep dedicated equipment in each room or area used for this protocol. To avoid contamination, do not move equipment between the Pre-PCR Clean Area and the Post-PCR Area.

Seal, vortex, and centrifuge

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

Handling the plate seal

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

Never reuse a seal. Always use a new seal.

Use MicroAmp™ Clear Adhesive Film to seal plates. An adhesive film applicator is optional.

- To minimize sample cross-well contamination and to ensure tight seals, use each seal only once. *Never reuse a seal.* Discard used seals immediately to avoid contaminating equipment or working surfaces with DNA.
- The seal can become loose due to high temperature in the thermal cycler. Always ensure tight sealing before vortexing a plate.
- Whenever a plate is taken out of the thermal cycler, before continuing on to the next step, ensure that the seal is tight, centrifuge the plate, then remove the seal and discard.
- Whenever a plate is taken out of the freezer, first thaw the plate, ensure that the seal is tight, centrifuge, and only then remove the plate seal.
- When reaction setup is completed, always use a new seal to seal the plate.
- When applying the seal to a plate, press the seal tightly onto the plate. Use an adhesive film applicator, if you have one. Using a plastic lid or a plastic tube rack is a potential source of contamination. Ensure that the seal is tight around all plate and well edges.

Sealing strip tubes

- Seal the strip tubes containing master mix before centrifuging in the benchtop mini centrifuge.
- Adhesive strips can be created by cutting a plate seal into strips wide enough and long enough to seal 8- or 12-well strip tubes.

Vortex

- Master Mix tubes—Vortex the master mix at high speed 3 times, 1 second each time.
- Vortex reagents—Vortex reagents 3 times, 1 second each time.
- Vortex enzyme—Briefly vortex enzymes, 1 second.
- Vortex plates—Vortex plates at high speed for 1 second. in all corners and in the center.

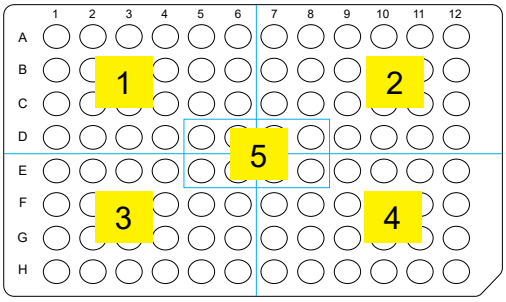


Figure 7 Vortex plates at the corners and center.

Centrifuge

When instructed to centrifuge plates or reagent vials, follow these guidelines unless otherwise instructed.

- **Plates**
  - Centrifuge at room temperature, except for the fragmentation step. During the fragmentation step, centrifuge the plates at 4°C in a refrigerated centrifuge.
  - Start the centrifuge, then allow it to reach 650 x *g*, then centrifuge at that speed for 1 minute.
- **Reagent Vials**—Centrifuge for 3 seconds using a benchtop minicentrifuge.
- **Enzyme Vials**—Centrifuge for 3 seconds using a benchtop minicentrifuge.

Thermal cyclers

The CytoScan™ Assay Automated Workflow has been optimized using the following thermal cyclers, 96-well plates, and adhesive films.

IMPORTANT! Use only the recommended 96-well plates and adhesive film, and only the thermal cyclers listed in Table 2. Using other plates and adhesive films that are incompatible with these thermal cyclers can result in loss of sample or poor results.

Table 2 Thermal cyclers for use with the CytoScan™ Assay Automated Workflow.

Laboratory	Thermal cyclers verified for use	Source	Validated or alternate
Pre-PCR Clean Area	Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811	Validated
	Applied Biosystems™ 2720 Thermal Cycler	4359659	Validated
	Applied Biosystems™ GeneAmp™ PCR System 9700, 96-Well Gold-Plated	4314878	Validated
	Applied Biosystems™ GeneAmp™ PCR System 9700, 96-Well Silver	N8050001	Validated
Post-PCR Area	Applied Biosystems™ ProFlex™ Thermal Cycler	4484075	Validated

Table 2 Thermal cyclers for use with the CytoScan Assay Automated Workflow. *(continued)*

Laboratory	Thermal cyclers verified for use	Source	Validated or alternate
Post-PCR Area	Applied Biosystems™ GeneAmp™ PCR System 9700, 96-Well Gold-Plated	4314878	Validated
	Applied Biosystems™ GeneAmp™ PCR System 9700, 96-Well Silver	N8050001	Validated
	Applied Biosystems™ Veriti™ 96-Well Thermal Cycler	4375786	Alternate
	Applied Biosystems™ Veriti™ Dx 96-well Thermal Cycler[1]	4452300	Alternate
	Bio-Rad™ DNA Engine™ PTC-200 Thermal Cycler	Bio-Rad™, PTC-200	Alternate
	Eppendorf™ Mastercycler™ pro S Thermal Cycler	Model 950030020 or 950030025	Alternate

[1] This thermal cycler is classified as US FDA Class I Medical Device for *in vitro* diagnostic use.

Table 3 Instrument-specific settings for alternate thermal cyclers.

Setting	Alternate thermal cycler		
	Applied Biosystems™ Veriti™ 96-Well Thermal Cycler	Bio-Rad™ DNA Engine™ PTC-200 Thermal Cycler	Eppendorf™ Mastercycler™ pro S
Lid teperature	103°C	103°C	103°C
Temperature mode	N/A	Calculated	Safe
TSP heated lid	N/A	N/A	Yes
Switch off lid at low block temperature	N/A	N/A	No

Thermal cycler protocol setup

Use only calibrated thermal cyclers. We recommend that thermal cyclers be serviced at least once per year to ensure that they are operating within the manufacturer's specifications. The thermal cycler protocols listed in Table 4 and Table 5 are used in this assay. Enter and store these potocols on the appropriate thermal cycler in the Pre-PCR Clean Area and the Post-PCR Area.

Table 4 Pre-PCR Clean Area.

Number of thermal cyclers required	Thermal cycler protocol name
1	CytoScan Digest
	CytoScan Ligate

Table 5 Post-PCR Area.

Number of thermal cyclers required	Thermal cycler protocol name
2 for 25 samples 4 for 49 samples	**CytoScan PCR**
1	**CytoScan Fragment**
	CytoScan Label
	CytoScan Hybridization

Program your thermal cyclers

Specific thermal cycler protocols are required for the CytoScan™ Assay Automated Workflow. Before you start processing samples, enter and save these protocols into the appropriate thermal cyclers.

IMPORTANT! Always use the heated lid option when programming thermal cycler protocols. See the appropriate thermal cycler user guide for programming information.

CytoScan Digest thermal cycler protocol

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

CytoScan Ligate thermal cycler protocol

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

CytoScan PCR thermal cycler protocol

You must use thermal cyclers with silver or gold-plated silver blocks. Do not use thermal cyclers with aluminum blocks.

Applied Biosystems™ GeneAmp™ PCR System 9700

- Ramp speed: Maximum
- Volume: 100 µL

Applied Biosystems™ ProFlex™ Thermal Cycler

- Ramp speed: 6.0°C/sec
- Lid temperature: 105°C
- Volume: 100 µL

Temperature	Time	Cycles
94°C	3 minutes	1 time
94°C 60°C 68°C	30 seconds 45 seconds 15 seconds	30 times
68°C	7 minutes	1 time
4°C	Hold (Can be held overnight)	

CytoScan Fragment thermal cycler protocol

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	Hold

CytoScan Label thermal cycler protocol

Temperature	Time
37°C	4 hours
95°C	15 minutes
4°C	Hold (Samples can remain at 4°C overnight.)

CytoScan Hybridization thermal cycler protocol

Temperature	Time
95°C	10 minutes
49°C	Hold

Running gels

TBE gels or E-Gel™ Agarose Gels can be used in this assay. Select the gel type in the software before processing samples on the NIMBUS™ Target Preparation Instrument.

TBE gels

- Run gels at 5 V/cm for 45 minutes or until the dye front reaches at least 75% of distance down the gel.
- Add ethidium bromide to the gel running buffer in the gel box. Add 2 drops of ethidium bromide per 1L of 1X TBE.

WARNING! E-Gel™ Agarose Gels and TBE gels contain ethidium bromide. Review the manufacturer's Safety Data Sheet for proper handling and disposal. Use good laboratory practices and always wear protective gloves when handling ethidium bromide. Dispose of the gel and gloves in accordance with national, state, and local regulations.

E-Gel™ Agarose Gels

Run E-Gel™ Agarose Gels according to the manufacturer's recommendations.

Hybridization

- Take the arrays out of the bags. Mark and arrange them on the laboratory bench in advance, so that they warm up to room temperature.
- Load only 6 to 8 arrays at a time. Remove the seal from the hybridization plate for only 6 to 8 samples at a time.
- Preheat the hybridization oven to 50°C at least 1 hour before use.

Washing arrays

It is important to work quickly when processing arrays for washing. Delays during this step impact data quality. To optimize this step, we suggest the following:

- Thirty minutes before hybridization is complete, prime the fluidics stations with the correct wash buffers. Start the fluidics protocol, then follow the directions on the LCD panel of the fluidics station.
- Load Stain Buffer 1, Stain Buffer 2, and the Array Holding Buffer in their respective positions on the fluidics station. Eject the wash block to avoid sensor time out.
- Process only 6 to 8 arrays at a time.
- Minimize delays when performing all steps after the arrays are removed from the oven, up to the time when washing starts.

Hybridization oven

Verify that the GeneChip™ Hybridization Oven 645 is calibrated before starting the hybridization step. Accurate hybridization temperature is critical for this assay. Service hybridization ovens at least once per year to ensure that they are operating within the manufacturer's specifications.

4 Genomic DNA general requirements

The general requirements for genomic DNA sources and extraction methods are described in this chapter. The success of this assay requires the amplification of PCR fragments between 150 bp to 2,000 bp in size throughout the genome. To achieve this, the genomic DNA must be of high quality, and must be free of contaminants that would affect the enzymatic reactions carried out.

For this assay, use the Applied Biosystems™ CytoScan™ Reagent Kit (24 sample, Cat. No. 901808). This kit contains the Genomic DNA (Ref 103) control that meets the requirements outlined in this section. The size of the starting genomic DNA can be compared with the Genomic DNA control to evaluate the quality. The Genomic DNA control can also be used as a routine experimental positive control for troubleshooting.

Assay performance can vary for genomic DNA samples that do not meet the general requirements described in this section. However, the reliability of any given result can be evaluated in the context of overall experimental design and goals.

General requirements and recommendations

- DNA must be double-stranded (not single-stranded).

  This can be verified using PicoGreen™ quantitation. This requirement relates to the restriction enzyme digestion step in the assay.
- DNA must be free of PCR inhibitors.

  Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (for example, EDTA). The genomic DNA extraction/purification method renders DNA that is generally salt-free because high concentrations of certain salts can also inhibit PCR and other enzyme reactions. Prepare DNA as described in Chapter 5, “CytoScan™ Assay Automated Workflow for 25 samples”.
- DNA must not be contaminated with other human genomic DNA sources, or with genomic DNA from other organisms.

  PCR amplification of the ligated genomic DNA is not human specific, so sufficient quantities of non-human DNA can also be amplified and could potentially result in compromised genotype calls. Contaminated or mixed DNA can manifest as high detection rates and low call rates.

- DNA must not be degraded.

  The genomic DNA fragment must have Nsp I restriction sites intact so that ligation can occur on both ends of the fragment and PCR can be successful. The approximate average size of genomic DNA can be assessed on a 0.8% or 1% TBE gel using an appropriate size ladder. Control Genomic DNA can be run on the same gel for side-by-side comparison. High quality genomic DNA runs as a major band at approximately 10–20 kb on the gel. Pre-amplification methods or pre-digestion with restriction enzymes other than Nsp I have not been tested by Thermo Fisher Scientific. If other methods are desired, we recommend conducting experiments to evaluate their performance with this assay.

Sources of human genomic DNA

The following sources of human genomic DNA have been successfully tested in our laboratories for DNA that meets the requirements described in "General requirements and recommendations" on page 47.

- Blood
- Cell line

Blood collection methods

The 2 blood collection methods that have been shown to be compatible with the assay are EDTA and heparin.

Genomic DNA extraction/purification methods

Genomic DNA extraction and purification methods that meet the general requirements outlined above should yield successful results. Methods that include boiling or strong denaturants are not acceptable, because the DNA would be rendered single-stranded. Genomic DNA extracted using the following methods have been tested at Thermo Fisher Scientific:

- QIAGEN™–Gentra™ Puregene™ Kit
- 5 PRIME–PerfectPure™ DNA Blood Kit

IMPORTANT! The CytoScan™ Assay Automated Workflow requires genomic DNA concentration of ≥50 ng/µL. Therefore, the elution volumes for each of the kits needs to be adjusted accordingly to achieve the desired concentration.

RNase treatment

The presence of RNA and free nucleotides can interfere with some quantitation methods using a spectrophotometer. To eliminate RNA contamination, perform RNase treatment during extraction using one of these kits.

- QIAGEN™–Gentra™ Puregene™ Kit—Perform RNase treatment as recommended in the extraction kit guide before elution of genomic DNA.
- 5 PRIME–PerfectPure™ DNA Blood Kit—Use only RNase-treated purification columns for extraction of genomic DNA.

The purified genomic DNA extracted using these 2 methods should meet the DNA quality specifications listed in the manufacturer's kit extraction guide.

Prepare genomic DNA QC on E-Gel™ *48* Agarose Gel, 1%

The following E-Base™ devices, E-Gel™ Agarose Gel formulations, and electrophoresis reagents are required for preparing genomic DNA.

Unless otherwise indicated, all materials are available through **thermofisher.com**. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Invitrogen™ Mother E-Base™ Device	EBM03
Invitrogen™ Daughter E-Base™ Device Optional for running multiple gels simultaneously.	EBD03
Invitrogen™ E-Gel™ *48* Agarose Gel, 1%	G800801
Invitrogen™ *Redi*Load™ Loading Buffer	750026
Invitrogen™ E-Gel™ 96 High Range DNA Marker	12352019
Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer	10482028
Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water	10977023

WARNING! E-Gel™ Agarose Gels and TBE gels contain ethidium bromide. Review the manufacturer's Safety Data Sheet for proper handling and disposal. Use good laboratory practices and always wear protective gloves when handling ethidium bromide. Dispose of the gel and gloves in accordance with national, state, and local regulations.

Dilute genomic DNA samples

Load a DNA mass of about 25 ng per well. If lower amounts are loaded, omit the loading dye in order to improve visualization.

1. Dilute the Ready-Load™ dye to 0.1X concentration, then use 3 µL of the diluted dye (0.1X concentration) for each sample.
2. Bring each sample to a total volume of 20 µL using nuclease-free water.

   For example, if the volume of genomic DNA required for 25 ng is 5 µL, add 3 µL of 0.1X Ready-Load™ dye and 12 µL of nuclease-free water, for a total volume of 20 µL. Strip tubes or 96-well PCR plates can be used for diluting genomic DNA samples.
3. Briefly vortex, then centrifuge the diluted DNA samples before loading onto the E-Gel™ Agarose Gel.

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 a E-Gel™ *48* Agarose Gel, 1%, then wipe away any buffer that comes out of the gel or is on the surface.
4. Insert the E-Gel™ Agarose Gel into the slot.

   12-well E-Gel™ Agarose Gels can also be used if running a smaller number of genomic DNA samples.
5. Load 20 µL of genomic DNA sample onto the E-Gel™ *48* Agarose Gel, 1%.
6. Dilute the E-Gel™ 96 High Range DNA Marker (1:3 dilution, 5 µL of marker in 10 µL of nuclease-free water), then load all 15 µL into each of the marker wells.
7. Fill all empty wells with 20 µL nuclease-free water.
8. Set the run time to 27 minutes.
9. Push the Power/Prg button again (the light changes from red to green).

   When the run time is reached, the system automatically shuts off (the dye is near the end of the lane). The gel is now ready for imaging.

Prepare genomic DNA

This assay has been optimized using UV absorbance to determine genomic DNA concentrations. Other quantitation methods, such as Quant-iT™ PicoGreen™ dsDNA Assay Kit, can give different readings.

This section contains information to prepare the genomic DNA for 25 or 49 samples.

Materials required

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 6 Equipment and consumables required for preparing the genomic DNA.

Quantity	Item
As required	Invitrogen™ MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	Invitrogen™ MicroAmp™ Adhesive Film Applicator
1	Cooling chamber, double block, chilled to 4°C, placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P20
1	Pipette, single-channel P100 or P200
1	Pipette, 12-channel, 2–20 µL
1	Pipette, 12-channel, 20–200 µL
As needed	Pipette tips for pipettes listed
2	Plates, 96-well, semi-skirted
As needed	Eppendorf™ Safe-Lock™ Tubes, microcentrifuge, 1.5 mL, natural
1	Plate centrifuge
1	Plate spectrophotometer or NanoDrop™ Spectrophotometer (Required only if no optical density (OD) measurements are available for samples.)
1	Vortexer
2	GeneMate™ 96-Well PCR Tube Storage Rack

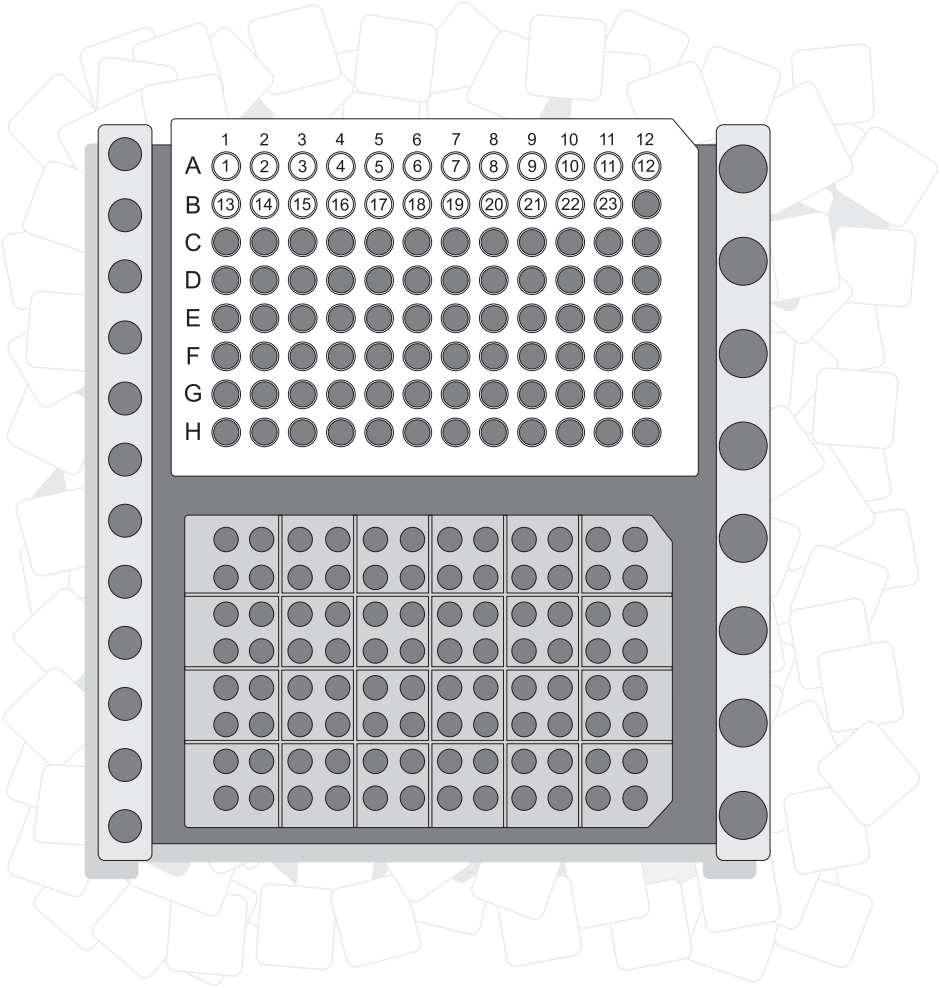
Table 7 Reagents required for preparing the genomic DNA.

Quantity	Reagent	CytoScan™ Reagent Kit	
		Module	Part No.
	Customer/test gDNA samples		
1	Genomic DNA (positive control)	Module 1	900421
1	Low EDTA TE Buffer	Module 2	902979

Prepare the genomic DNA for 25 samples

Set up the work area for preparing gDNA for 25 samples

1. Place a double cooling block on ice.
2. Place a 96-well plate in the upper half of the cooling block.



Note: The illustrations in this section depict the setup recommended for 25 samples: 23 genomic DNA samples, plus 1 positive control and 1 negative control.

- If running less than 25 samples or less, follow the plate layout in this section.
- If running 49 samples or more than 25 samples, see “Prepare the genomic DNA for 49 samples” on page 56 for instructions.

Dilute the stock genomic DNA samples to a working concentration

IMPORTANT! Do *not* dilute the Genomic DNA (control gDNA) provided in the CytoScan™ Reagent Kit. The Genomic DNA control is already at a working concentration.

1. Thaw the stock genomic DNA (gDNA) samples.
   a. Place the gDNA samples on the benchtop at room temperature until thawed.
   b. When thawed, place in the cooling block on ice.
2. Vortex the gDNA samples for 3 seconds.
3. Centrifuge at 650 x *g* for 1 minute, then place in the cooling block.
4. If sample concentration is unknown, take an optical density (OD) measurement of each sample now.
   Consult the spectrophotometer handbook for information on how to determine the sample concentration.

   IMPORTANT! To avoid contaminating samples with PCR product, take an aliquot of only each sample, not stock, to the spectrophotometer.
5. Prepare working stocks of each gDNA sample by diluting them to a final concentration of 50 ng/µL. Use Low EDTA TE Buffer to prepare the working stocks.
6. Seal the plate, vortex for 3 seconds, then centrifuge at 650 x *g* for 1 minute.
7. Place the plate back in the upper half of the cooling block.
   The plate is now ready for transfer into the digest/ligate plate.

Aliquot the prepared genomic DNA and controls

Set up a digestion/ligation plate for 25 samples

1. Mark a new 96-well plate for 25 samples: 23 gDNA samples, 1 positive control, and 1 negative control.

   The digestion and ligation reactions are performed in this plate.

Note: The suggested sample plate layout is shown in Figure 8, but if desired, the controls can be placed in any microplate well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

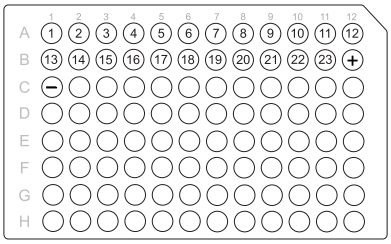


Figure 8 96-well plate labeled for digestion and ligation.

2. Place the labeled plate on the lower half of the cooling block.

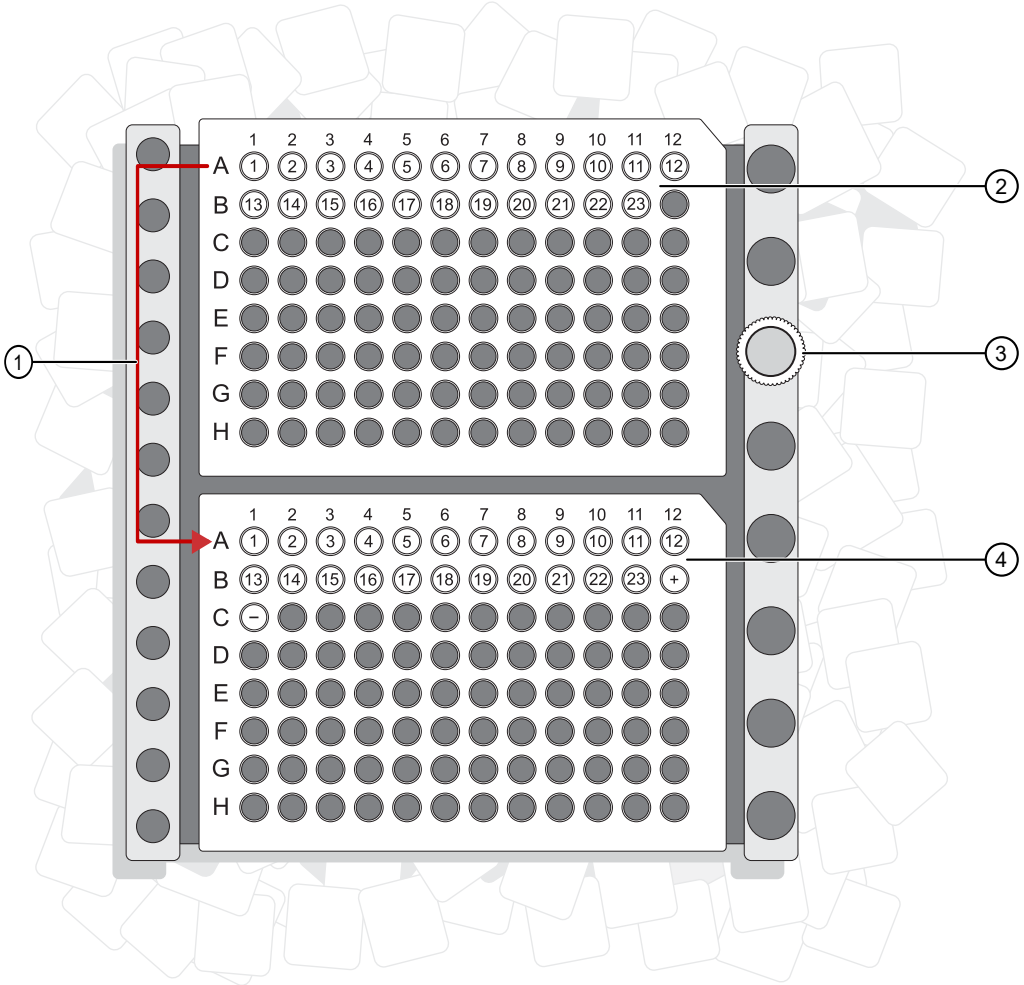


Figure 9 Setup for aliquoting diluted gDNA and controls to a 96-well plate labeled for digestion/ligation.

(1) Working stocks of gDNA samples (50 ng/µL) are aliquoted from upper plate to lower plate.
(2) Upper plate—96-well plate with working stocks of gDNA samples at 50 ng/µL concentration.
(3) Genomic DNA (positive control) from the CytoScan™ Reagent Kit.
(4) Lower plate—96-well plate labeled for digestion and ligation.

Aliquot the genomic DNA working stocks and controls

Note: 5 µL of the 50 ng/µL working stock is equivalent to 250 ng genomic DNA per well.

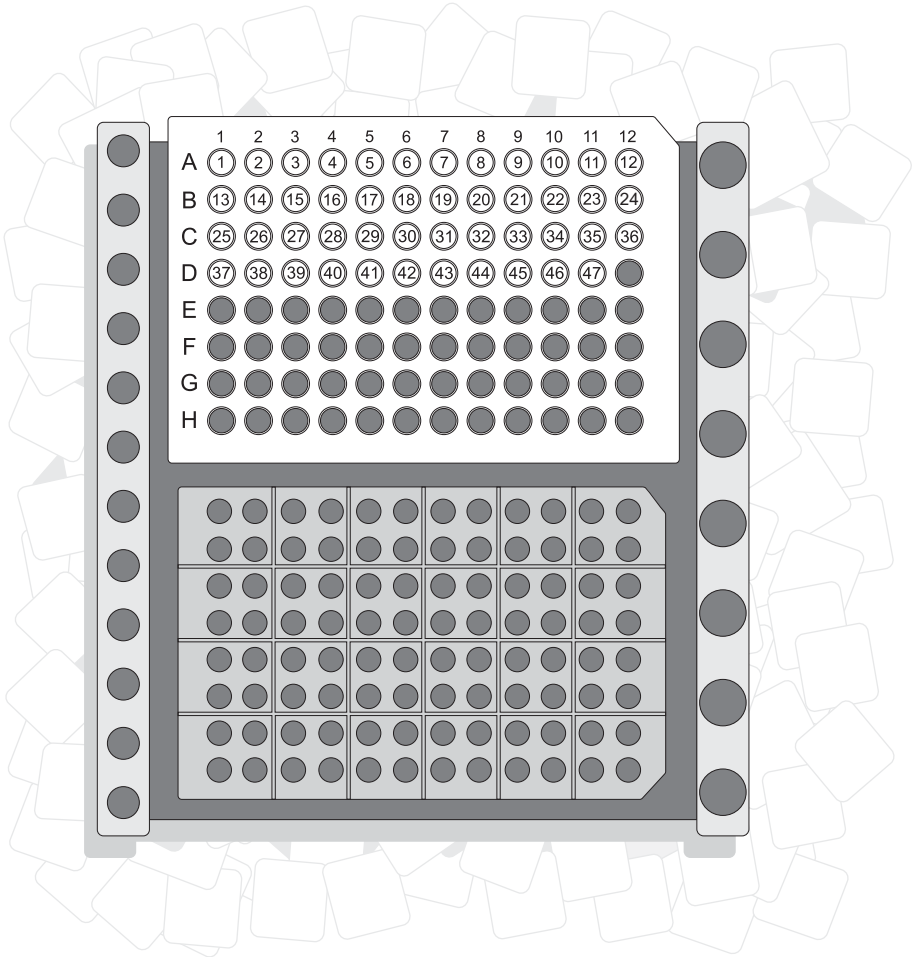
1. Thaw the Genomic DNA control from the CytoScan™ Reagent Kit.
   a. Place on the benchtop at room temperature until thawed.
   b. When thawed, place in the cooling block on ice.
2. If needed, vortex the genomic DNA working stock for 3 seconds, then centrifuge for 1 minute.

3. Transfer a 5 µL aliquot of the samples to designated wells of the digest/ligate plate. (Figure 9.)
4. For the controls, aliquot 5 µL of:
   a. Genomic DNA control from the CytoScan™ Reagent Kit as the positive control (+).
   b. Low EDTA TE Buffer as the negative control (–).
5. Tightly seal the digest/ligate plate with a new seal, then centrifuge for 1 minute at 2,000 rpm.
6. Do one of the following:
   - Proceed to "Stage 1—Restriction enzyme digestion" on page 62.
   - Store the prepared sample plate at –20°C.

Prepare the genomic DNA for 49 samples

Set up the work area for preparing gDNA for 49 samples

1. Place a double cooling block on ice.
2. Place a 96-well plate in the upper half of the cooling block.



Note: The illustrations in this section depict the setup recommended for 49 samples: 47 genomic DNA samples, plus 1 positive control and 1 negative control.

- If running less than 49 samples, but more than 25 samples, follow the plate layout in this section.
- If running 25 samples or less, see "Prepare the genomic DNA for 25 samples" on page 52 for instructions.

Dilute the stock genomic DNA samples to a working concentration

IMPORTANT! Do *not* dilute the Genomic DNA (control gDNA) provided in the CytoScan™ Reagent Kit. The Genomic DNA control is already at a working concentration.

1. Thaw the stock genomic DNA (gDNA) samples.
   a. Place the gDNA samples on the benchtop at room temperature until thawed.
   b. When thawed, place in the cooling block on ice.
2. Vortex the gDNA samples for 3 seconds.
3. Centrifuge at 650 x *g* for 1 minute, then place in the cooling block.
4. If sample concentration is unknown, take an optical density (OD) measurement of each sample now.

   Consult the spectrophotometer handbook for information on how to determine the sample concentration.

   IMPORTANT! To avoid contaminating samples with PCR product, take an aliquot of only each sample, not stock, to the spectrophotometer.
5. Prepare working stocks of each gDNA sample by diluting them to a final concentration of 50 ng/µL. Use Low EDTA TE Buffer to prepare the working stocks.
6. Seal the plate, vortex for 3 seconds, then centrifuge at 650 x *g* for 1 minute.
7. Place the plate back in the upper half of the cooling block.
   The plate is now ready for transfer into the digest/ligate plate.

Aliquot the prepared genomic DNA and controls

Set up a digestion/ligation plate for 49 samples

1. Mark a new 96-well plate for 49 samples: 47 gDNA samples, 1 positive control, and 1 negative control.

   The digestion and ligation reactions will be performed in this plate.

Note: The suggested sample plate layout is shown on Figure 10, but if desired, the controls can be placed in any microplate well from A1 through E1 because the NIMBUS™ Instrument processes all samples.

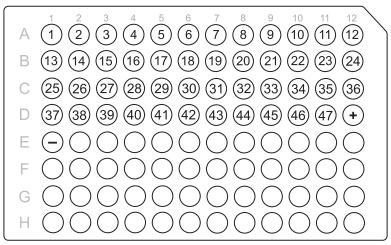


Figure 10 96-well plate labeled for digestion and ligation.

2. Place the labeled plate on the lower half of the cooling block.

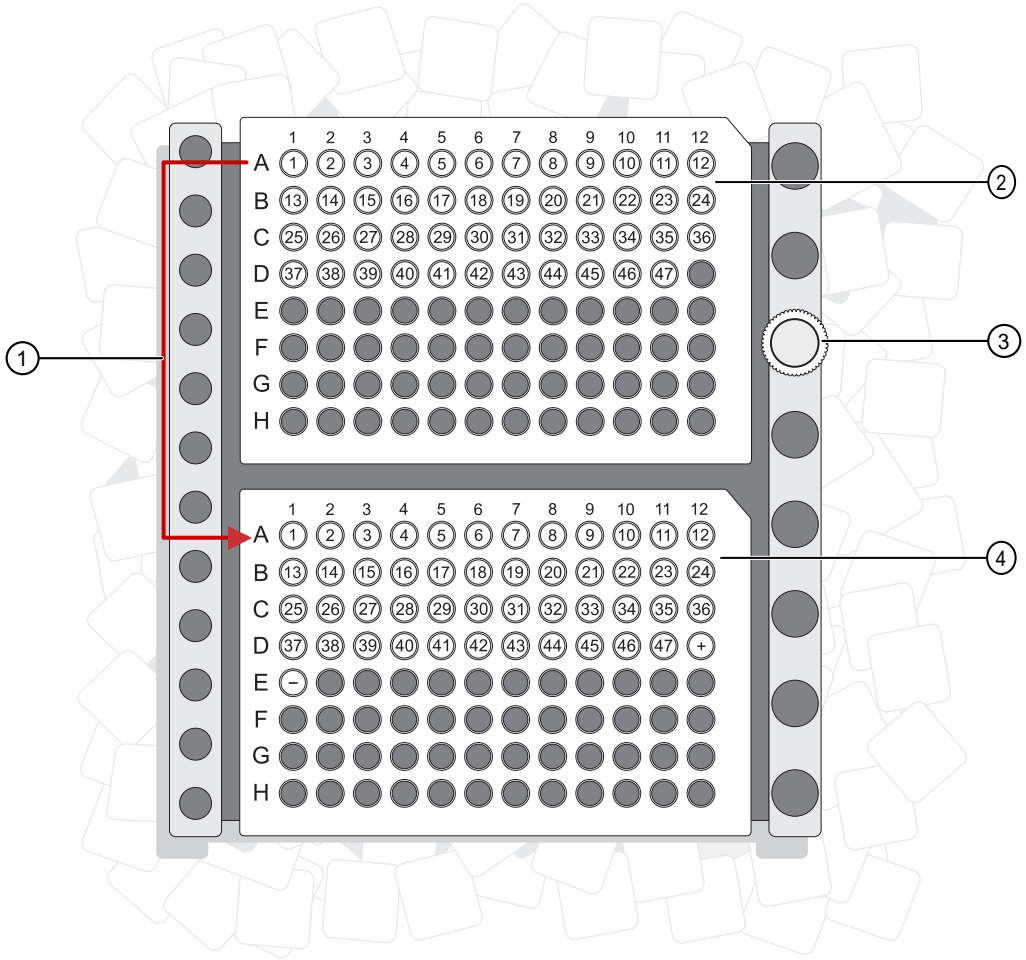


Figure 11 Setup for aliquoting diluted gDNA and controls to a 96-well plate labeled for digestion and ligation.

① Working stocks of gDNA samples (50 ng/µL) are aliquoted from upper plate to lower plate.
② Upper plate—96-well plate with working stocks gDNA samples at 50 ng/µL concentration.
③ Genomic DNA control from the CytoScan™ Reagent Kit (positive control).
④ Lower plate—96-well plate labeled for digestion and ligation.

Aliquot the genomic DNA working stocks and controls

Note: 5 µL of the 50 ng/µL working stock is equivalent to 250 ng genomic DNA per well.

1. Thaw the Genomic DNA control from the CytoScan™ Reagent Kit.
   a. Place on the benchtop at room temperature until thawed.
   b. When thawed, place in the cooling block on ice.
2. If needed, vortex the genomic DNA working stock for 3 seconds, then centrifuge for 1 minute.
3. Transfer a 5 µL aliquot of the samples to designated wells of the digest/ligate plate. (Figure 11.)

4. For the controls, aliquot 5 µL of:
   a. Genomic DNA control from the CytoScan™ Reagent Kit as the positive control (+).
   b. Low EDTA TE Buffer as the negative control (–).
5. Tightly seal the digest/ligate plate with a new seal, then centrifuge for 1 minute at 2,000 rpm.
6. Do one of the following:
   - Proceed to "Stage 1—Restriction enzyme digestion" on page 62.
   - Store the prepared sample plate at –20°C.

5 CytoScan™ Assay Automated Workflow for 25 samples

The Applied Biosystems™ CytoScan™ Assay Automated Workflow processes 25 or 49 samples (including controls) using the NIMBUS™ Target Preparation Instrument with CytoScan™ Assay-specific deck configuration.

This chapter provides instructions for processing 25 samples in parallel. The illustrations in this chapter are based on running 25 samples: 23 genomic DNA samples, plus 1 positive and 1 negative control. Use these illustrations as guidelines when processing 25 samples.

Stage 3C to Stage 8A are performed using the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument.

If processing 49 samples, see Chapter 6, “CytoScan™ Assay Automated Workflow for 49 samples”, which includes important guidelines for plate layouts.

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.

Stage 1—Restriction enzyme digestion

Materials required for Stage 1—Restriction enzyme digestion

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 8 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P10
1	Pipette, single-channel P100 or P200
1	Pipette, 12-channel, 2-20 µL
1	Pipette, 12-channel, 20-200 µL
As required	Pipette tips for pipettes
1	Thermal cycler
1	8-well or 12-well strip tube, 0.2 mL
Optional	8-tube or 12-tube strip tube caps
2	Eppendorf™ Safe-Lock™ Tubes, microcentrifuge, 1.5 mL, natural
1	Vortexer
2	GeneMate™ 96-Well PCR Tube Storage Rack

Table 9 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	Nsp I	Module 1	901718
1	Nsp I Buffer	Module 1	901719
1	100X BSA	Module 1	901720
1	Chilled Nuclease-Free Water	Module 2	902976
1	Genomic DNA sample plate (23 samples normalized to 50 ng/uL, 1 positive control, and 1 negative control)	—	—

Prepare the equipment, consumables, and reagents

Power on the thermal cycler

Power on the thermal cycler to preheat the lid. Leave the block at room temperature.

Set up the work area for digestion

1. Place a double cooling block and the Nuclease-Free Water on ice.
2. Place a 12-well strip tube on the upper half of the cooling block

3. Label a 1.5 mL Eppendorf™ tube as “Dig”, then place in the cooling block.

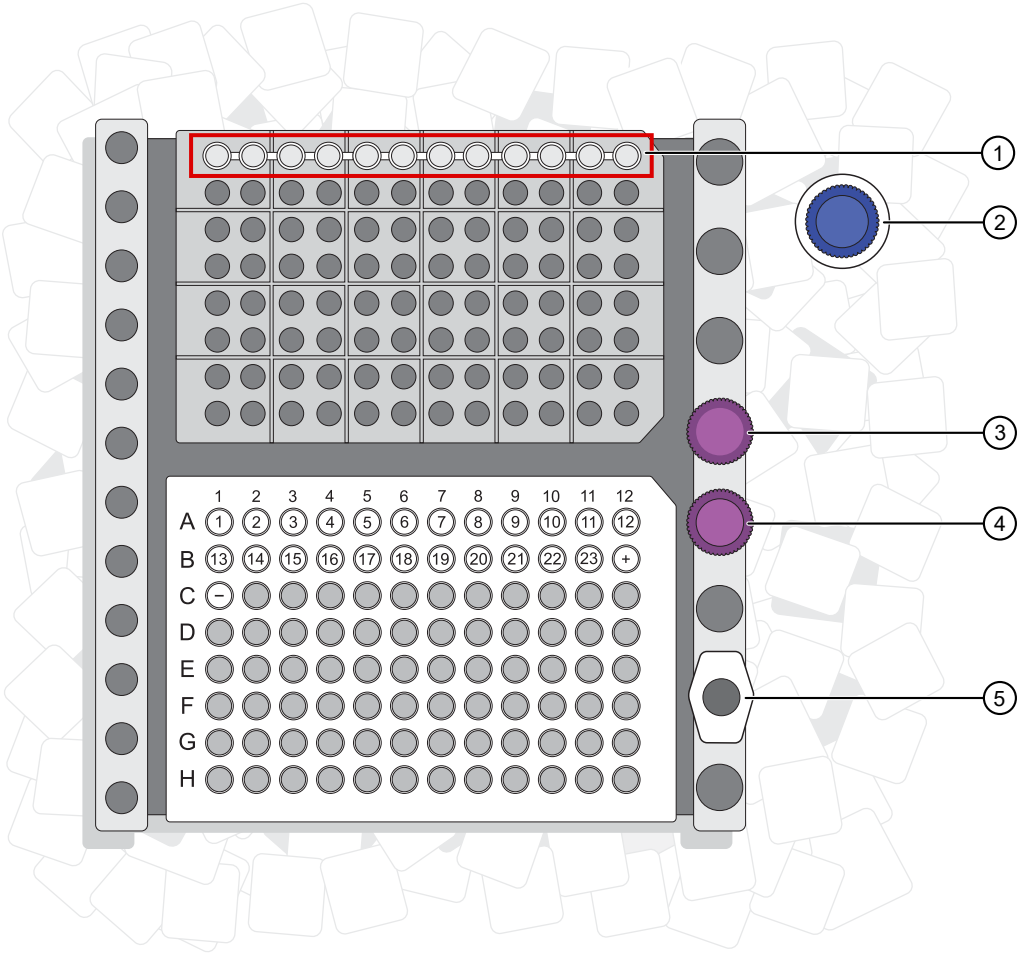


Figure 12 Setup for digestion. Nsp I enzyme not pictured; still at –20°C.

① 8-well or 12-well strip tube to aliquot Digestion Master Mix.
② Nuclease-Free Water.
③ Nsp I Buffer.
④ 100X BSA.
⑤ Digestion Master Mix tube labeled “Dig”.

4. Cut an adhesive seal into strips wide enough to seal 8-well or 12-well strip tubes.

Prepare the genomic DNA and the reagents

1. Prepare the genomic DNA plate.
   a. If the genomic DNA plate is frozen, allow it to thaw at room temperature.
   b. Centrifuge at 2,000 rpm for 1 minute.
   c. Place the plate on the bottom half of the colling block on ice.

2. Thaw the following reagents at room temperature. When thawed, immediately place on the cooling block on ice.
   - Nsp I Buffer
   - 100X BSA

   IMPORTANT! Leave the Nsp I enzyme at –20°C until ready to use.

3. Prepare the Nsp I Buffer and 100X BSA.
   a. Vortex 3 times, 1 second each time.
   b. Briefly centrifuge for 3 seconds.
   c. Place in the cooling block on ice.
4. Place Nuclease-Free Water on ice.

Prepare the Digestion Master Mix

For this procedure, keep all reagents, tubes, and the cooling block on ice.

1. To the 1.5 mL Eppendorf™ tube labeled “Dig”, add the following reagents based on the volumes shown in Table 10.
   - Chilled Nuclease-Free Water
   - 10X Nsp I Buffer
   - 100X BSA
2. Place the master mix in the cooling block.
3. Remove the Nsp I enzyme from the freezer, then immediately place in a cooler chilled to –20°C.
4. Vortex at high speed for 1 second.
5. Briefly centrifuge the enzyme for 3 seconds. Keep it in the –20°C cooler.
6. Immediately add the enzyme to the master mix.

Table 10 Digestion Master Mix.

Reagent	25 Samples (20% overage)
Chilled Nuclease-Free Water	332.6 µL
Nsp I Buffer	57.6 µL
100X BSA	5.8 µL
Nsp I	28.8 µL
Total volume	**424.8 µL**

7. Return the enzyme to the –20°C cooler.
8. Vortex the master mix at high speed 3 times, 1 second each time.

9. Briefly centrifuge for 3 seconds.

10. Place in the cooling block.

Add the Digestion Master Mix to samples

Divide the master mix into 8-well or 12-well strip tubes, then dispense the master mix from the strip tubes into the samples using a multichannel pipette.

1. Divide the Digestion Master Mix equally into the strip tubes on ice.

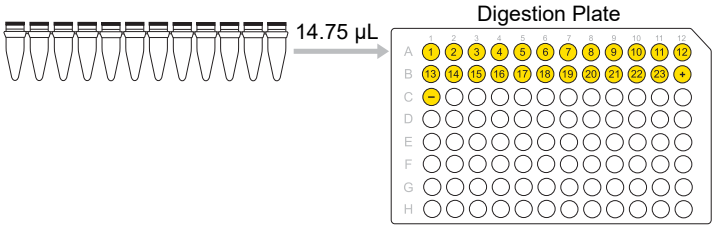
2. Seal the strip tube with an adhesive seal strip (or strip tube caps).

3. Centrifuge, then place back on a cooling block on ice. Remove the seal, then discard.

4. Unseal the sample plate, then discard the seal.

5. Using a multichannel P20 pipette, aliquot 14.75 µL of Digestion Master Mix to each sample and controls in row A.

Reagent	Volume
Genomic DNA (50 ng/µL)	5.00 µL
Digestion Master Mix	14.75 µL
Total volume	**19.75 µL**



6. Seal the plate tightly with a new seal.

Load samples onto the thermal cycler

1. Vortex the plate at high speed for 1 second in all corners and in the center according to the guidelines in “Seal, vortex, and centrifuge” on page 40, then centrifuge at 2,000 rpm for 1 minute.

2. Ensure that the lid of the thermal cycler is preheated.

3. Load the plate onto the thermal cycler.

4. Run the **CytoScan Digest** thermal cycler protocol.

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

5. Discard the remaining Digestion Master Mix and return any remaining reagents to –20°C.

6. When the **CytoScan Digest** thermal cycler protocol is finished, remove the plate from the thermal cycler. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.

7. Do one of the following:

   - Place the plate in a cooling block on ice, then proceed immediately to "Stage 2—Ligation" on page 68.
   - If not proceeding directly to Ligation, ensure that the plate is sealed tightly and store the plate at –20°C.

Stage 2—Ligation

Materials required for Stage 2—Ligation

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 11 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P10
1	Pipette, single-channel P20
1	Pipette single-channel P100 or P200
1	Pipette, 12-channel, 2-20 µL
1	Pipette, 12-channel, 20-200 µL
As needed	Pipette tips for pipettes listed above
1	Thermal cycler
1	8-well or 12-well strip tube, 0.2 mL
Optional	8-tube or 12-tube strip tube caps
1	Eppendorf™ Safe-Lock™ Tubes, microcentrifuge, 1.5 mL, natural
1	Vortexer
2	GeneMate™ 96-Well PCR Tube Storage Rack

Table 12 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	Adaptor, Nsp I	Module 1	902702
1	DNA Ligase Buffer		901722
1	DNA Ligase		901723

Prepare the equipment, consumables, and reagents

Power on the thermal cycler

Power on the thermal cycler to preheat the lid. Leave the block at room temperature.

Set up the work area for ligation

1. Place a double cooling block on ice.
2. Label a 1.5 mL Eppendorf™ tube as “Lig”, then place in the cooling block.
3. Place an 8-well or 12-well strip tube on the upper half of the cooling block.
4. Cut an adhesive seal into strips wide enough to seal 8-well or 12-well strip tubes.

Thaw the reagents and digested samples

IMPORTANT! Leave the DNA Ligase enzyme at –20°C until ready to use.

1. Thaw the following reagents at room temperature. When the reagents are thawed, immediately place on the cooling block on ice.
   - Adaptor, Nsp I.
   - DNA Ligase Buffer (requires about 20 minutes to thaw).
2. If the digested samples are frozen, allow them to thaw at room temperature.

Prepare the digested samples and reagents

1. Prepare the digested samples.
   a. Centrifuge at 2,000 rpm for 1 minute.
   b. Place in the lower half of the cooling block on ice.
2. Prepare the reagents.
   a. Vortex the DNA Ligase Buffer and the Adaptor, Nsp I at high speed 3 times, 1 second each time.

   IMPORTANT! Vortex the buffer as long as necessary before use to ensure that any precipitate is resuspended and the buffer is clear.

b. Briefly centrifuge for 3 seconds.

c. Place in the cooling block.

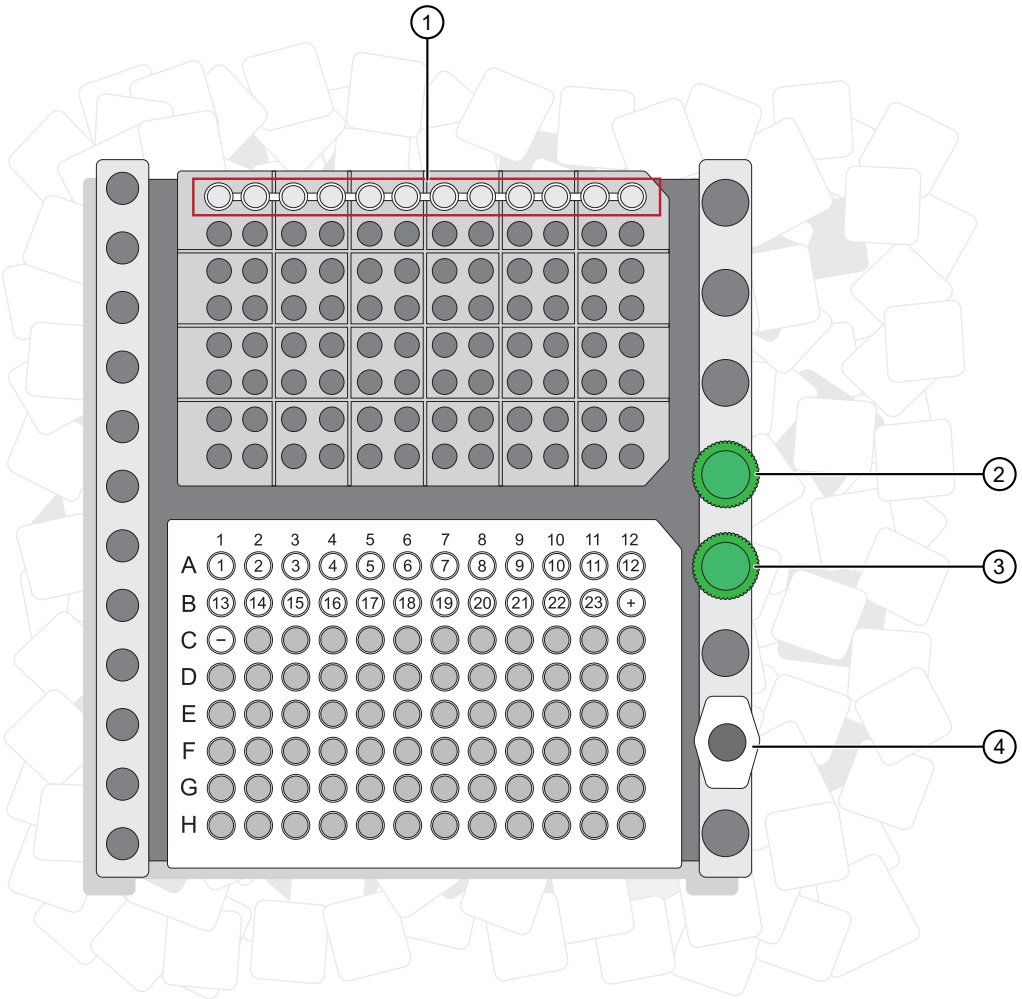


Figure 13 Setup for ligation. (DNA Ligase enzyme not pictured; still at –20°C.)

① 8-well or 12-well strip tubes to aliquot Ligation Master Mix.
② Adaptor, Nsp I.
③ DNA Ligase Buffer.
④ Ligation Master Mix in tube labeled “Lig”.

Prepare the Ligation Master Mix

For this procedure, keep all reagents and tubes on ice.

1. To the 1.5 mL Eppendorf™ tube labeled “Lig”, add the following reagents based on the volumes shown in Table 13:
   - DNA Ligase Buffer
   - Adaptor, Nsp I

2. Remove the DNA Ligase from the freezer, then immediately place in the cooler chilled to –20°C.
3. Vortex at high speed for 1 second.
4. Briefly centrifuge the DNA Ligase for 3 seconds, then place it in the –20°C cooler.
5. Immediately add the DNA Ligase to the master mix, then place it back in the –20°C cooler.

Table 13 Ligation Master Mix.

Reagent	25 Samples (25% overage)
DNA Ligase Buffer	75.0 µL
Adaptor, Nsp I	22.5 µL
DNA Ligase	60.0 µL
Total volume	**157.5 µL**

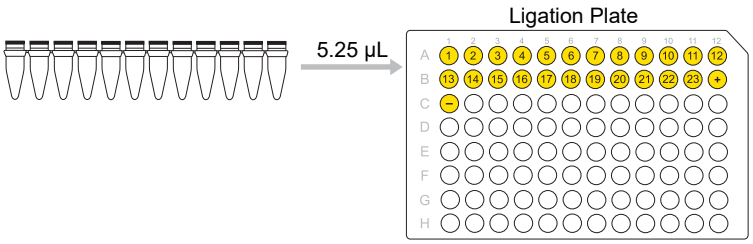
6. Vortex the Ligation Master Mix at high speed 3 times, 1 second each time.
7. Briefly centrifuge for 3 seconds.
8. Place the master mix in the cooling block on ice.
9. Proceed immediately to "Add the Ligation Master Mix to reactions" on page 71.

Add the Ligation Master Mix to reactions

When working with more than 8 samples, we strongly recommend dividing the master mix into strip tubes and dispensing the master mix from the strip tubes into the samples using a multichannel pipette.

1. Divide the Ligation Master Mix equally into the 8-well or 12-well strip tubes on ice.
2. Seal the strip tube with an adhesive seal strip (or strip tube caps), then briefly centrifuge. Place back in the cooling block on ice, then remove the seal and discard.
3. Unseal the digested sample plate and discard the seal.
4. Using a multichannel P20 pipette, aliquot 5.25 µL of Ligation Master Mix to each digested sample and control.

Reagent	Volume
Digested DNA	19.75 µL
Ligation Master Mix	5.25 µL
Total volume	**25.00 µL**



Load the samples onto the thermal cycler

1. Seal the plate tightly with a new seal.
2. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2,000 rpm for 1 minute.
3. Ensure that the thermal cycler lid is preheated.
4. Load the plate onto the thermal cycler.
5. Run the **CytoScan Ligate** thermal cycler protocol.

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

6. Return the remaining reagents to the freezer and discard the remaining master mix.
7. When the **CytoScan Ligate** thermal cycler protocol is finished, remove the plate from the thermal cycler. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.
8. Do one of the following:
   - If following the recommended 3-day workflow, proceed immediately to "Stage 3A—PCR reaction setup" on page 73.
   - Samples can be stored in a cooling block on ice for up to 60 minutes.
   - The sample plate can also be left in the thermal cycler at 4°C hold overnight.
   - If not proceeding directly to the next step, ensure that the plate is sealed tightly, then store the plate at –20°C. The plate can be frozen at –20°C for up to 10 days.

Stage 3A—PCR reaction setup

Tip: Before starting Stage 3A, ask someone in the Post-PCR Area to queue up the **CytoScan PCR** protocol on 2 thermal cyclers in preparation for the PCR step.

Materials required for Stage 3A—PCR reaction setup

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 14 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
2	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P20
1	Pipette, single-channel P100
1	Pipette, single channel P200
1	Pipette, single-channel P1000
1	Pipette, 12-channel, 2-20 µL
1	Pipette, 12-channel, 20-200 µL
As required	Pipette tips for the pipettes
4	Plates, 96-well, semi-skirted
2	GeneMate™ 96-Well PCR Tube Storage Rack
1	Reagent reservoir, 25 mL
1	Tube, centrifuge 15 or 50 mL
1	Vortexer
2	Thermal cyclers

Table 15 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	PCR Primer	Module 1	902674
1	Chilled Nuclease-Free Water	Module 2	902976
1	CytoScan™ Amplification Kit, Cat. No. 902975: • dNTP Mixture (2.5 mM each) • GC-Melt Reagent • 50X Titanium™ *Taq* DNA Polymerase • 10X Titanium™ *Taq* PCR Buffer	The CytoScan™ Amplification Kit is not included in the CytoScan™ Reagent Kit and must be ordered separately.	

Gels and related materials required

Verifying the PCR reaction is required for this stage.

About controls

To determine the presence of contamination, always include 1 PCR negative control with every set of samples in a run.

IMPORTANT! It is crucial to dilute the ligated DNA with chilled Nuclease-Free Water before PCR.

Dilute the ligated samples

If samples are frozen, thaw at room temperature (~30 minutes). Centrifuge 650 x *g* for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.

1. Place Nuclease-Free Water on ice 20 minutes before use.
2. Place a double cooling block on ice.
3. Place a reagent reservoir on the upper half of the cooling block on ice.
4. Pour chilled Nuclease-Free Water into the reagent reservoir.
5. When the **CytoScan Ligate** thermal cycler protocol is finished, remove the plate.
6. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.
7. Place the plate in the lower half of the cooling block on ice.
8. Unseal the ligated sample plate and discard the seal.

9. Using a P200 pipette, add 75 µL of Nuclease-Free Water to each reaction.

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

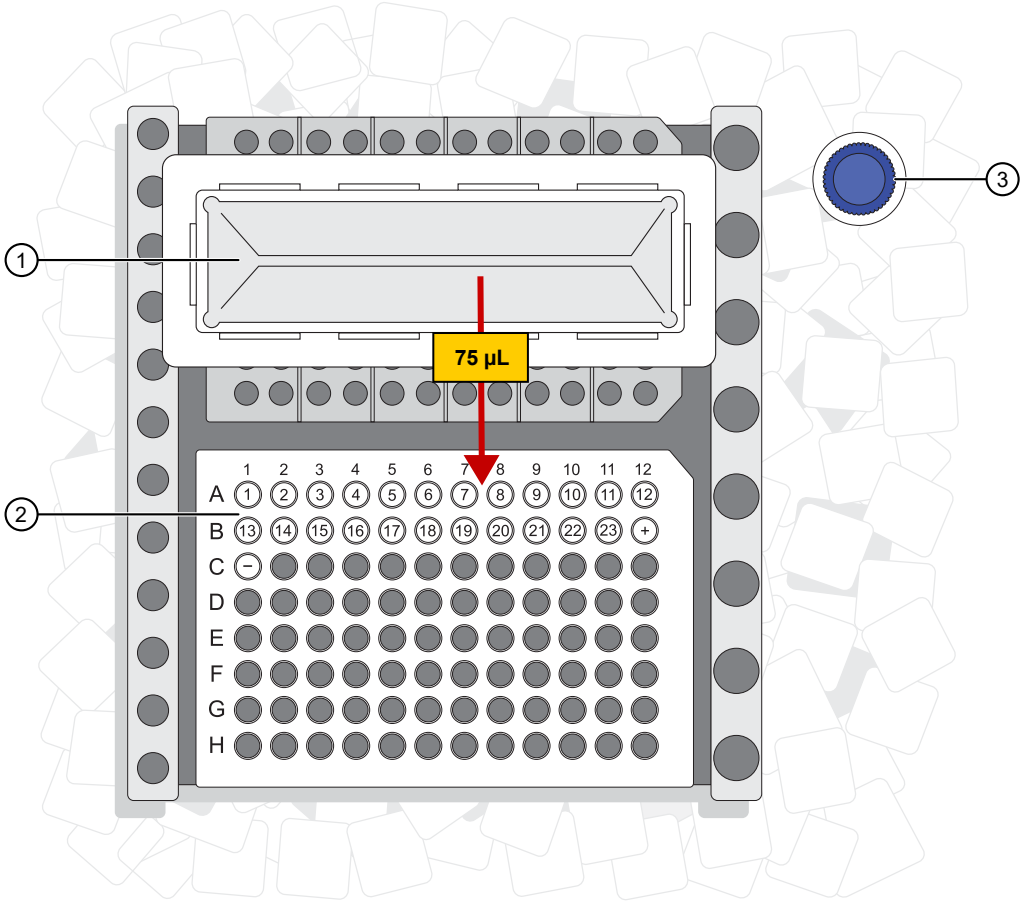


Figure 14 Dilute the ligated samples.

① Chilled Nuclease-Free Water in the reservoir.
② Ligated samples
③ Nuclease-Free Water

10. Tightly seal the plate with a new seal.

11. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2,000 rpm for 1 minute.

12. If not proceeding with PCR set up, store the plate at –20°C.

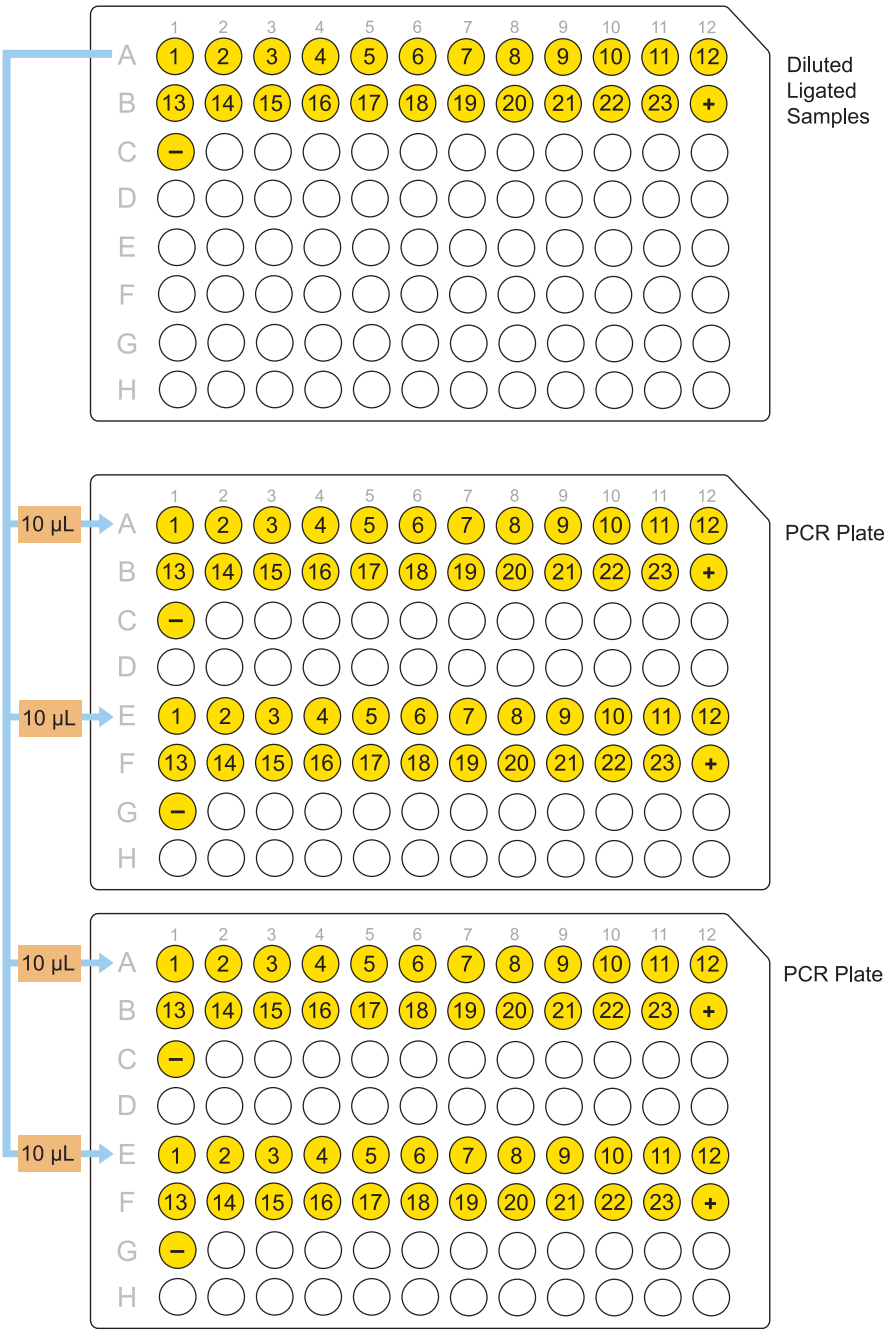
STOPPING POINT The plate can be frozen at –25°C to –15°C for up to 10 days.

Transfer diluted ligated samples to the PCR plate

1. Place 1 single and 1 double cooling block on ice.
2. Keep the diluted ligated sample plate on the single cooling block.

   If the diluted ligated samples are frozen, thaw them at room temperature. Ensure that the plate is sealed tightly, then vortex at high speed for 1 second in all corners and in the center according to the guidelines in “Seal, vortex, and centrifuge” on page 40. Then centrifuge at 2,000 rpm for 1 minute. Immediately place the plate on the upper half of the cooling block.
3. Label 2 new plates as “PCR”, then place the plates on the double cooling block, one on the upper half and the second on the lower half.
4. Unseal the diluted ligated sample plate, then discard the seal.

5. Using a multichannel P20 pipette, transfer 10 µL of each diluted ligated sample to the corresponding wells of a new PCR plate.



6. Seal the plates tightly with a new seal, then centrifuge at 2,000 rpm for 1 minute.

7. If not proceeding immediately to the PCR stage, store the plate with the remaining samples at –20°C.

   Left-over samples can be stored at –20°C for up to 10 days.

Thaw the reagents and samples

IMPORTANT! Leave the 50X Titanium™ *Taq* DNA Polymerase at –20°C until ready to use.

Allow the following reagents to thaw at room temperature. Immediately place on the cooling block on ice when reagents are thawed.

- 10X Titanium™ *Taq* PCR Buffer
- dNTP Mixture (2.5 mM each)
- PCR Primer

Prepare the samples and reagents

1. Label the 15 mL centrifuge tube “PCR”.
   For more than 24 samples, use a 50-mL tube.
2. Place on ice:
   - Chilled Water, Nuclease-Free
   - GC-Melt Reagent
   - Place the reagent reservoir on the bottom half of the cooling block on ice.
3. If the diluted ligated samples aliquoted into the PCR plate are frozen, thaw them at room temperature. Ensure that the plate is sealed tightly and vortex at high speed for 1 second in all corners and in the center according to the guidelines in “Seal, vortex, and centrifuge” on page 40, then centrifuge at 2,000 rpm for 1 minute.

4. Immediately place on the chamber as shown in the following image.

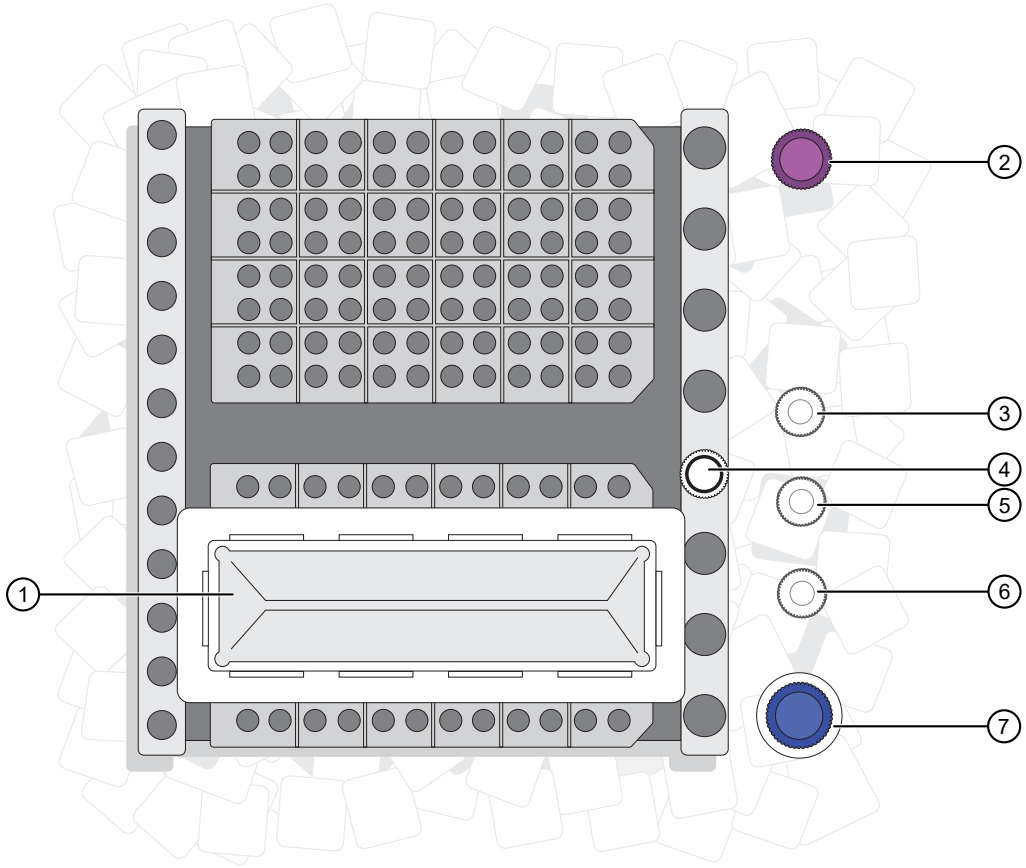


Figure 15 Setup for PCR (Titanium™ *Taq* DNA polymerase not shown; still at –20°C).

1. Reagent reservoir.
2. PCR Master Mix tube.
3. dNTP Mixture (2.5 mM each).
4. PCR Primer.
5. GC-Melt Reagent.
6. Titanium™ *Taq* PCR Buffer.
7. Nuclease-Free Water.

5. Prepare the reagents (except the enzyme):
   a. Vortex the reagents at high speed 3 times, 1 second each time. Briefly centrifuge for 3 seconds.
   b. Place in the cooling block.

Power on 2 thermal cyclers (Post-PCR Area)

Have someone in the Post-PCR Area power on 2 thermal cyclers to preheat the lid. Leave the block at room temperature.

To avoid contamination, do not go from the Pre-PCR Clean Area to the Post-PCR Area, and then back again.

Prepare the PCR Master Mix

IMPORTANT! Accurate pipetting of all components is critical for obtaining the correct size distribution of PCR products.

1. Keep the 15-mL centrifuge tube labeled "PCR" on ice, then add the reagents in the order shown in the following table, *except for the 50X Titanium™ Taq DNA polymerase*.
2. Remove the 50X Titanium™ *Taq* DNA polymerase from the freezer, then immediately place in a cooler that is chilled to –20°C.
3. Vortex at high speed for 1 second.
4. Briefly centrifuge the 50X Titanium™ *Taq* DNA polymerase for 3 seconds.
5. Immediately add the 50X Titanium™ *Taq* DNA polymerase to the master mix, then return the tube to the –20°C cooler.

Table 16 PCR Master Mix.

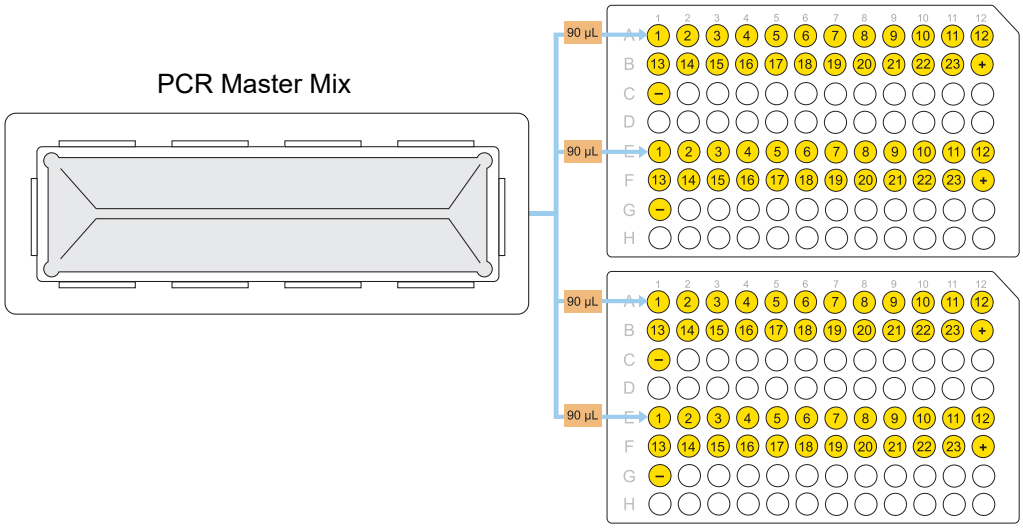
Reagent	25 Samples (15% overage)
Chilled Nuclease-Free Water	4,360.8 µL
10X Titanium™ *Taq* PCR Buffer	1,104.0 µL
GC-Melt Reagent	2,208.0 µL
dNTP Mixture (2.5 mM each)	1,545.6 µL
PCR Primer	496.8 µL
50X Titanium™ *Taq* DNA polymerase Do not add until ready to aliquot PCR Master Mix to ligated samples.	220.8 µL
Total volume	**9,936.0 µL**

6. Vortex the master mix at high speed 3 times, 1 second each time.
7. Pour the master mix into the reagent reservoir. Keep the cooling block on ice.

Add the PCR Master Mix to each sample

1. Unseal the PCR sample plates and discard the seal.
2. Using a multichannel P200 pipette, aliquot 90 µL PCR Master Mix to each sample and control on the PCR plate.

   To avoid contamination, change pipette tips after each dispense. Ensure that each pipette tip picks up 90 µL.



After adding the master mix, the total volume in each well is 100 µL.

Reagent	Volume
Ligated and diluted DNA	10 µL
PCR Master Mix	90 µL
Total volume	**100 µL**

3. Tightly seal the plates with a new seal according to the guidelines in "Seal, vortex, and centrifuge" on page 40.

4. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40.

5. Repeat vortexing 1 more time, then centrifuge at 2,000 rpm for 1 minute.

6. Keep the plates in the cooling block on ice until ready to load onto the thermal cyclers.

Stage 3B—PCR

Equipment required and location

Stage 3B—PCR requires 2 pre-heated thermal cyclers located in the Post-PCR Area. Ensure that the thermal cycler lid is preheated. Ensure that the block is room temperature (15°C to 30°C).

Load the plates and run the CytoScan PCR thermal cycler protocol

1. Transfer the plates on ice to the Post-PCR Area.
2. Ensure that the thermal cycler lid is preheated.
   Keep the block at room temperature.
3. Load the plates onto the thermal cyclers.
4. Run the **CytoScan PCR** thermal cycler protocol.

   IMPORTANT! Use only silver or gold-plated silver blocks.

Temperature	Time	Cycles
94°C	3 minutes	1X
94°C	30 seconds	30X
60°C	45 seconds	
68°C	15 seconds	
68°C	7 minutes	1X
4°C	HOLD (Can be held overnight)	
Volume: 100 µL		

   After the **CytoScan PCR** thermal cycler protocol finishes, the plate can be left on hold at 4°C in the thermal cycler for up to 24 hours.
5. When the **CytoScan PCR** thermal cycler protocol is finished, leave the plate in the thermal cycler.
   If proceeding to purification, process the plate within 2.5 hours.
6. After removing the plate from the thermal cycler, keep the plate on a 96-well plate rack.
7. Ensure that the plate is tightly sealed, then centrifuge at 600 x *g* for 1 minute.
   If not proceeding immediately to the next step, the plate can be stored at –20°C for up to 10 days.

Stage 3C—PCR product check

This stage verifies the PCR reaction by using a TBE gel or an E-Gel™ Agarose Gel. This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration.

First, start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument, then follow the instructions for:

- "PCR product check using TBE gels" on page 85.

  or

- "PCR product check using E-Gel™ Agarose Gel" on page 92.

Materials required for Stage 3C—PCR product check

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 17 Materials required for running a TBE gel.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Electrophoresis gel box
1	Electrophoresis power supply
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1	Reservoir 4 ROW PYR PP 73 mL
1	25 bp DNA Ladder
1	Gels, 2% TBE (precast or house-made)
1	1X TBE Buffer
1	Ethidium bromide solution
1	5X RapidRun™ Loading Dye
1	96-well Full Skirt PCR Plate, clear

Table 18 Materials required for running an E-Gel™ Agarose Gel.

Quantity	Item
1	NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific Cleanup screen
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Mother E-Base™ Device
1	Daughter E-Base™ Device (optional for running multiple gels simultaneously)
1 rack	Conductive 50 µL filter tips in frames
1 rack	Conductive 300 µL filter tips in frames
2	E-Gel™ *48* Agarose Gels, 2%
1	*Redi*Load™ Loading Buffer
1	TrackIt™ Cyan/Orange Loading Buffer
1	25 bp DNA Ladder

Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument

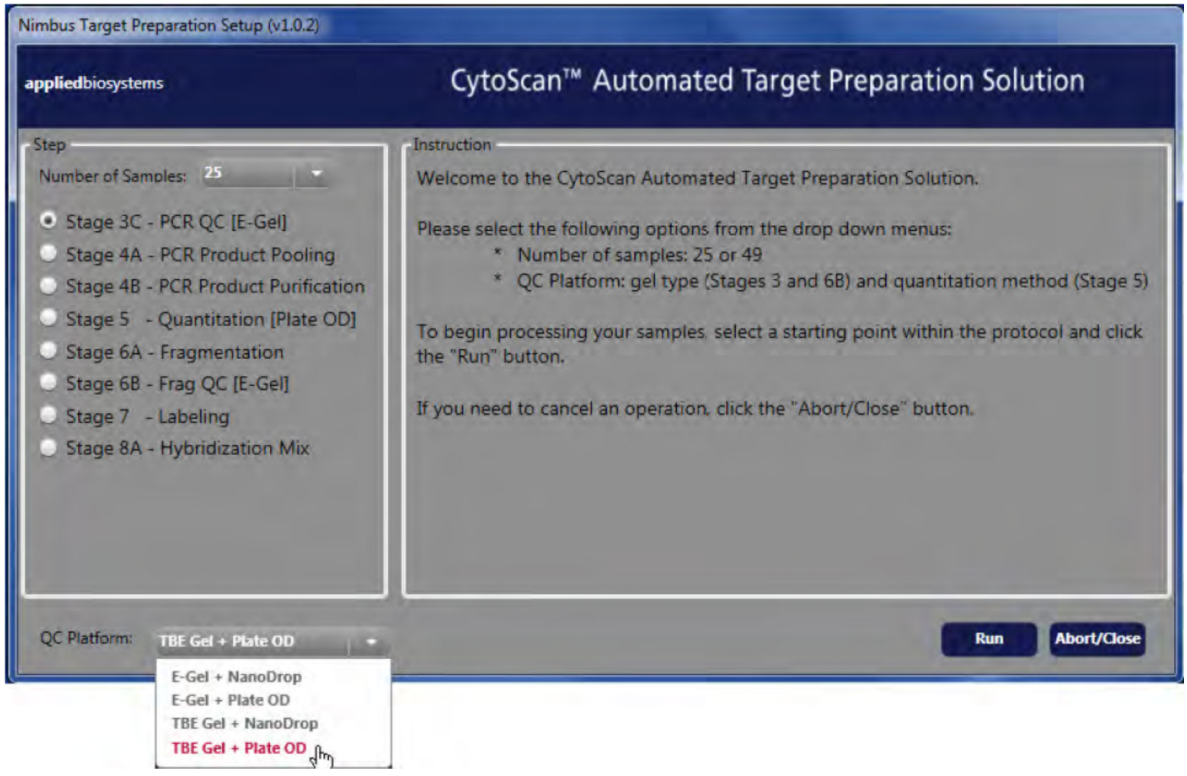
1. Power on the NIMBUS™ Instrument.
2. On the desktop, double-click the CytoScan™ icon to open the **Welcome** screen.

PCR product check using TBE gels

WARNING! E-Gel™ Agarose Gels and TBE gels contain ethidium bromide. Review the manufacturer's Safety Data Sheet for proper handling and disposal. Use good laboratory practices and always wear protective gloves when handling ethidium bromide. Dispose of the gel and gloves in accordance with national, state, and local regulations.

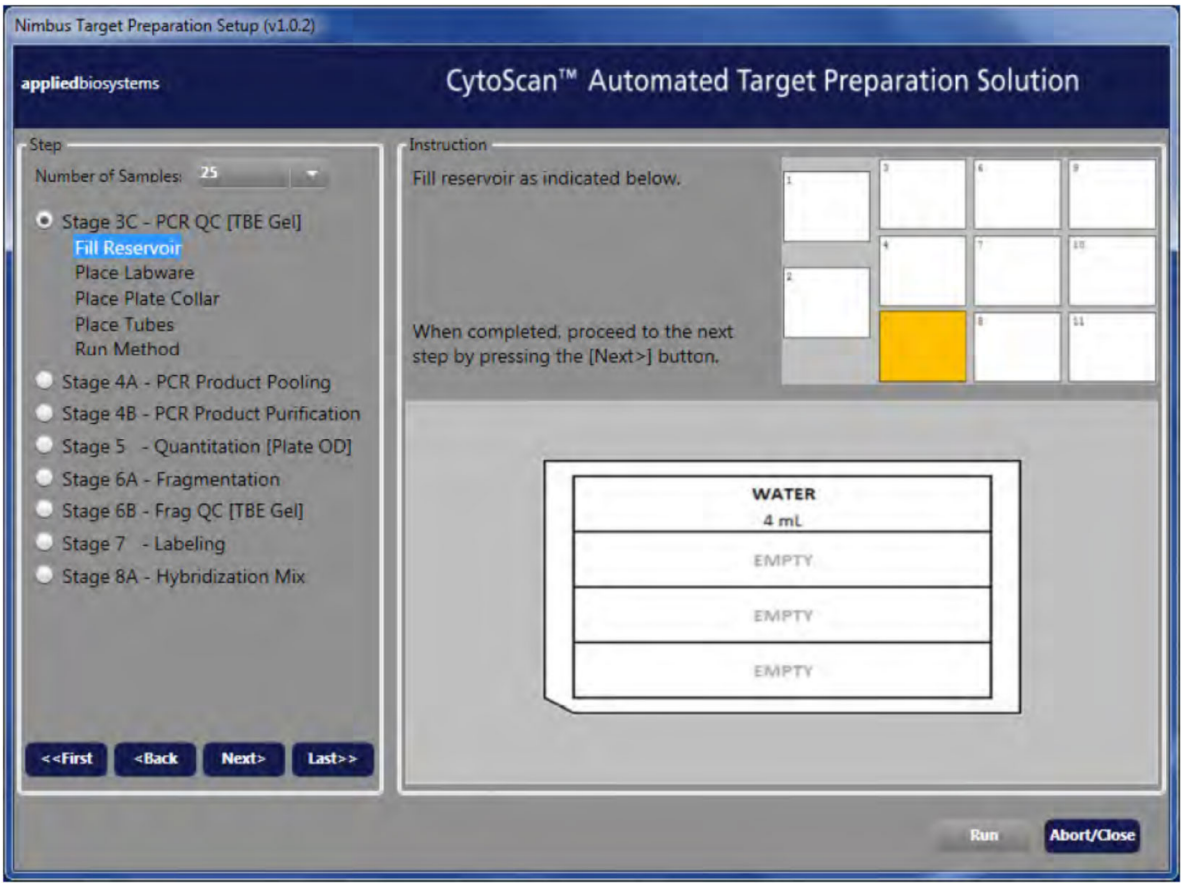
Note: If samples are frozen, thaw at room temperature (~30 minutes). Centrifuge 650 x *g* for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.

1. Remove the plate from the thermal cycler.
2. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.
3. Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument.
   See "Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument" on page 84.
4. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25.
   - **Stage 3C–PCR QC**.
   - **QC Platform**—TBE Gel + a quantitation method (for example, "TBE Gel + Plate OD").



5. Click **Run** to start the method, then click **Yes** to confirm.

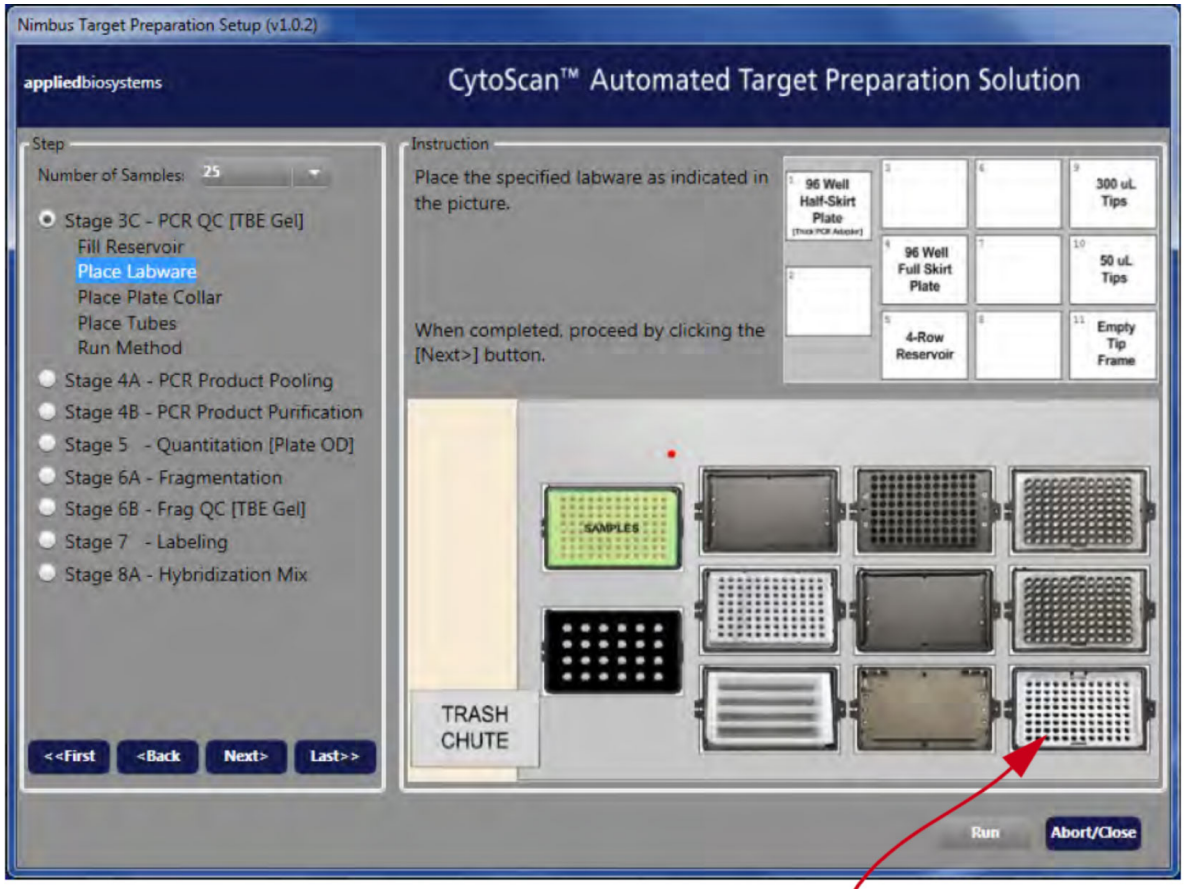
6. Fill the reservoir as shown in the on-screen instructions, then click **Next**.



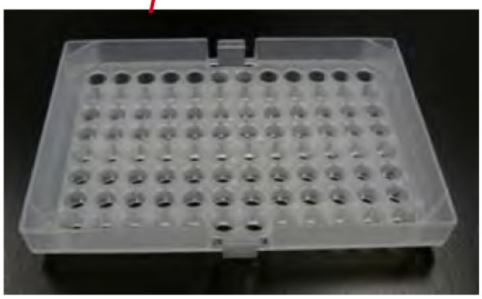
7. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

Note: Place an empty tip frame on position 11.

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.



Empty tip frame for position 11

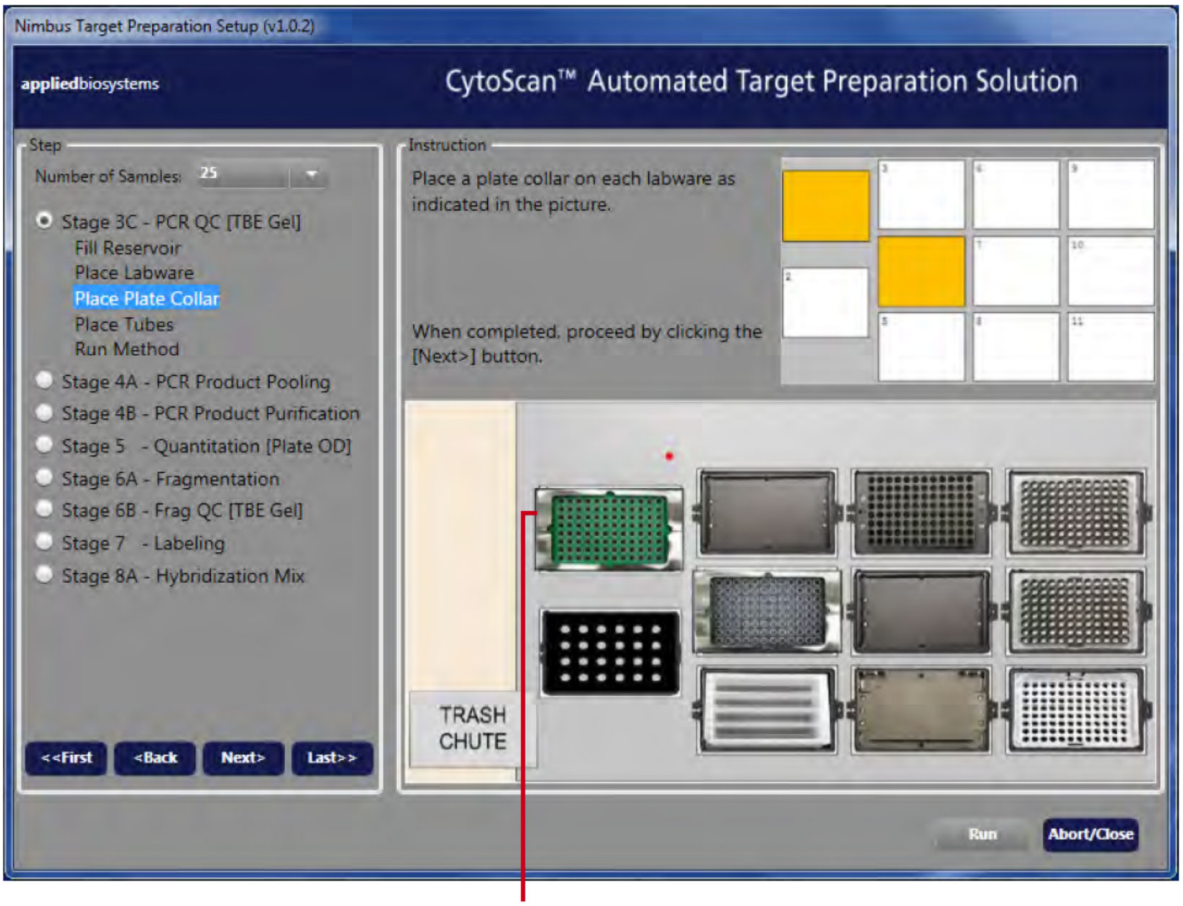


8. Unseal one of the PCR plates and discard the seal. Place the plate on the NIMBUS™ Instrument deck as shown in the previous image, then click **Next**.

   IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

9. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**.

   Labware at deck positions 1 and 4 require a plate collar.



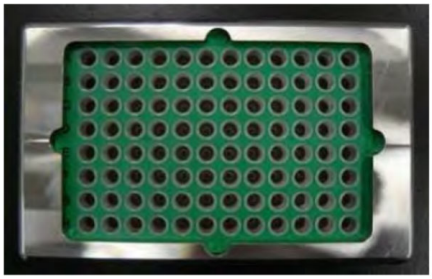
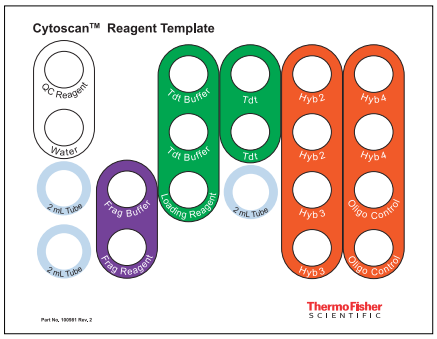
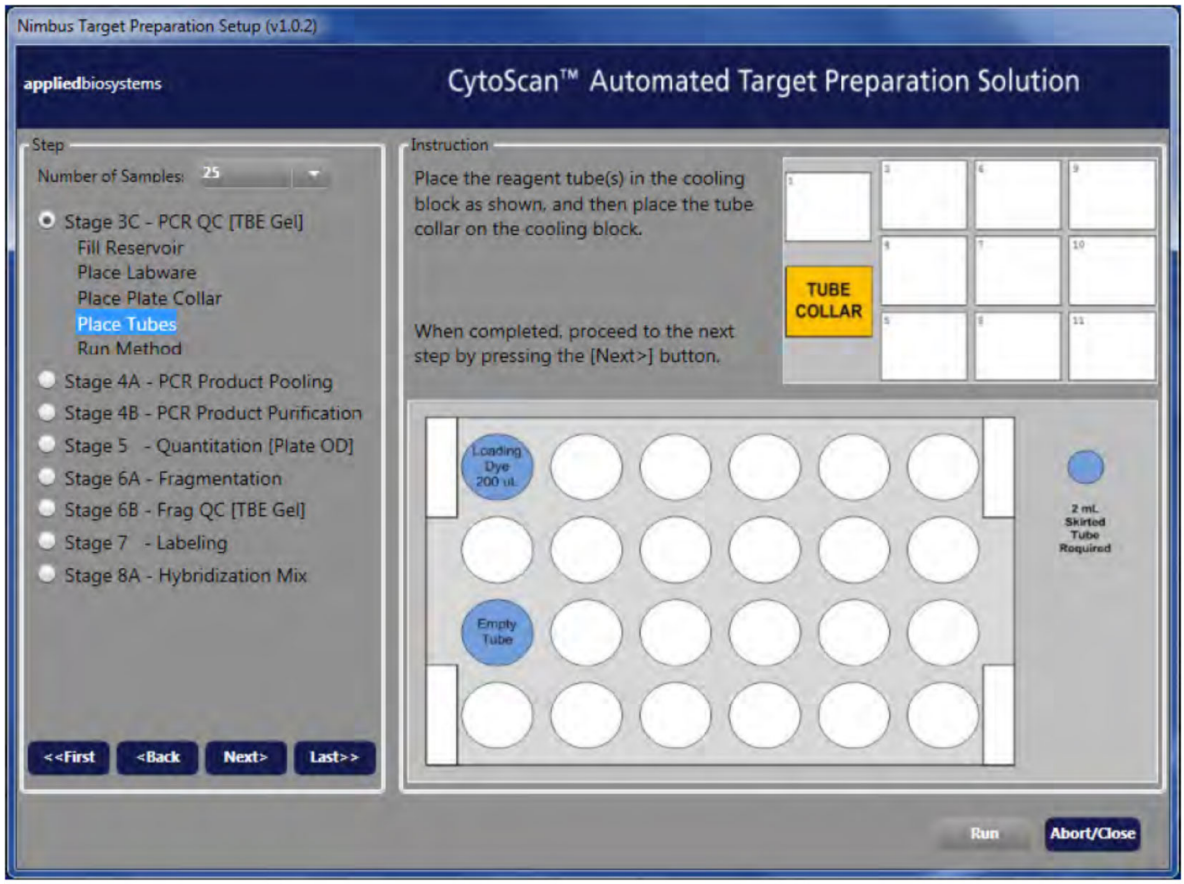


Plate collar on the sample plate

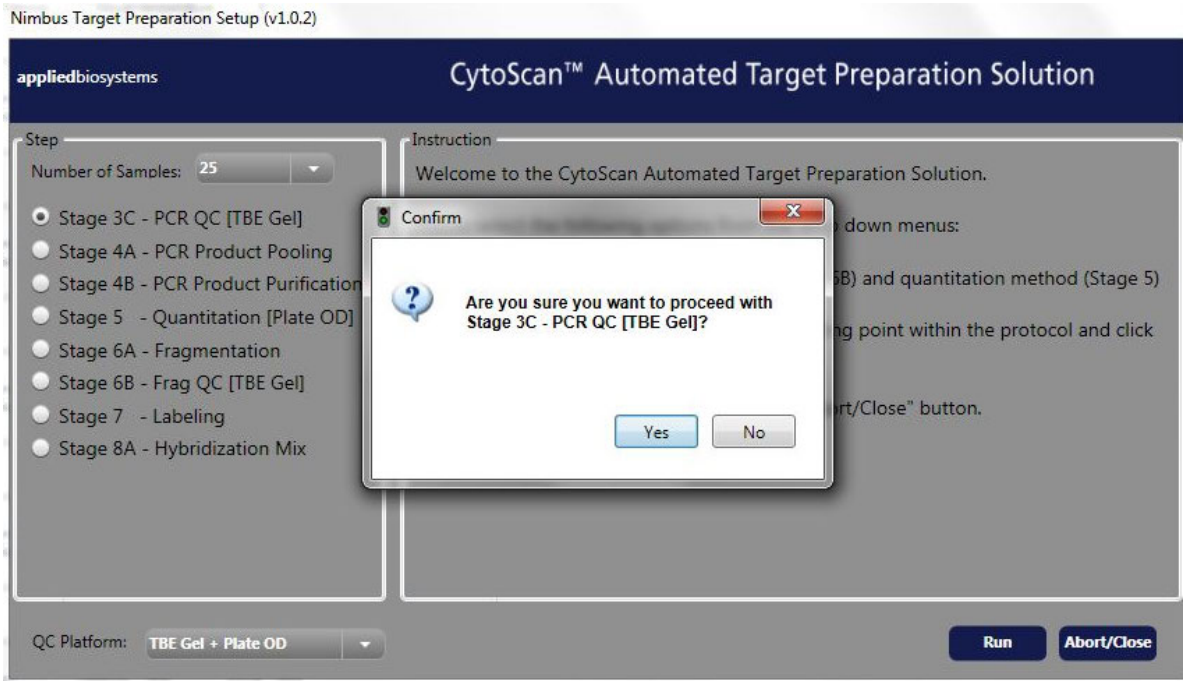
10. Place the tubes as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen and in the following image, then click **Next**.

IMPORTANT! Use the CytoScan™ Reagent Template, Cat. No. 100981 Rev. 2 for this protocol. The template accommodates the fragmentation protocol and reagents.

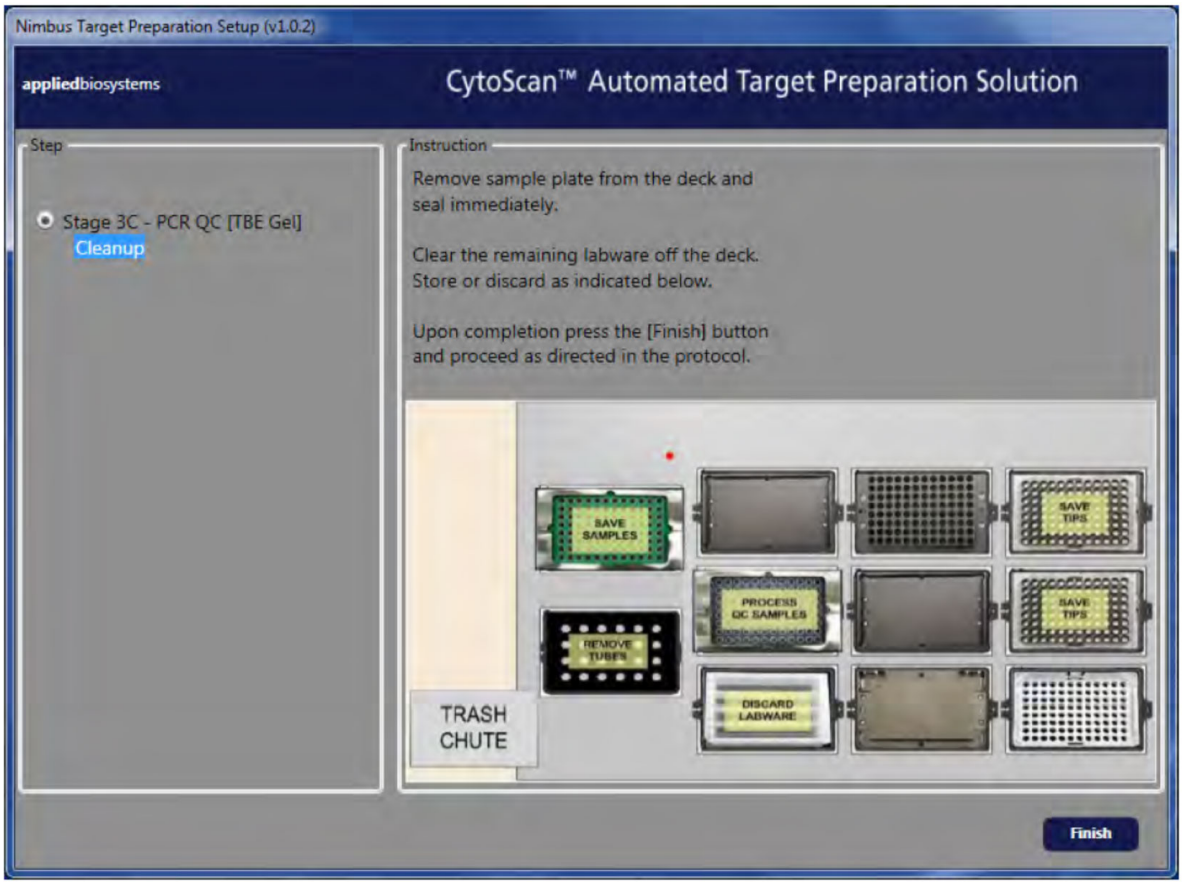




11. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



12. Remove the PCR plate from the NIMBUS™ Instrument deck, then seal the plate tightly with a new seal. If not proceeding to Stage 4A—PCR product pooling, store it at –20°C.

13. Remove the gel QC plate from the deck, then seal the plate tightly with a new seal.

14. Remove labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

15. Click **Finish**, then click **Yes** to confirm.
    The method closes.

16. Vortex the gel QC plate, then centrifuge briefly in a plate centrifuge.

Note: Add ethidium bromide to the gel running buffer. Add 2 drops of ethidium bromide per liter of 1X TBE.

Run the TBE gel

1. Load 8 µL of the samples from the QC Sample Plate onto a 2% TBE gel.

2. Load 5 µL 25 bp DNA Ladder to the first and last wells of the gel.

3. Run the gel at 5 V/cm for 45 minutes or until the dye front reaches at least 75% of distance down the gel.
   Run gels at 5 V/cm (5 volts X distance in cm between electrodes). For example, run a 33-cm electrophoresis box at 165 V; run a 16-cm electrophoresis box at 80 V.

4. Verify that the PCR product distribution is between approximatey150 bp to 2,000 bp.

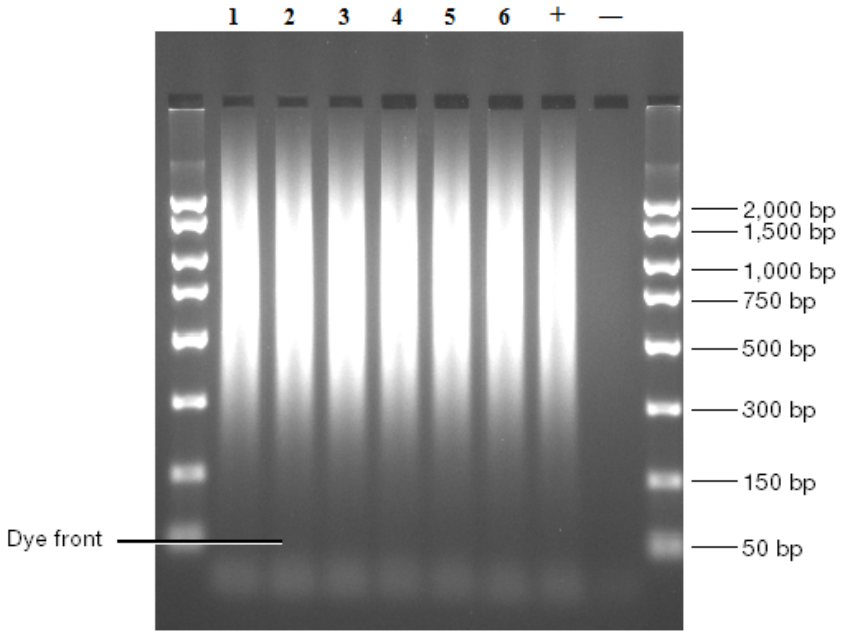


Figure 16 Example of PCR products run on 2% TBE gel at 5 V/cm for 45 minutes. Average product distribution is between about 150 to 2,000 bp.

5. Do one of the following:
   - If the PCR has been verified, proceed to "Stage 4A—PCR product pooling" on page 100.
   - If not proceeding directly to the next stage, seal the plate of PCR product, then store the plate at –20°C.

PCR product check using E-Gel™ Agarose Gel

WARNING! E-Gel™ Agarose Gels and TBE gels contain ethidium bromide. Review the manufacturer's Safety Data Sheet for proper handling and disposal. Use good laboratory practices and always wear protective gloves when handling ethidium bromide. Dispose of the gel and gloves in accordance with national, state, and local regulations.

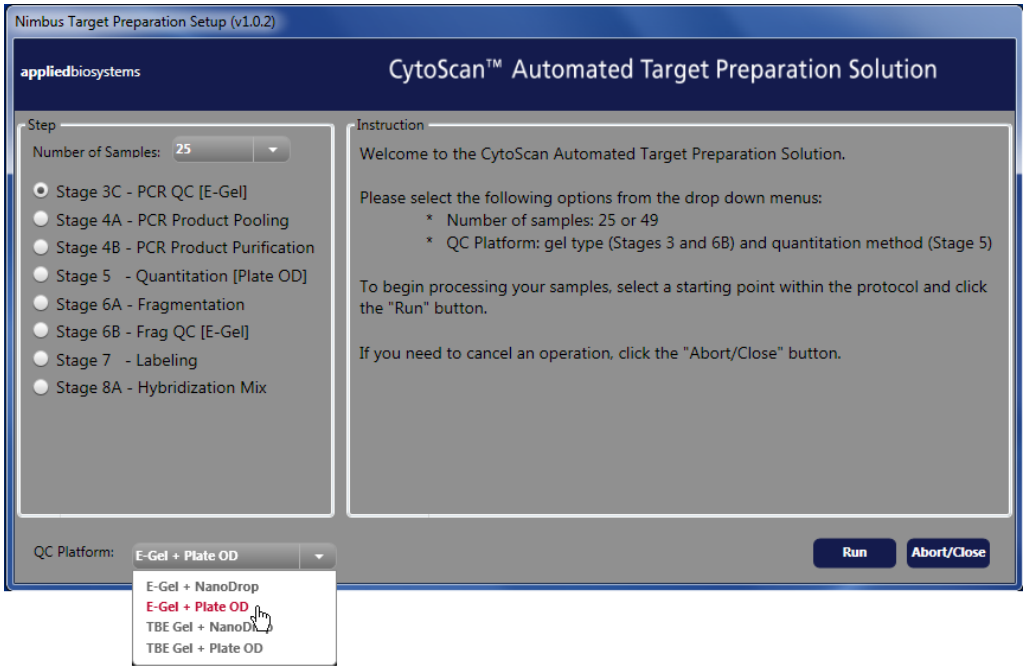
Dilute the TrackIt™ Cyan/Orange Loading Buffer

1. Add 50 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.95 mL Nuclease-Free Water (total volume is 50 mL).
2. Mix well, then store at room temperature.

Prepare a QC gel plate for PCR product

Note: If samples are frozen, thaw at room temperature (~30 minutes). Centrifuge 650 x *g* for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.

1. Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument.
   See "Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument" on page 84.
2. On the **Welcome** screen, select the following options.
   - **Number of samples**—25.
   - **Stage 3C–PCR QC**.
   - **QC Platform**—E-Gel and a quantitation method (for example, "E-Gel + Plate OD").

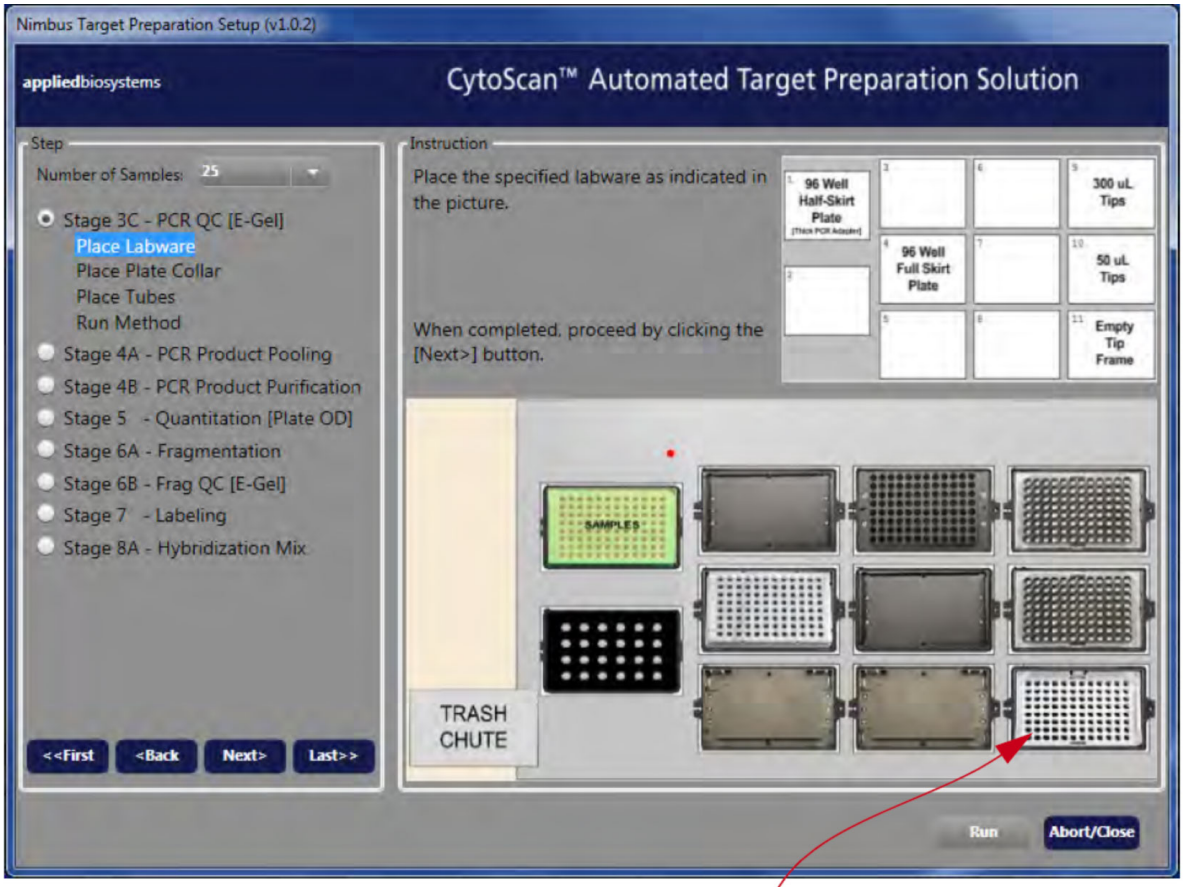


3. Click **Run** to start the method, then click **Yes** to confirm.

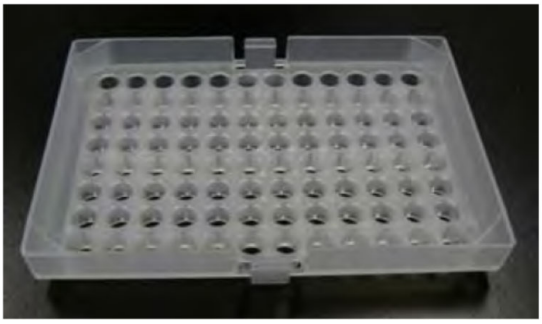
4. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

Note: Place an empty tip frame on position 11.

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.



Empty tip frame for position 11



5. Unseal one of the PCR plates, then discard the seal. Place the plate on the NIMBUS™ Instrument deck as shown, then click **Next**.

IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**.

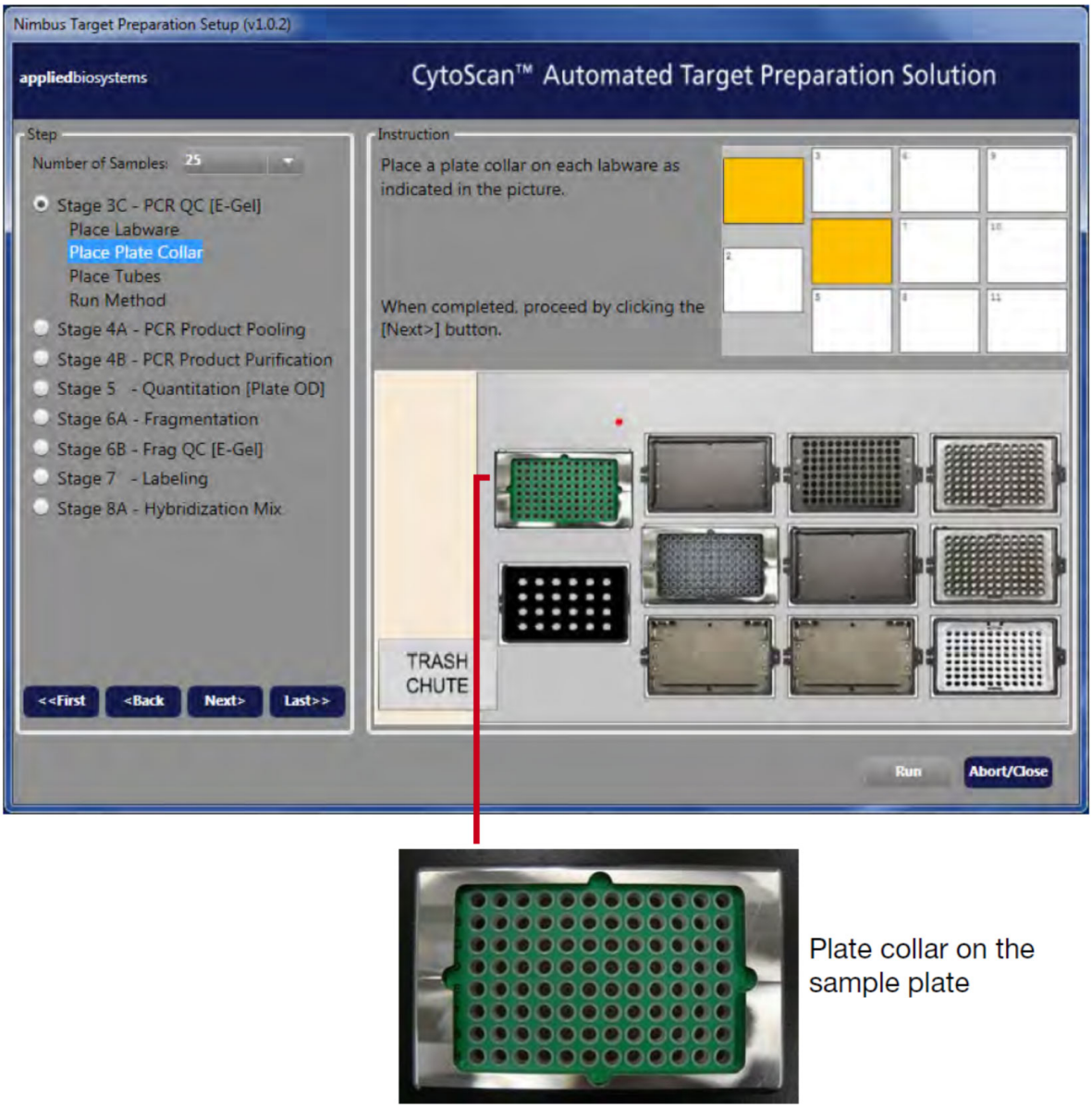
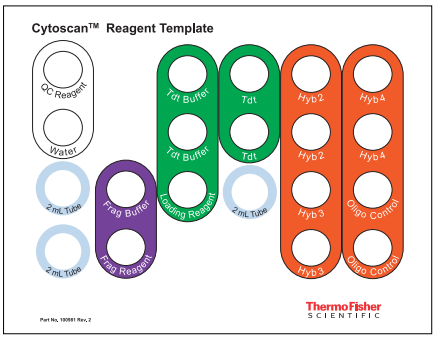
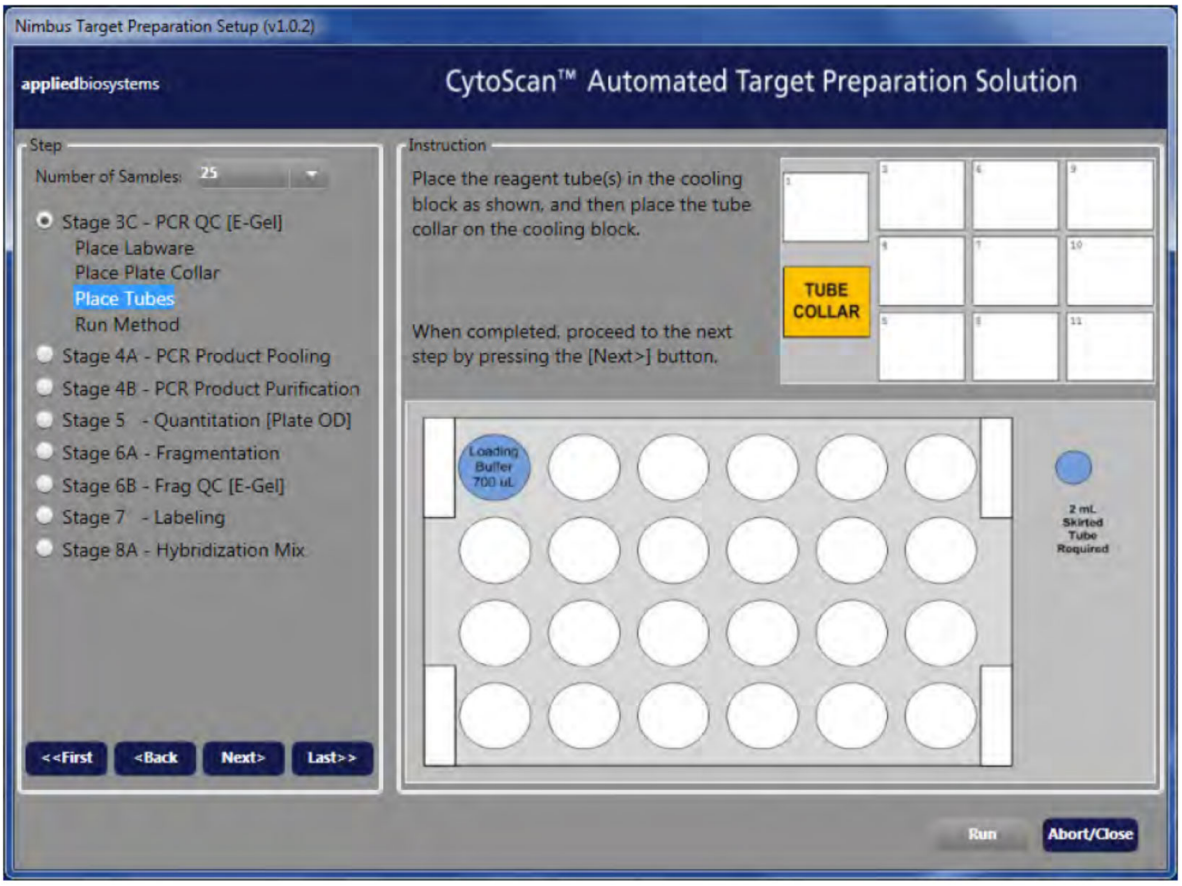


Plate collar on the sample plate

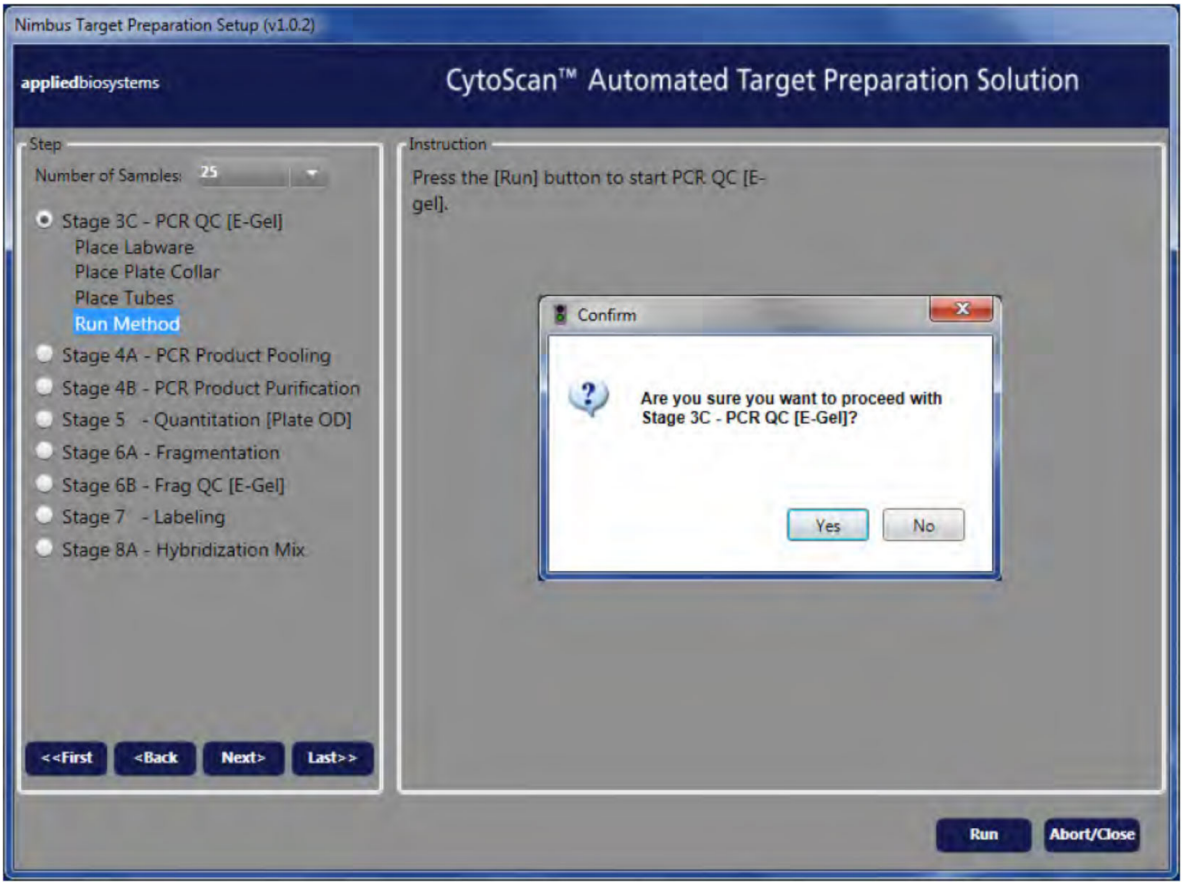
7. Place the tube(s) in the tube rack as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.

IMPORTANT! Use the CytoScan™ Reagent Template, Cat. No. 100981 Rev. 2 for this protocol. The template accommodates the fragmentation protocol and reagents.

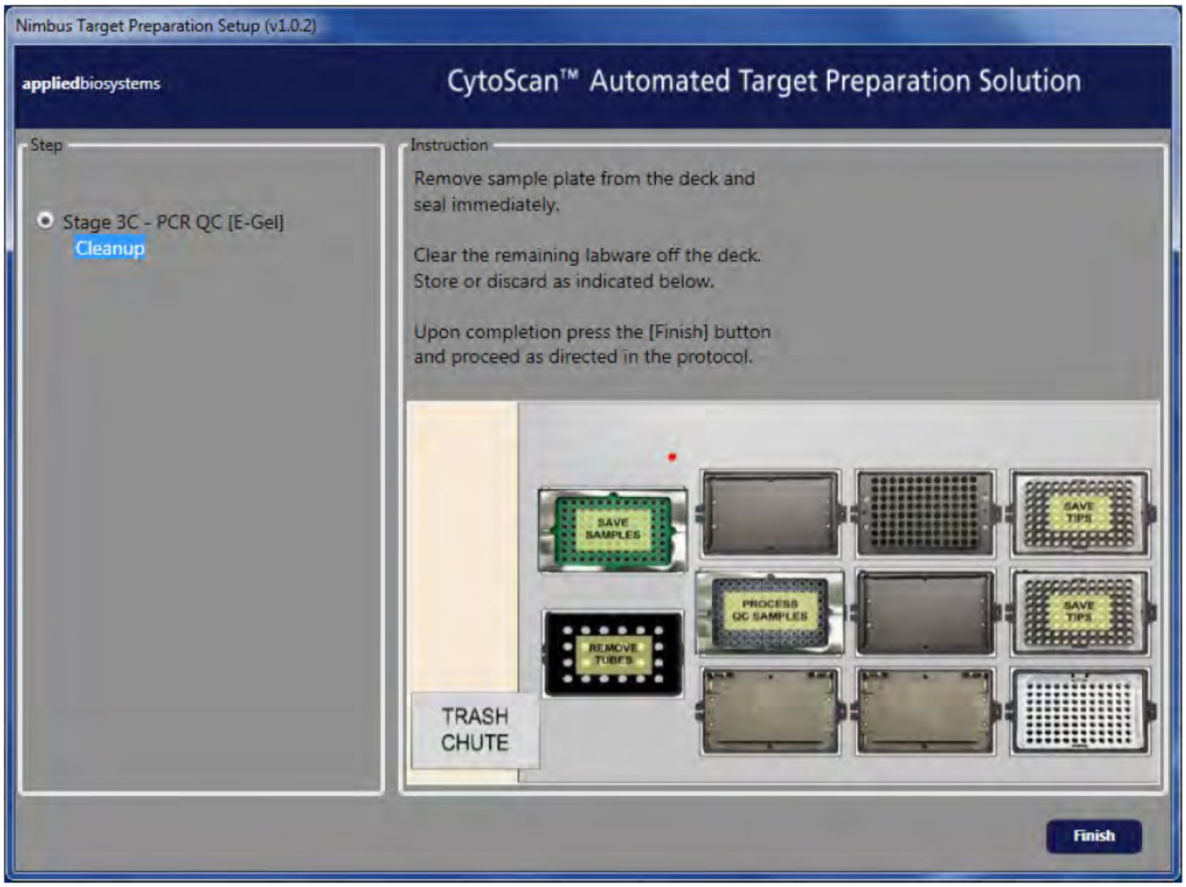




8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Remove the PCR plate from the NIMBUS™ Instrument deck, then seal the plate tightly with a new seal. If not proceeding to the PCR Product Pooling step, store the plate at –20°C.

10. Remove the gel QC plate from the NIMBUS™ Instrument deck, then seal the plate tightly with a new seal.

11. Remove the labware as shown on the **Cleanup** screen.

12. Click **Finish**, then click **Yes** to confirm.
    The method closes.

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, 2% into the slot (12-well E-Gel™ Agarose Gels can also be used if running a smaller number of samples).

5. Briefly vortex, then centrifuge the diluted samples before loading.

6. Load 20 µL of the diluted PCR product from the PCR QC gel plate onto the E-Gel™ *48* Agarose Gels, 2%.

7. Dilute the 25 bp DNA Ladder (1:6 dilution, 4 µL in 20 µL of Nuclease-Free Water), then load all 15 µL into each of the marker wells (as needed). Fill empty wells with 20 µL water.

8. Set the run time to 21 minutes.

9. Push the Power/Prg button again (the light changes from red to green).

   The system automatically shuts off when the run time is reached (the dye is near the end of the lane). The gel is then ready for imaging.

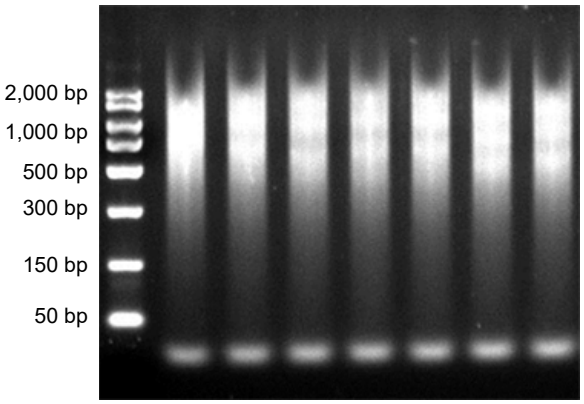


Figure 17 Gel image of the PCR product (from genomic DNA control) on E-Gel™ *48* Agarose Gels, 2%.

10. Do one of the following:

    - If the PCR has been verified, proceed to "Stage 4A—PCR product pooling" on page 100.
    - If not proceeding directly to the next stage, seal the plate of PCR product, then store the plate at –20°C.

Stage 4A—PCR product pooling

This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration. 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.

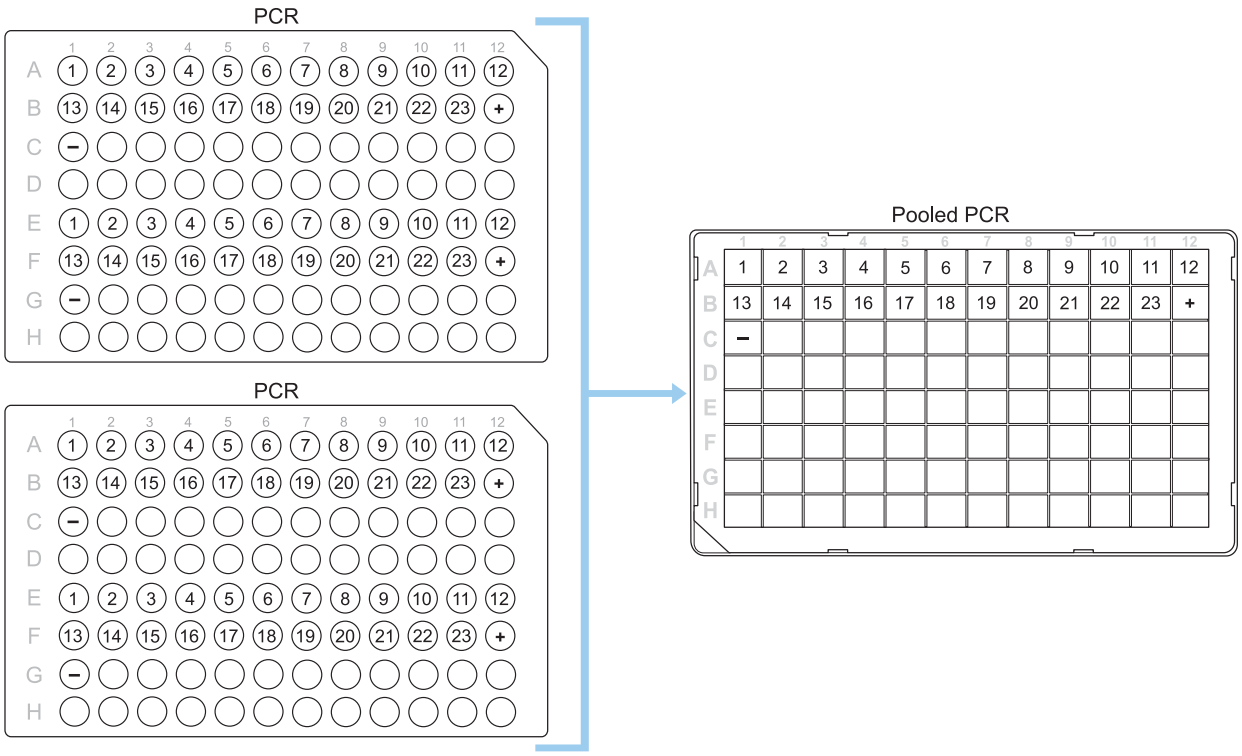


Figure 18 Pooling PCR products for 25 samples.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 4A—PCR product pooling

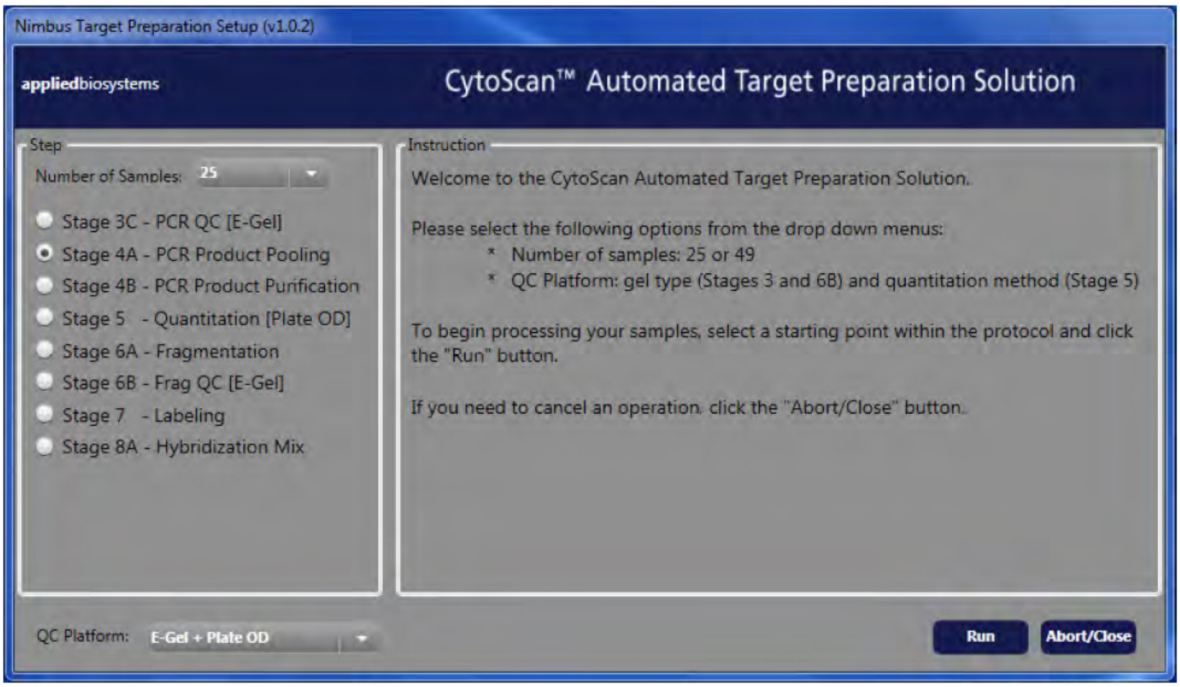
IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 19 Materials required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
1	2.2 mL Square Well Storage Plate
1 rack	Conductive 300-µL filter tips in frames
4	PCR tube racks

Pool the PCR products

1. On the **Welcome** screen, select the following options.
   - **Number of samples**—25
   - **Stage 4A–PCR Product Pooling**

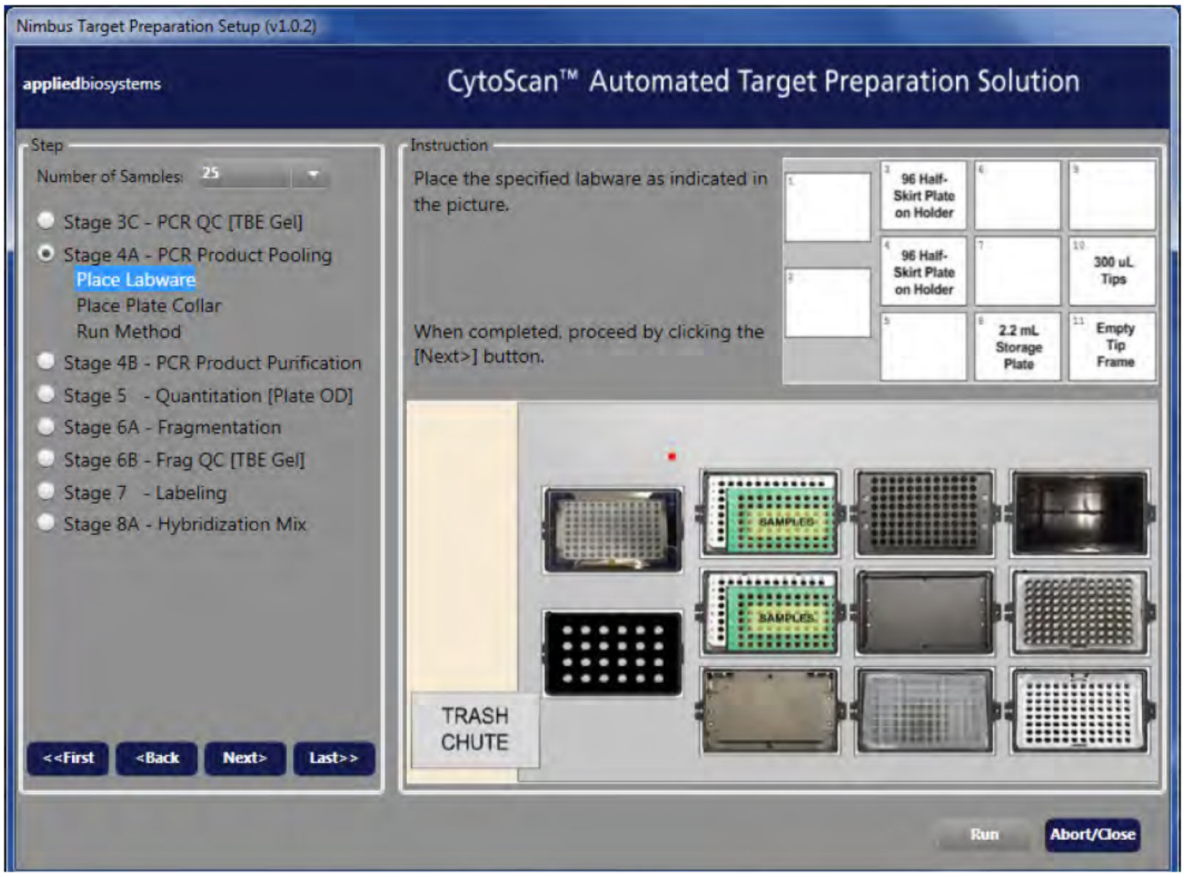


2. Click **Run** to start the method, then click **Yes** to confirm.

3. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions.

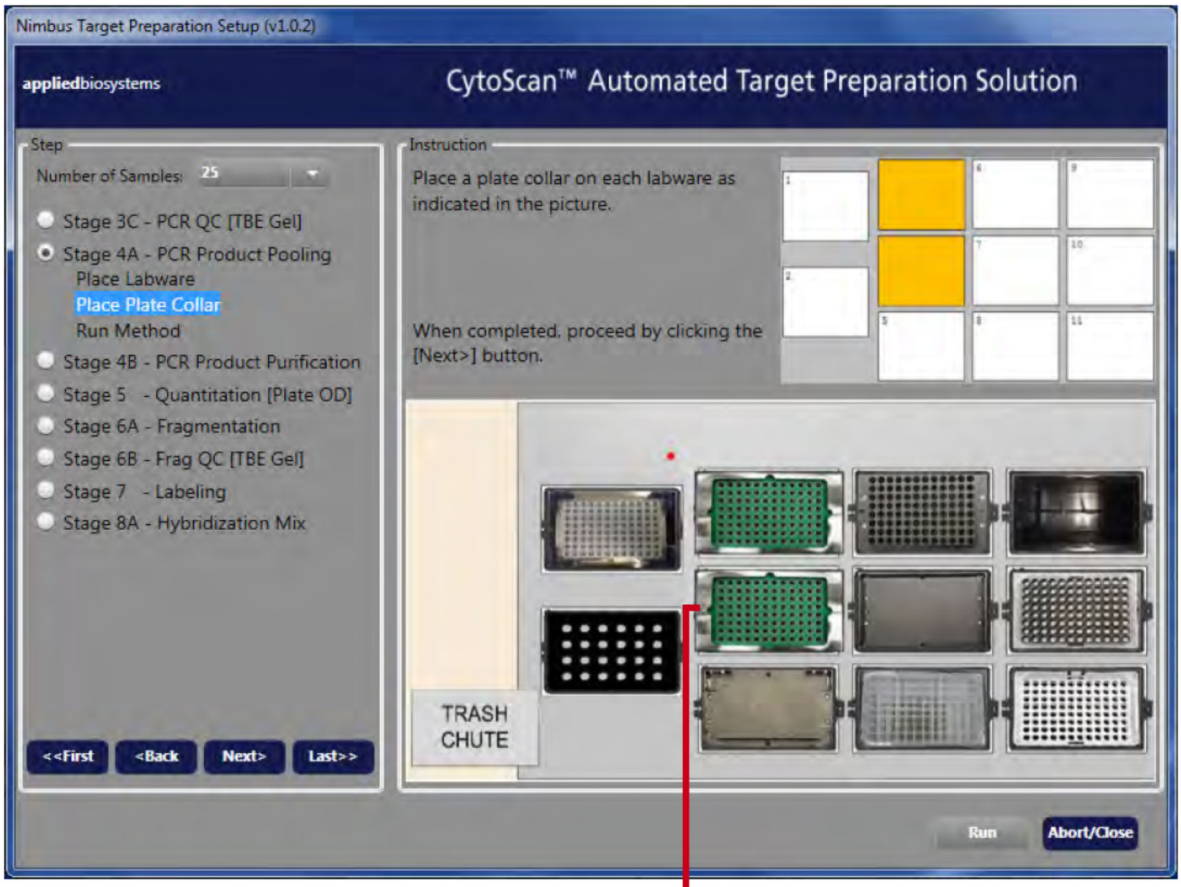
Note: Place an empty tip frame on deck position 11.

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.



4. Unseal each PCR plate, then place on the GeneMate™ PCR tube storage rack. Discard the seal. Place both plates on the NIMBUS™ Instrument deck as shown, then click **Next**.

5. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 3 and 4 require a plate collar.



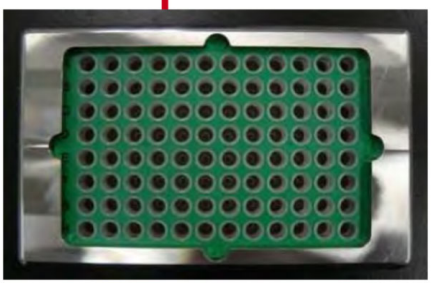
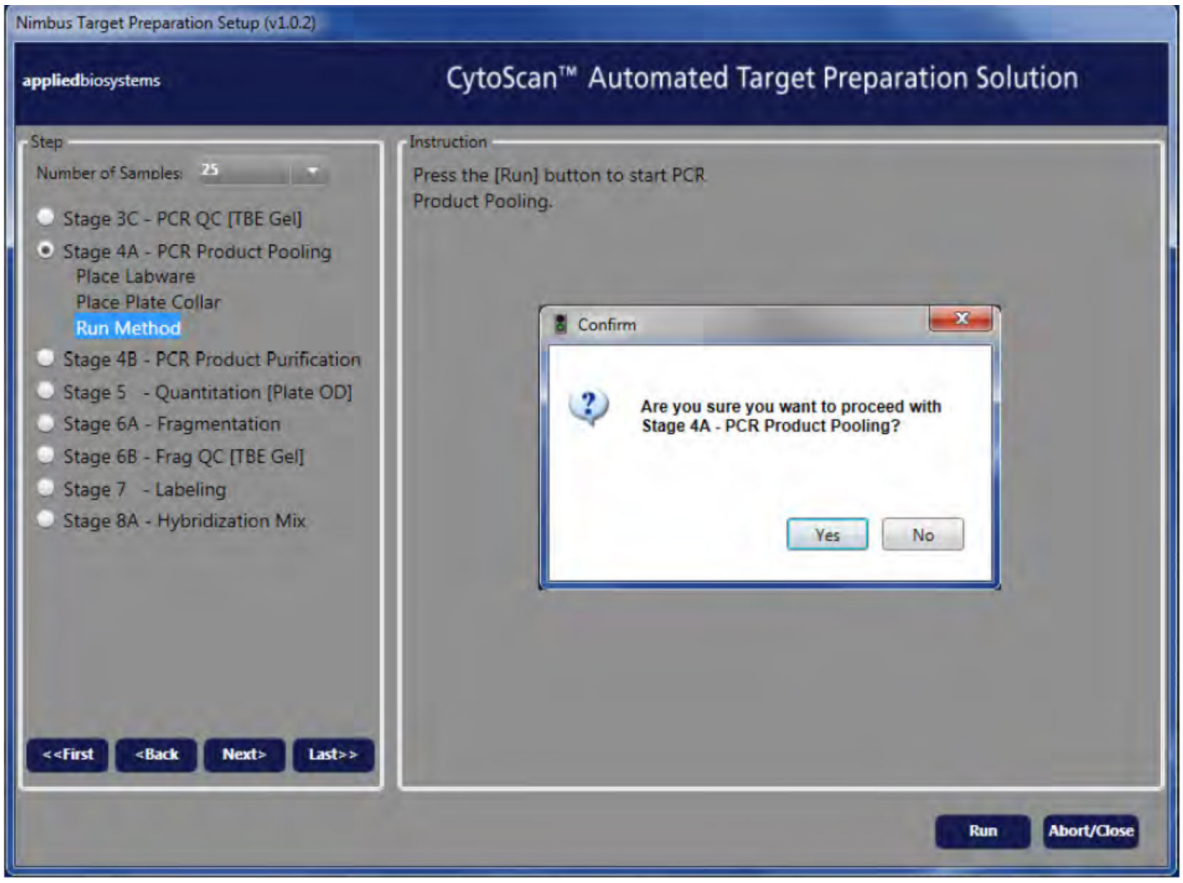
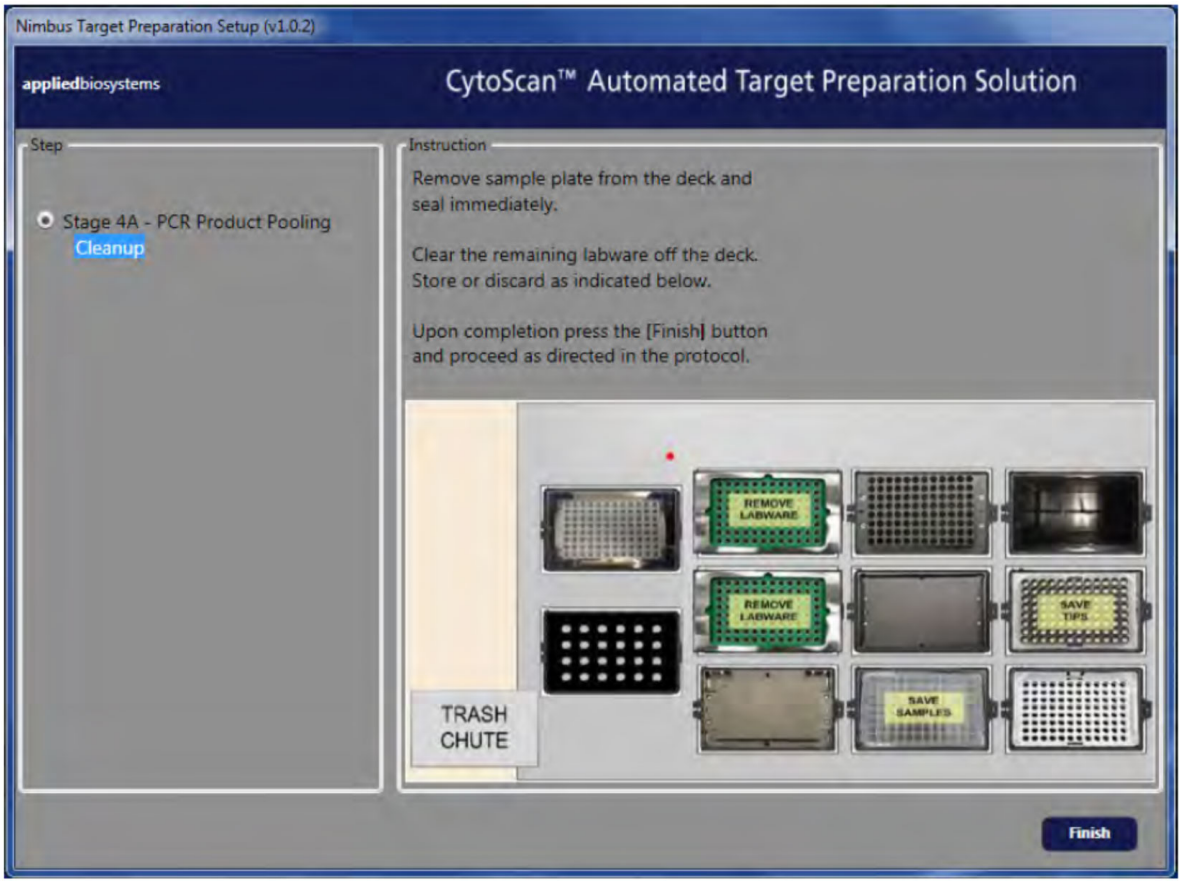


Plate collar on a sample plate

6. Click **Run** to start the method , then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



7. Remove labware from the NIMBUS™ Instrument deck as shown in the on-screen instructions.
8. Click **Finish**, then click **Yes** to confirm.
   The method closes.
9. Proceed to "Stage 4B—PCR product purification" on page 106.

Stage 4B—PCR product purification

This stage uses the NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 4B—PCR product purification

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 20 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Micro Plate 96 PCR Adapter and Plate Retainer (fixed on the Thermoshake device)
1	96S Super Magnet Plate
1	PCR Tube Rack
2 racks	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	Plates, 96-well, semi-skirted
1	Reservoir 4 ROW PYR PP 73 mL
2	2.2 mL Square Well Storage Plate
1	1.2 mL Square Well Round Bottom Storage Plate

Table 21 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	◯Purification Beads	Module 4	901807
1	◯Elution Buffer	Module 5	901738
1	◯Purification Wash Buffer		901372
As required	Absolute ethanol	—	—

Prepare the Purification Wash Buffer

1. Add 45 mL of absolute ethanol to the Purification Wash Buffer bottle.

   IMPORTANT! Ensure that the correct amount of ethanol has been added to the Purification Wash Buffer bottle.

2. Cap the bottle tightly, then invert 10 times.

3. Enter the preparation date on the bottle label and put a checkmark in the checkbox.

Prepare the Purification Beads

1. Thoroughly mix the Purification Beads stock by inverting the bottle 10 times.

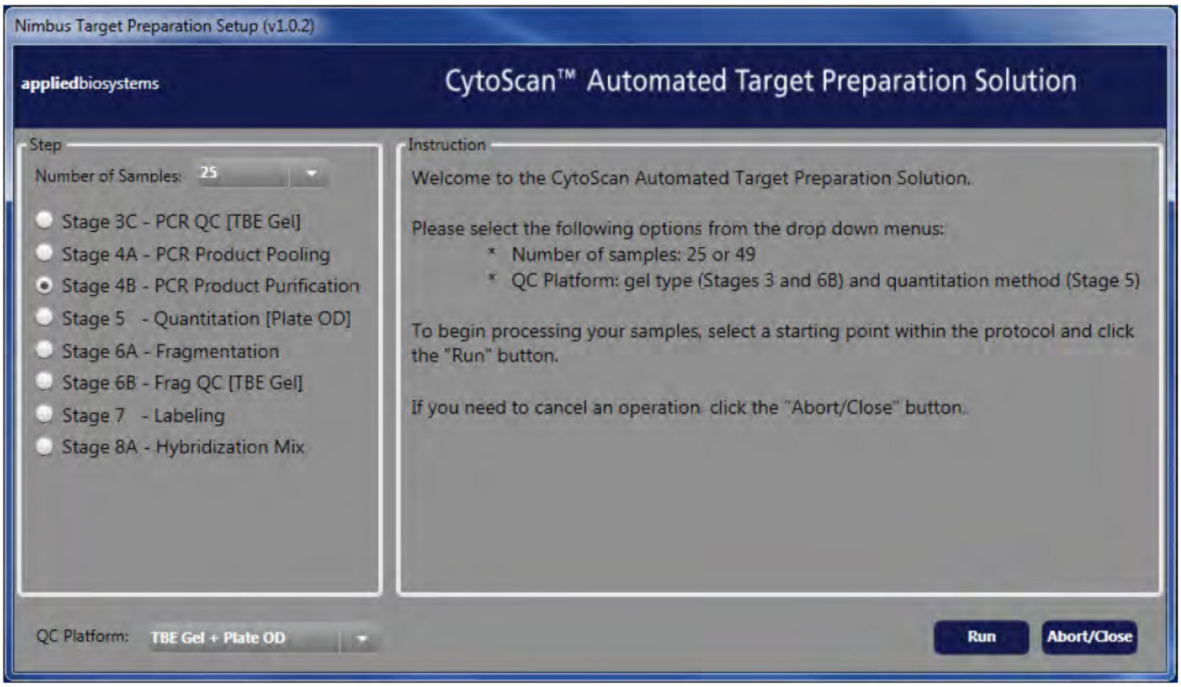
2. Examine the bottom of the bottle to ensure that the solution appears homogeneous.

Purify the pooled PCR products

Note: Controls can be placed in any well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

PCR wells (4):	100 µL from each well	= 400 µL
Total volume in each well of the deepwell plate		**= 400 µL/well – 3 µL aliquoted for PCR gel in PCR QC step**

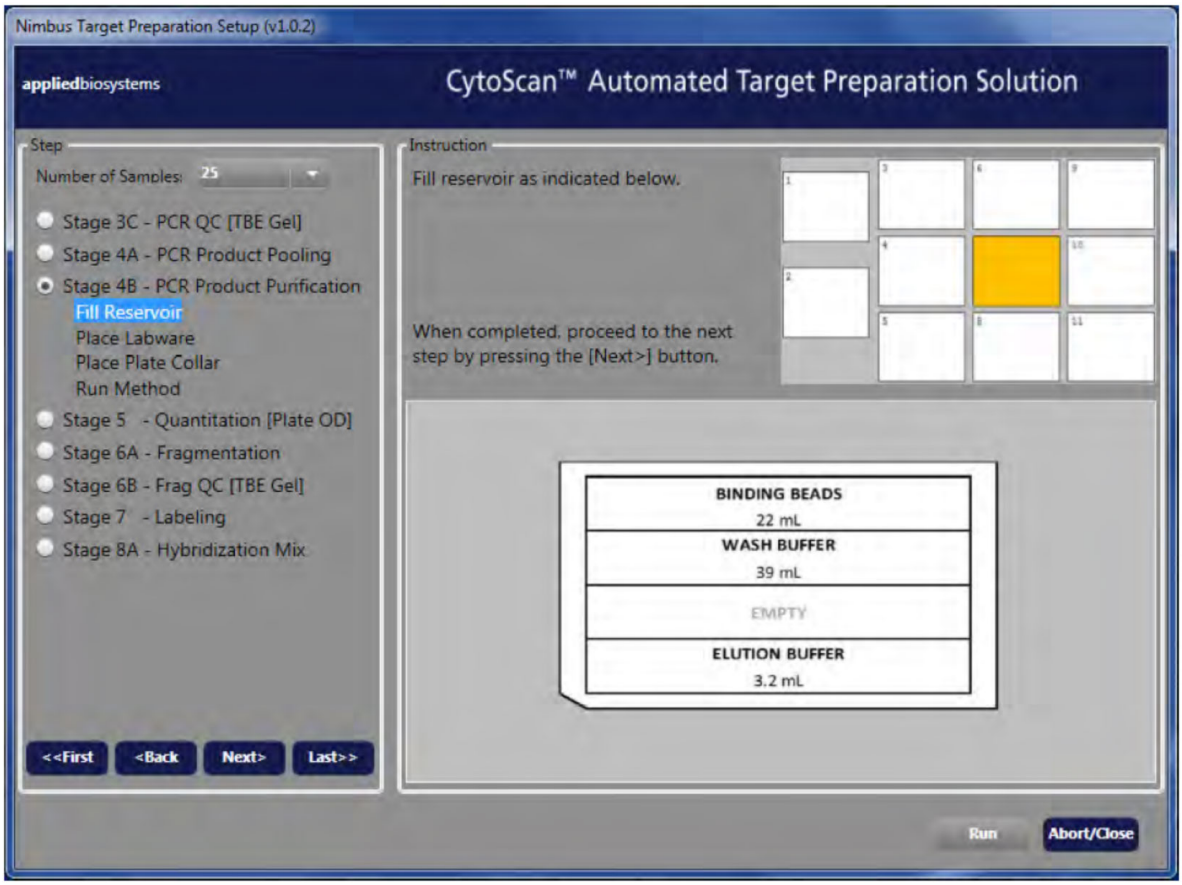
1. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 4B–PCR Product Purification**



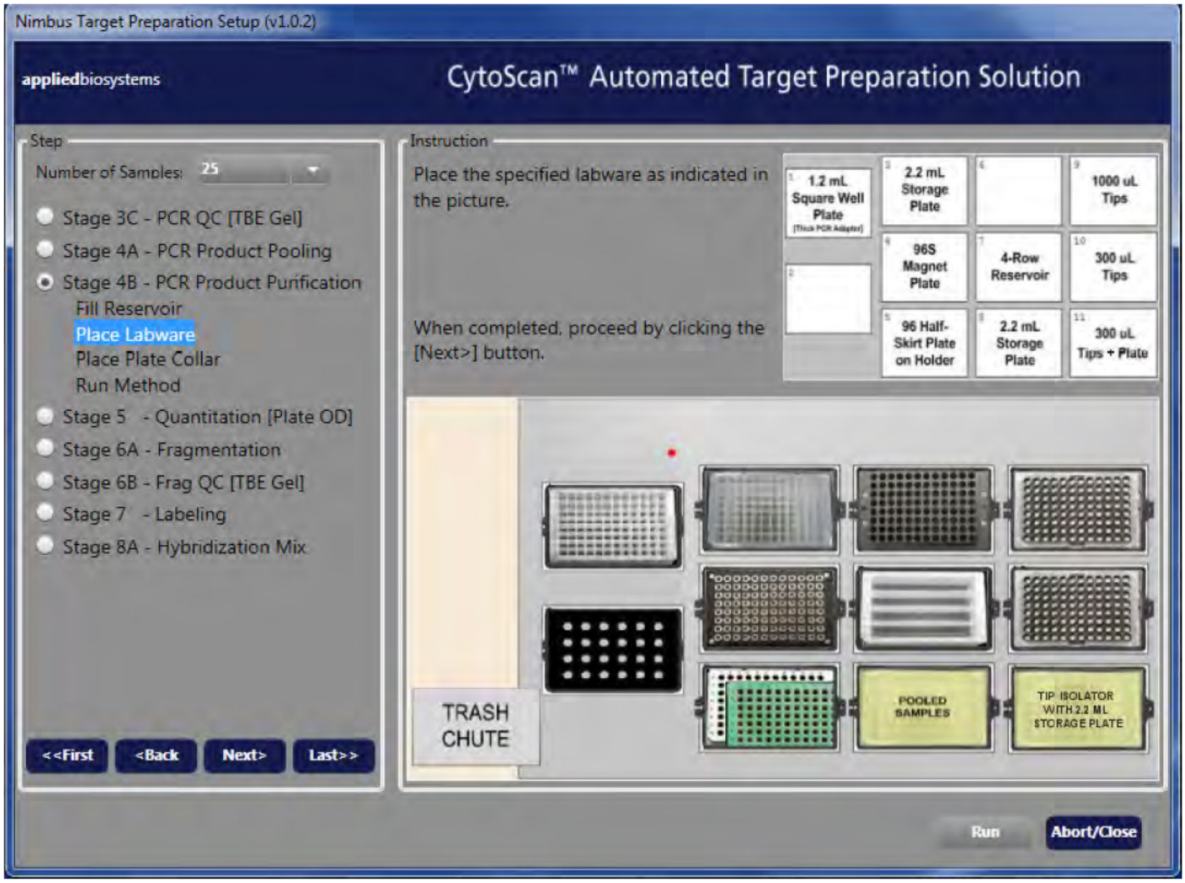
2. Click **Run** to start the method, then click **Yes** to confirm.

3. Transfer the entire content of each reagent bottle into its respective reservoir position. Ensure that the reservoir contains the minimum volumes shown in the on-screen instructions, then click **Next**.

Note: Each Elution Buffer bottle contains 3.3 mL of buffer. Transfer the entire contents of the Elution Buffer bottle. Ensure that the buffer is evenly spread across the reservoir.


Nimbus Target Preparation Setup (v1.0.2)
appliedbiosystems
CytoScan™ Automated Target Preparation Solution
Step
Number of Samples: 25
Stage 3C - PCR QC [TBE Gel]
Stage 4A - PCR Product Pooling
Stage 4B - PCR Product Purification
Fill Reservoir
Place Labware
Place Plate Collar
Run Method
Stage 5 - Quantitation [Plate OD]
Stage 6A - Fragmentation
Stage 6B - Frag QC [TBE Gel]
Stage 7 - Labeling
Stage 8A - Hybridization Mix
<<First
<Back
Next>
Last>>
Instruction
Fill reservoir as indicated below.
When completed, proceed to the next step by pressing the [Next>] button.
BINDING BEADS
22 mL
WASH BUFFER
39 mL
EMPTY
ELUTION BUFFER
3.2 mL
Run
Abort/Close

4. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions.



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.

5. Place a 2.2 mL Square Well Storage Plate under the tip isolator and place a rack of 300-µL tips on top as shown, then click **Next** when deck setup is completed.

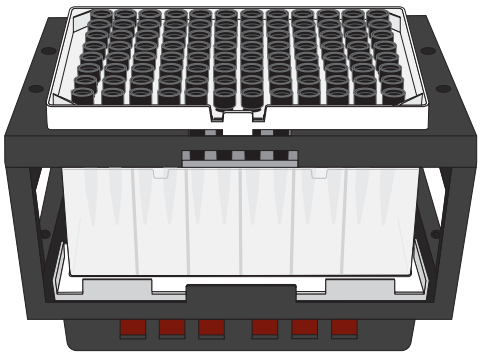
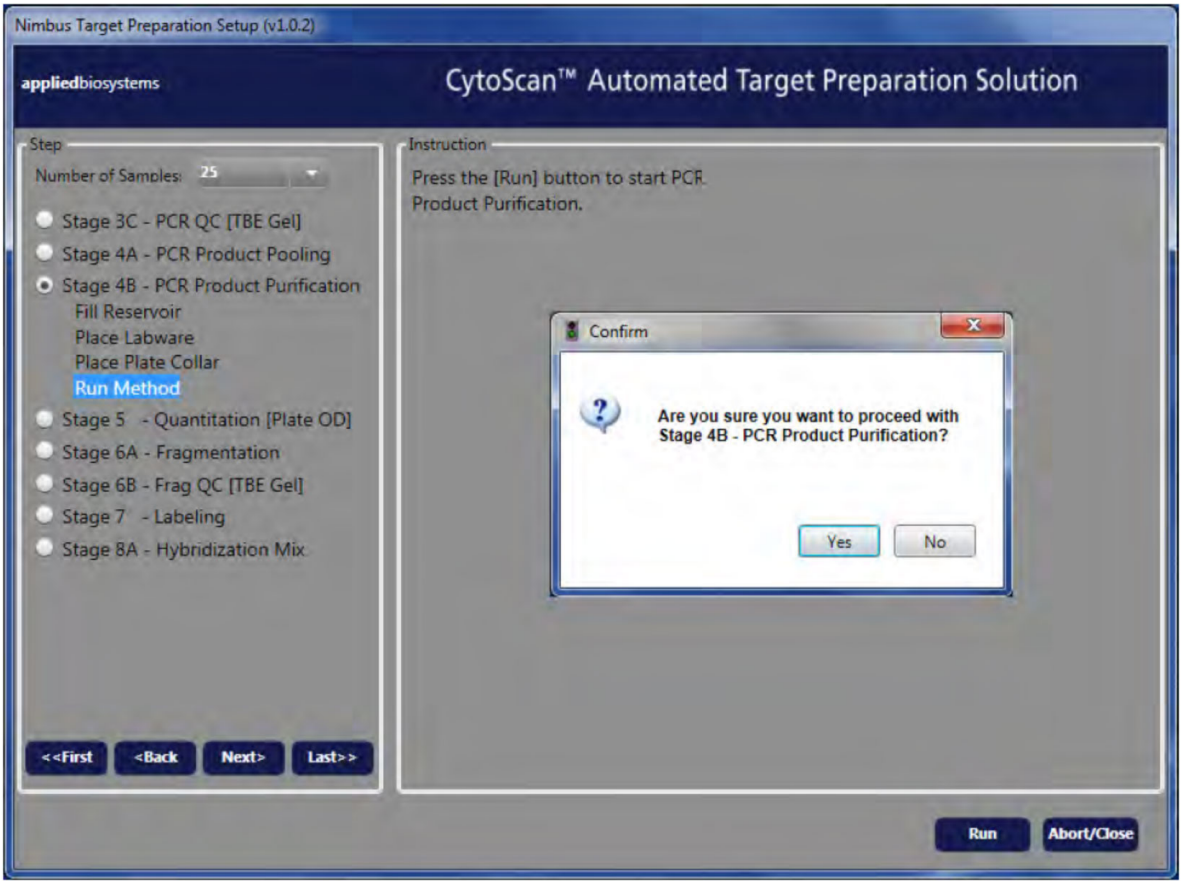


Figure 19 Tip Isolator with 2.2 mL Square Well Storage Plate underneath and 300-µL tips on top.

6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 5 and 7 require a plate collar.

Plate collar on a sample plate

7. Click **Run** to start the method, then click **Yes** to confirm.



Timers show the duration and elapsed time for each step during PCR purification.

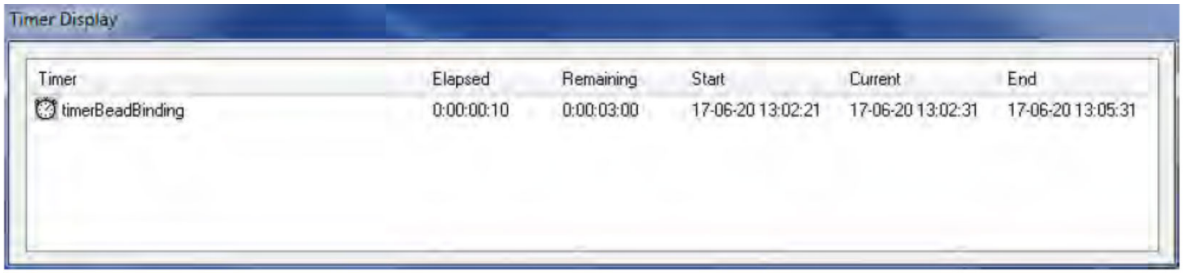


Figure 20 Example timer.

The NIMBUS™ Instrument transfers 47 µL of eluted sample to the appropriate well of a new 96-well plate.

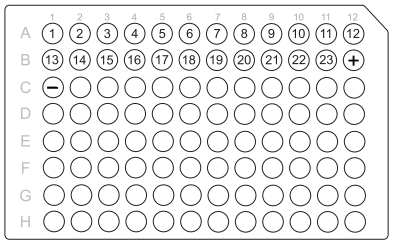


Figure 21 Purified PCR product sample format.

8. After the purification program is complete, visually examine the purified sample plate to determine whether the wells contain the same volume.

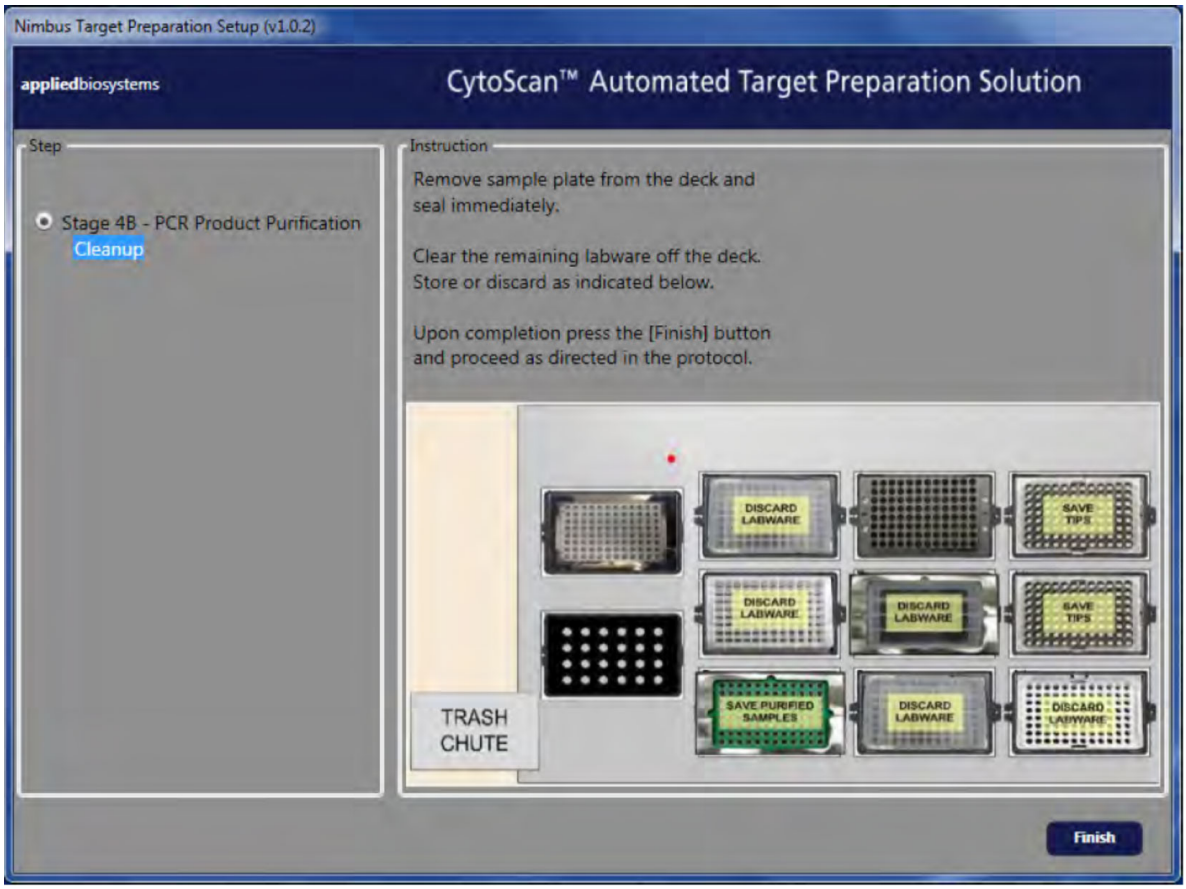
   If a well has lower volume:

   a. Measure the volume using a pipette.

   b. If the volume is less than 47 µL: With the Cleanup Plate still on the magnet, check if there is residual volume in the corresponding well. If so, carefully retrieve the eluent using a manual pipette.

9. After purification is complete, tightly seal the plate, then vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2,000 rpm for 1 minute.

10. Remove labware from the NIMBUS™ Instrument deck as shown on **Cleanup** screen.



11. Click **Finish**, then click **Yes** to confirm.
    The method closes.

12. Proceed to “Stage 5—Quantitation” on page 115.

Stage 5—Quantitation

This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific **Cleanup** screen. Perform quantitation using one of these methods:

- "Quantitation procedure using the SpectraMax® Plus 384 Microplate Spectrophotometer" on page 116.
- "Quantitation procedure using the NanoDrop™ Spectrophotometer" on page 122.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 5—Quantitation

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 22 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Marker, fine point, permanent
1	Mini microcentrifuge
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
2	96-well Full Skirt PCR Plate, clear (if using the NanoDrop™ Spectrophotometer)
1	Corning™ UV-Transparent Microplate (if using the SpectraMax® Plus 384 Microplate Spectrophotometer)
1	Spectrophotometer
1	Reservoir 4 ROW PYR PP 73 mL

Table 23 Reagent required.

Quantity	Reagent	CytoScan™ Reagent Kit	
		Module	Part No.
As required	Nuclease-Free Water	Module 4	901781

Important information about this stage

IMPORTANT! To ensure the best results, carefully read the following information before you start this stage of the assay.

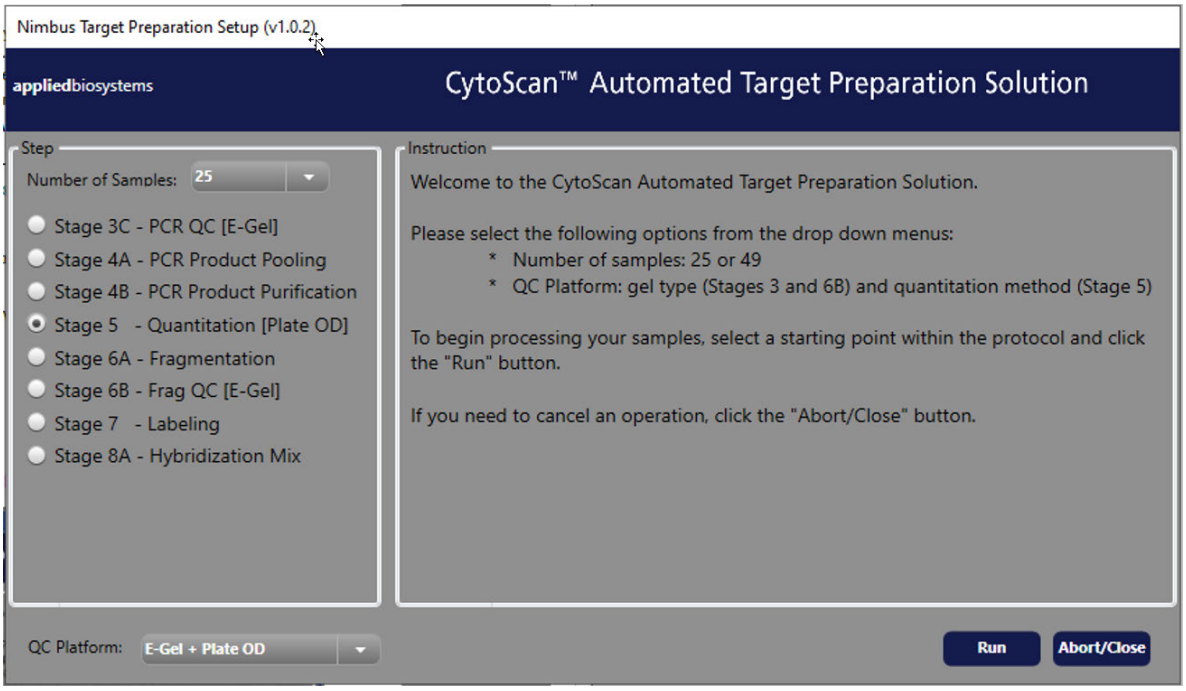
- The accuracy of the OD measurement is critical. Carefully follow this procedure and be sure the OD measurement is within the linear range of the instrument.
- Calibrate the spectrophotometer regularly to ensure correct readings.
- This assay has been optimized using a UV spectrophotometer for quantitation.
- The controls can be placed in any microplate well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

Quantitation procedure using the SpectraMax® Plus 384 Microplate Spectrophotometer

1. Power on the SpectraMax® Plus 384 Microplate Spectrophotometer, then allow it to warm for at least 10 minutes before use.

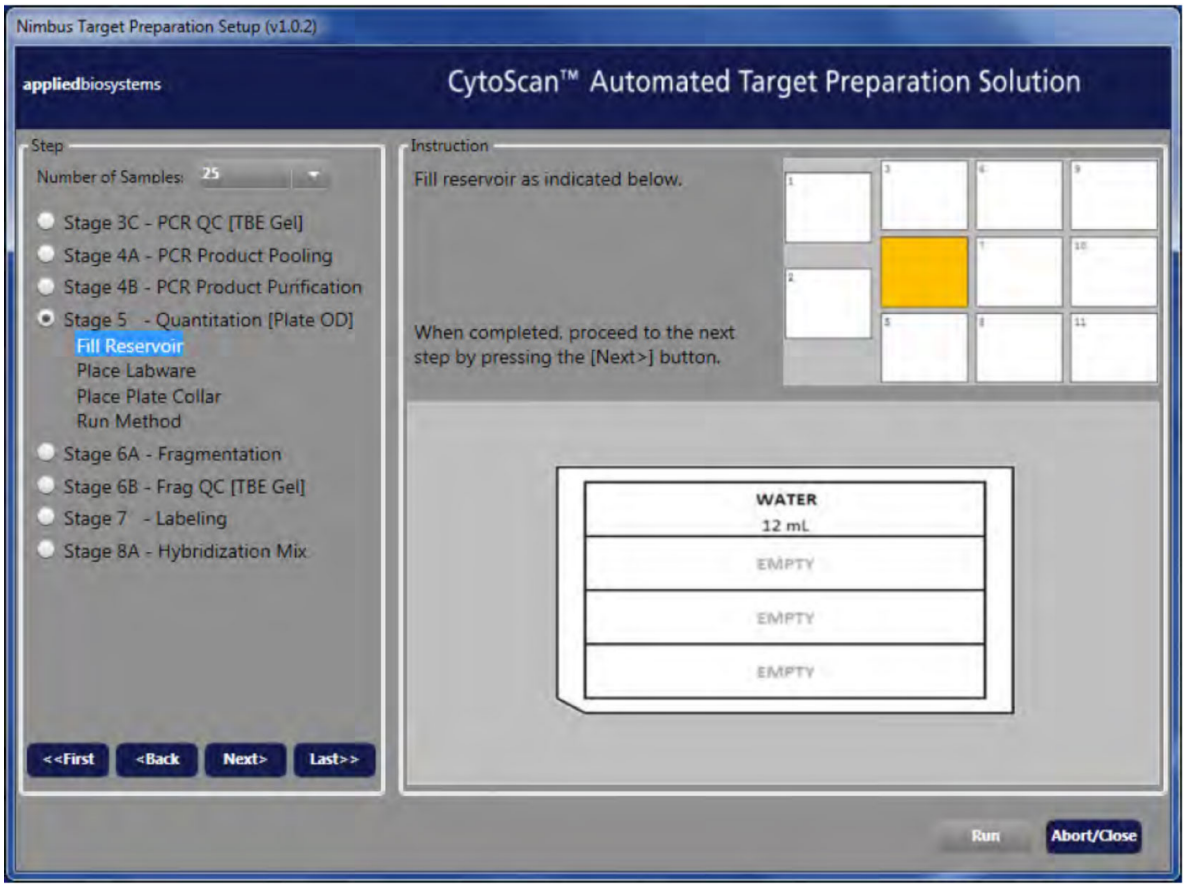
2. Ensure that the purified sample plate is tightly sealed. Vortex and centrifuge the plate at 2,000 rpm for 1 minute, then put in a plate holder.

3. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 5–Quantitation**
   - **QC Platform**—For example, “E-Gel + Plate OD”.



4. Click **Run** to start the method, then click **Yes** to confirm.

5. Fill the reservoir as shown in the on-screen instructions, then click **Next**.

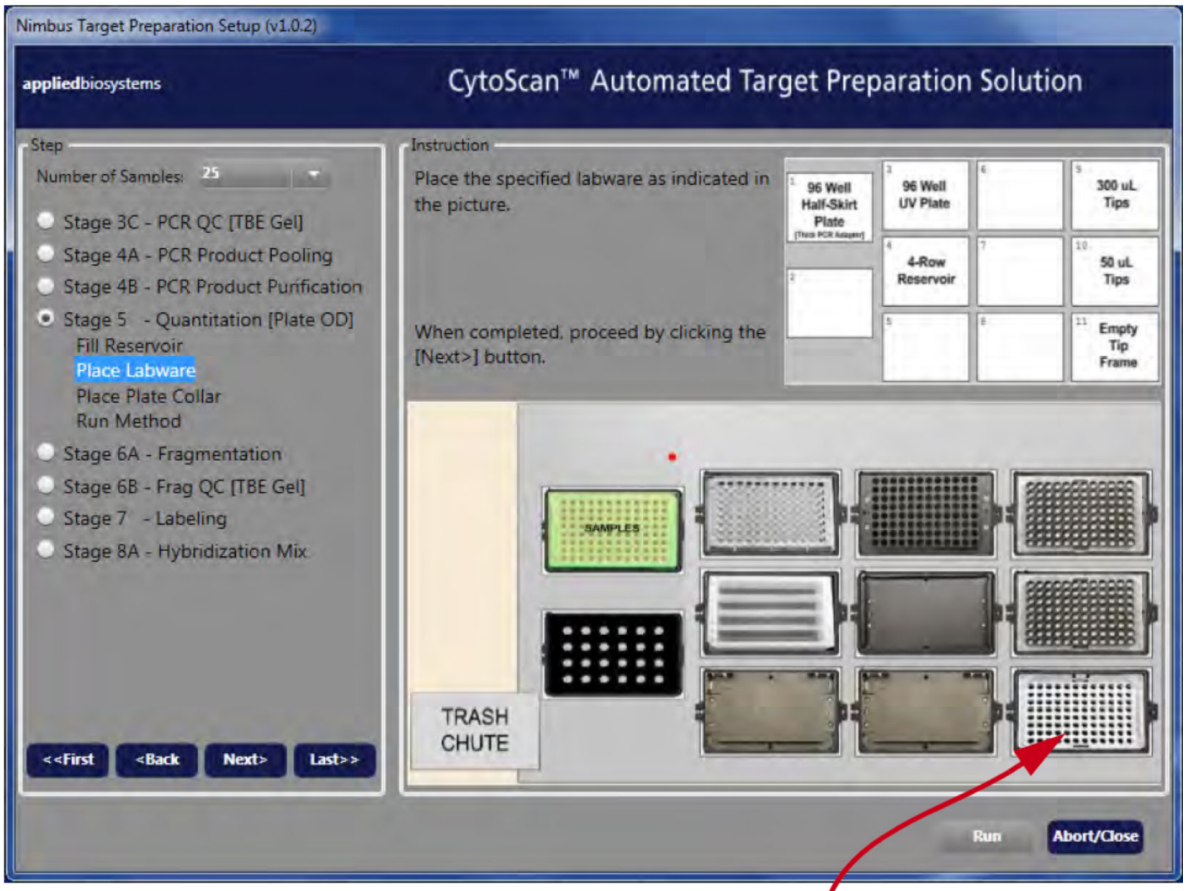


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

Note: Place an empty tip frame on deck position 11.

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.



Empty tip frame for position 11

7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1 and 4 require a plate collar.

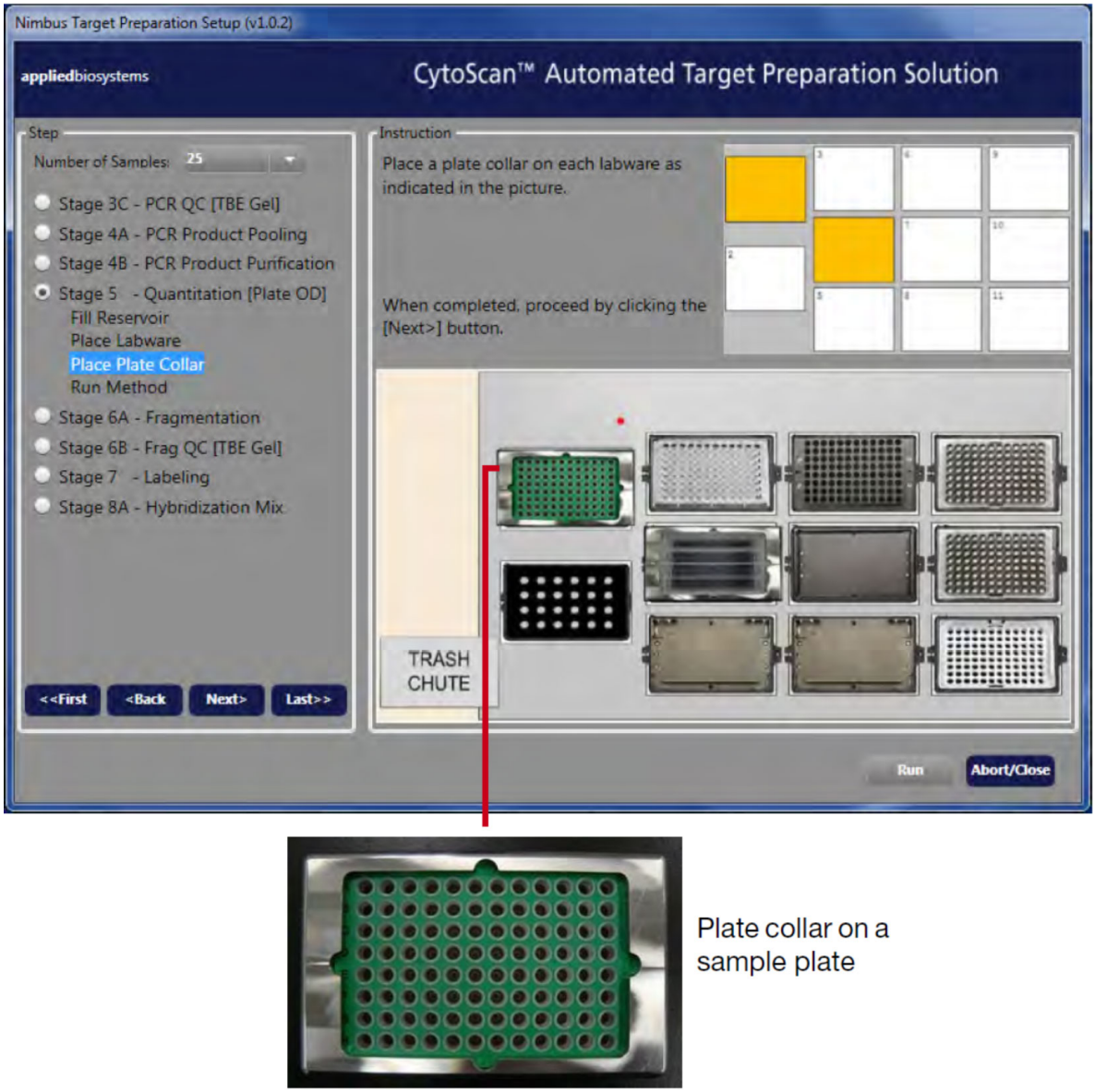
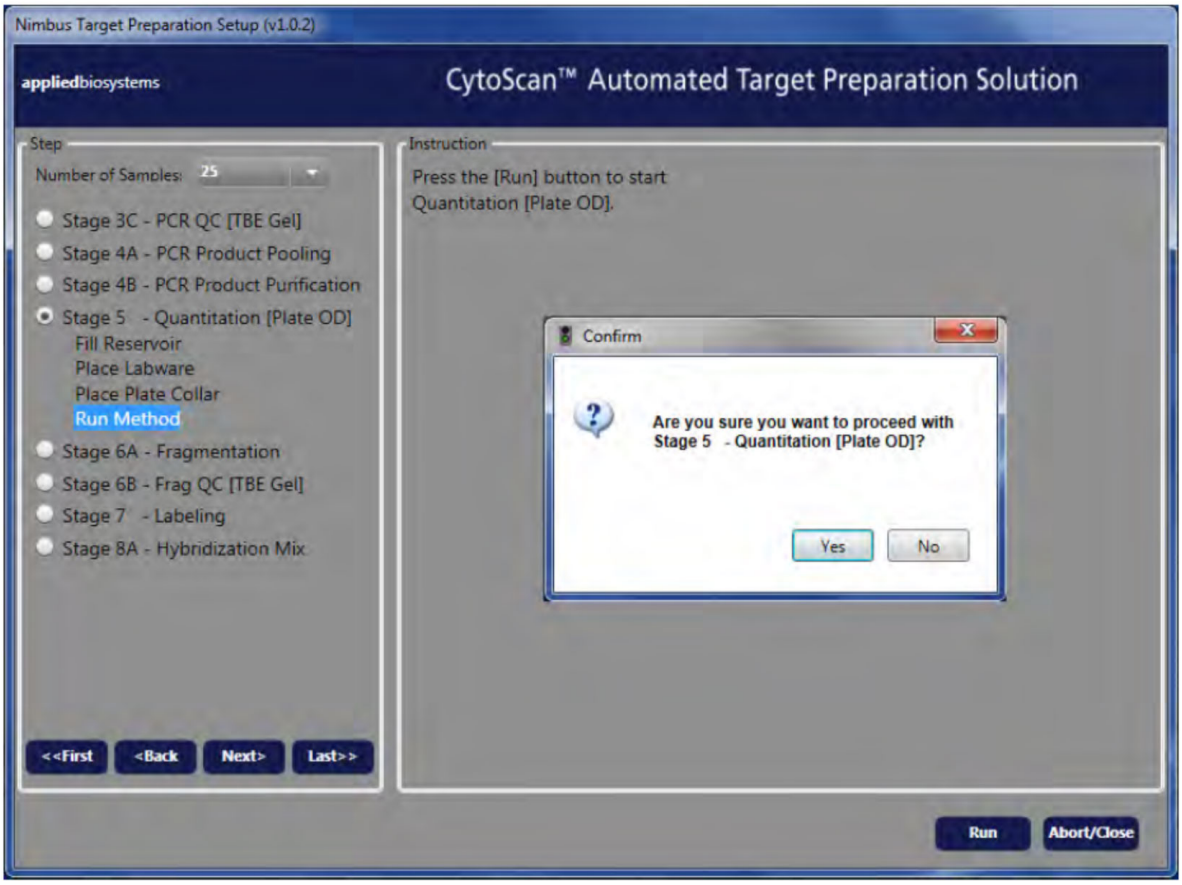
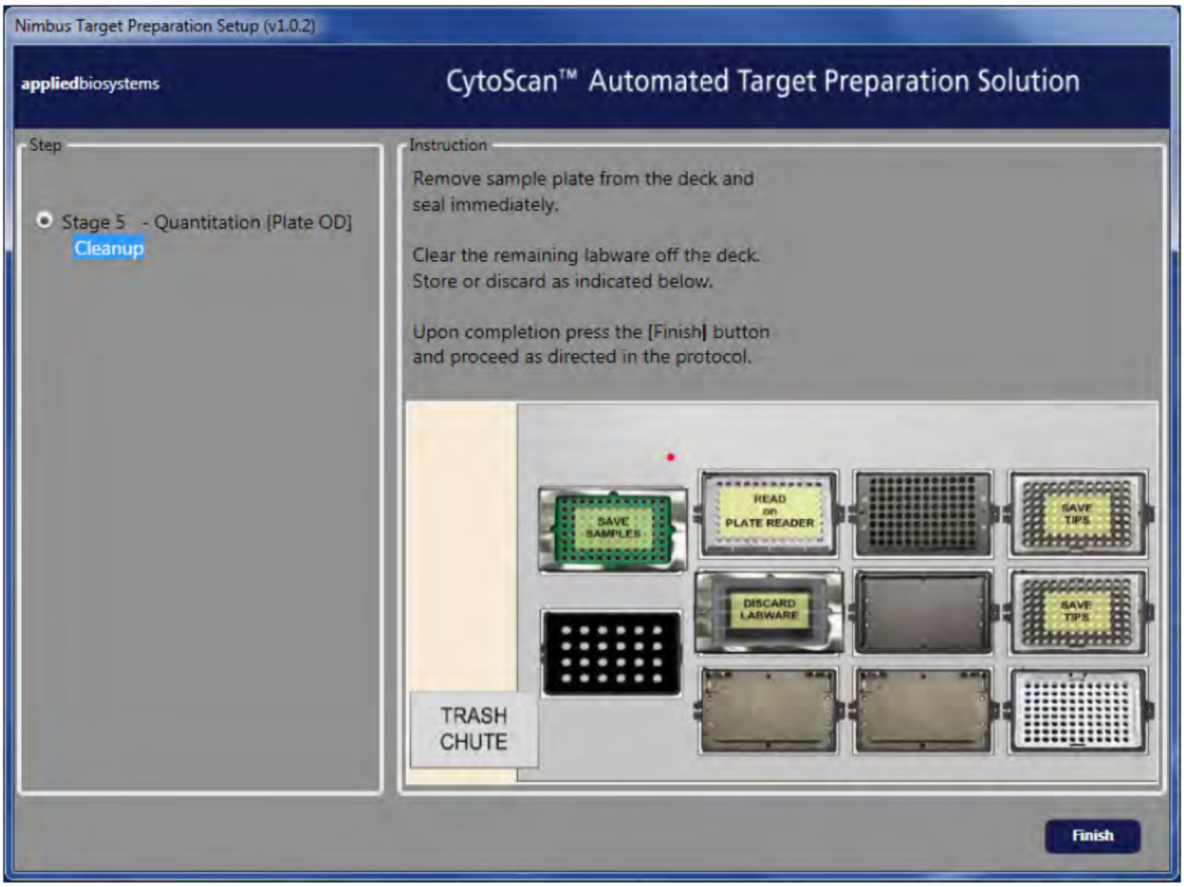


Plate collar on a sample plate

8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Tightly seal the plate with purified samples with a new seal, then store at –20°C. The plate can be stored at –20°C for up to 10 days.

10. Seal the UV plate, and using a laboratory tissue on the adapter surface, vortex, then centrifuge at 2,000 rpm for 1 minute.

11. Remove labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

12. Click **Finish**, then click **Yes** to confirm.

    The method closes.

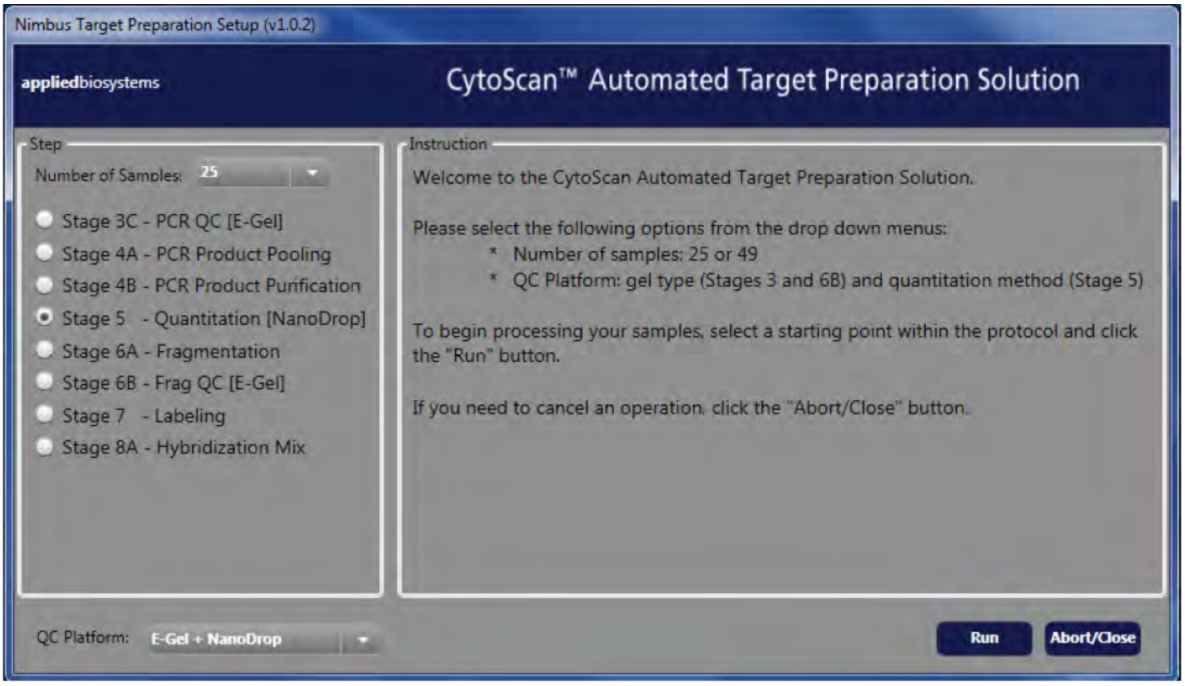
Quantitate the diluted PCR product

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.
   OD_{280} and OD_{320} are used as controls.
2. Determine the OD_{260} 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.

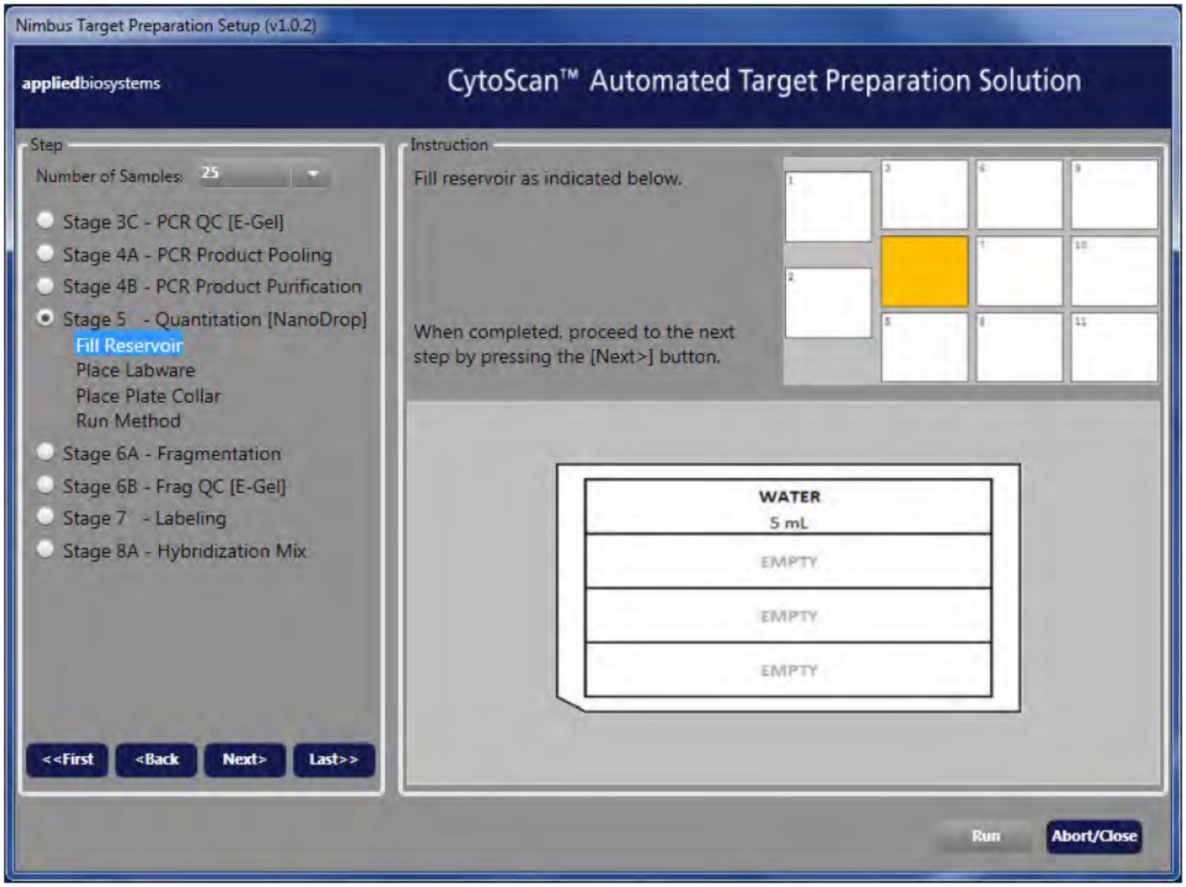
Quantitation procedure using the NanoDrop™ Spectrophotometer

1. Power on the NanoDrop™ Spectrophotometer, then allow it to warm for at least 10 minutes before use.
2. Ensure that the plate is tightly sealed. Vortex and centrifuge the purified samples at 2,000 rpm for 1 minute, then put in a plate holder.
3. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 5–Quantitation**
   - **QC Platform**—For example, “E-Gel + Nanodrop”.



4. Click **Run** to start the method, then click **Yes** to confirm.

5. Fill the reservoir as shown in the on-screen instructions, then click **Next**.

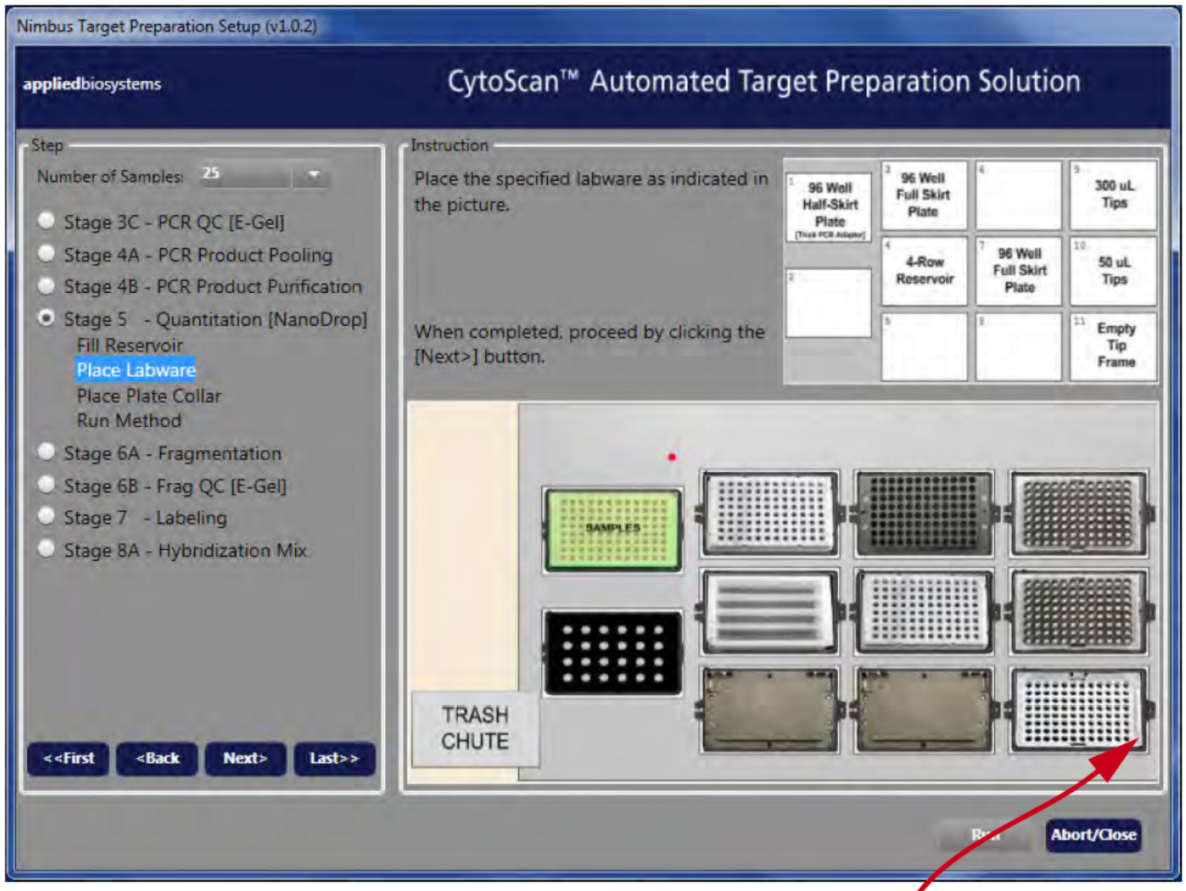


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

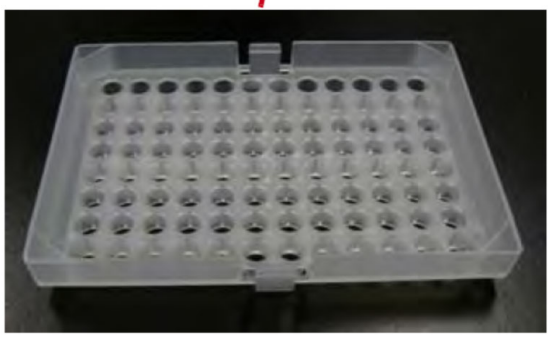
IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

Note: Place an empty tip frame on deck position 11.

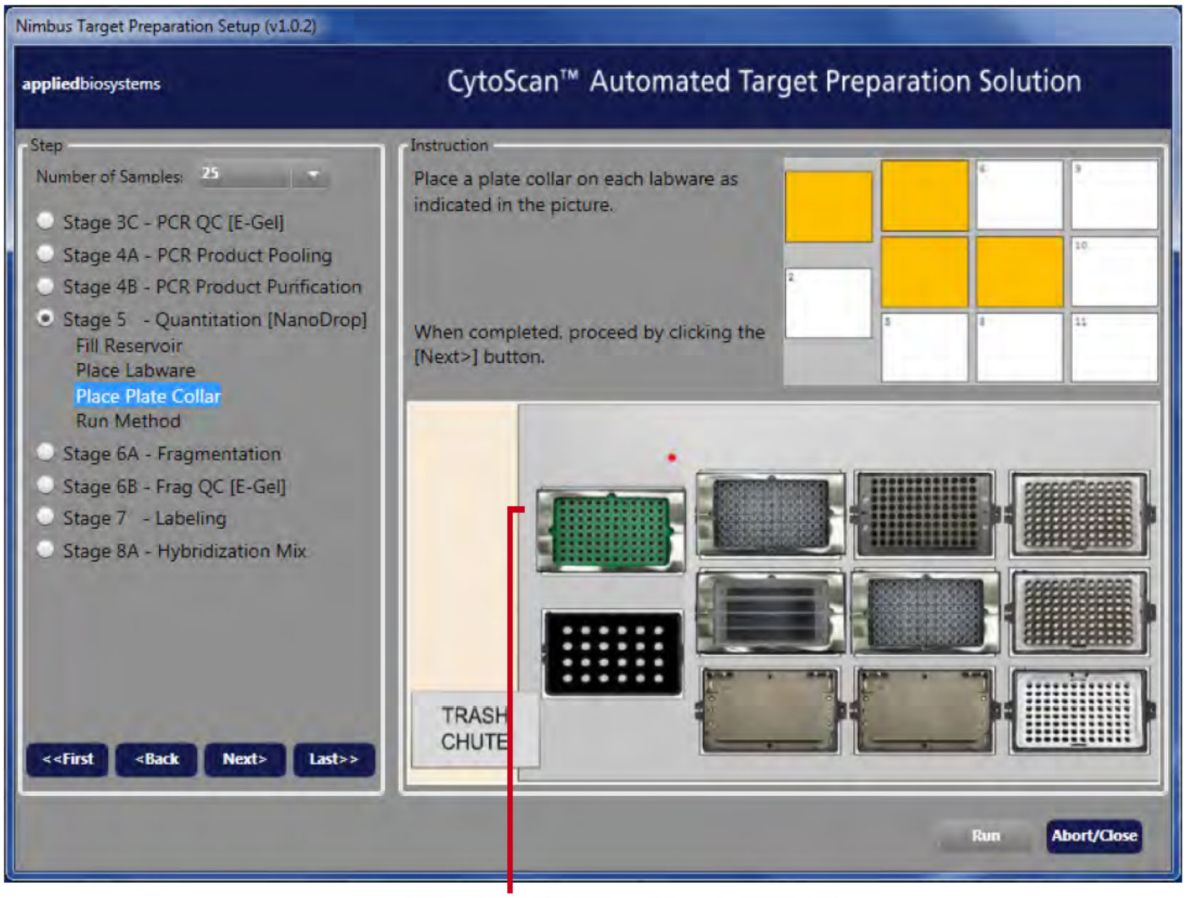
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 3, 4, and 7 require a plate collar.



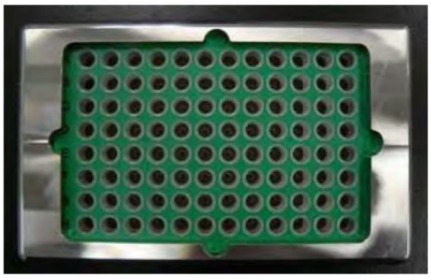
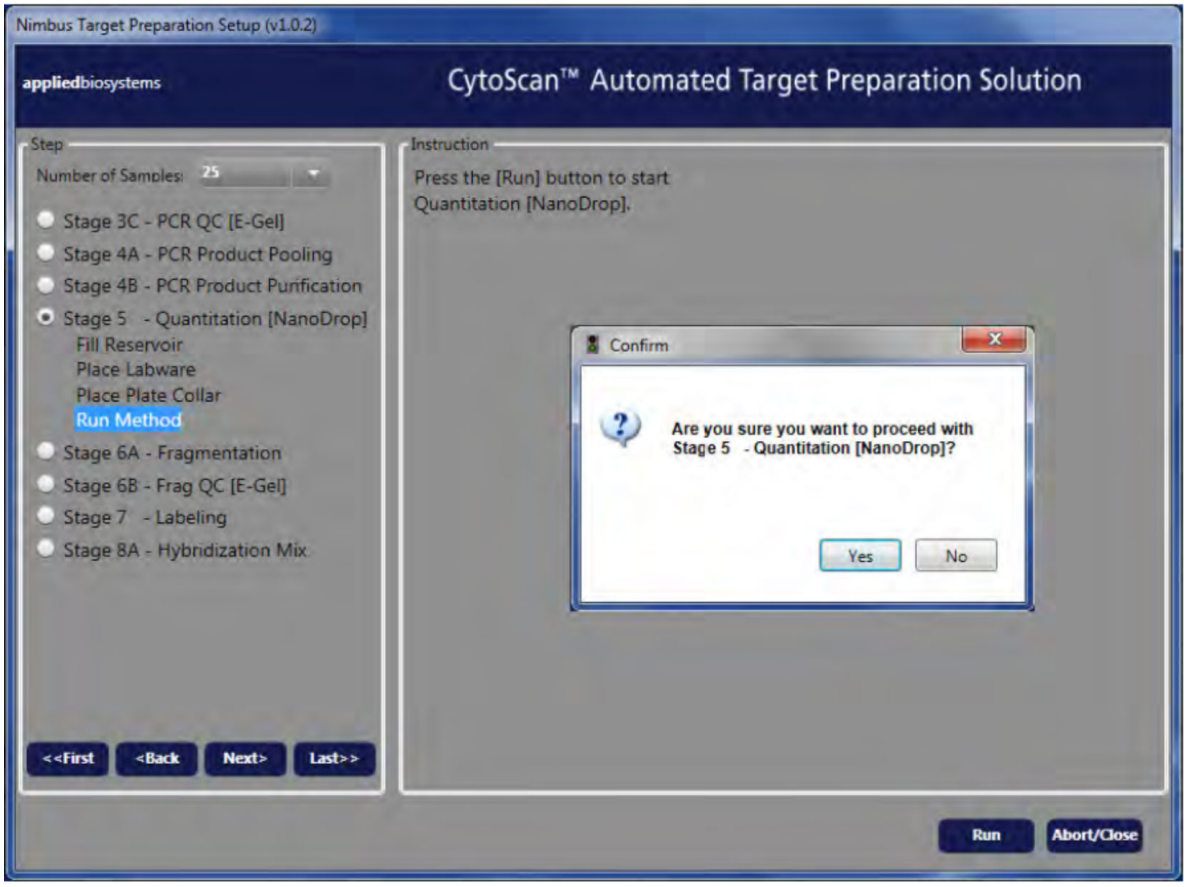
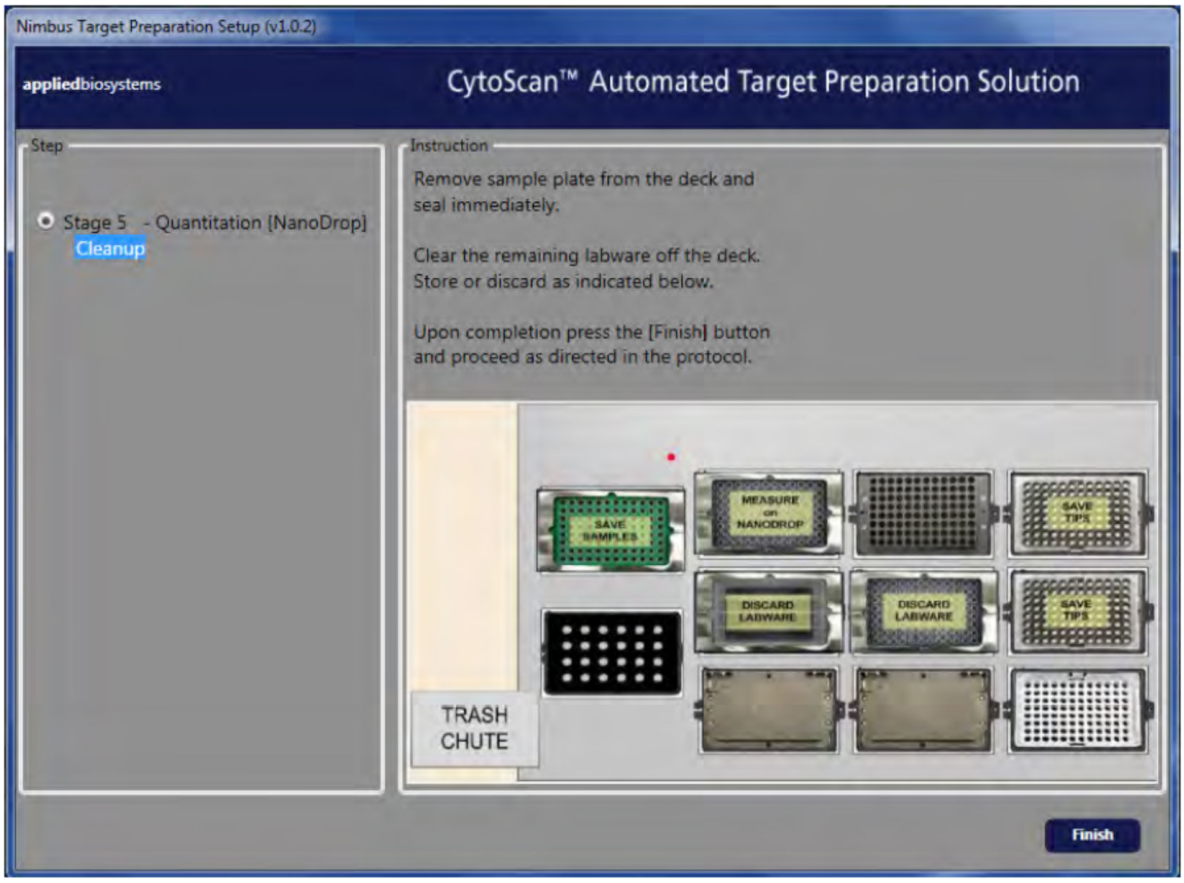


Plate collar on a sample plate

8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Tightly seal the plate with purified samples with a new seal, then store at –20°C. The plate can be stored at –20°C for up to 10 days.

10. Seal the UV plate, and using a laboratory tissue on the adapter surface, vortex, then centrifuge at 2,000 rpm for 1 minute.

11. Remove labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

12. Click **Finish**, then click **Yes** to confirm.
    The method closes.

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.
      OD_{280} and OD_{320} 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).

Acceptable DNA yield

The average purification yield samples should be ≥3.0 µg/µL. If the average yield is <3.0 µg/µL, see the troubleshooting section. We do not recommend further processing of samples with yields <2.5 µg/µL.

The following OD ranges are based on the use of a conventional UV spectrophotometer plate reader and assume a path length of 1 cm.

- The OD_{260}/OD_{280} ratio should be >1.8.
- The OD_{320} measurement should be very close to zero (≤0.1).

If your OD readings are not within the acceptable range, see to Appendix B, “Troubleshooting”.

What to do next

Do one of the following:

- Proceed immediately to “Stage 6A—Fragmentation” on page 130.
- If not proceeding immediately to the next step, seal the plate of purified samples, and store at –20°C. The plate can be stored at –20°C for up to 10 days.

Stage 6A—Fragmentation

This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration. The NIMBUS™ Instrument:

- Prepares the Fragmentation Master Mix.
- Aliquots the Fragmentation Master Mix into a distribution plate.
- Adds the Fragmentation Master Mix to the samples and performs mixing.

Note:

- Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.
- To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..
- Controls can be placed in any well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

Materials required for Stage 6A—Fragmentation

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 24 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Refrigerated plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	96-well Full Skirt PCR Plate, clear (Axygen™)

Table 24 Equipment and consumables required. *(continued)*

Quantity	Item
1	Thermal cycler
2	Tube, screw cap, no ribs, 2 mL
1	Vortexer
2	PCR Tube Racks

Table 25 Reagents required.

Quantity	Reagent	CytoScan™ Reagent Kit	
		Module	Part No.
1	Chilled Nuclease-Free Water	Module 2	902976
1	Fragmentation Reagent	Module 3	902428
1	Fragmentation Buffer		903001

Important information about this stage

The degree of fragmentation is critical. Perform this stage carefully to ensure uniform, reproducible fragmentation. To ensure the best results, carefully read the following information before you start this stage of the assay.

IMPORTANT! All additions, dilutions, and mixing must be performed at 4°C. Ensure that all reagents reach equilibrium before use.

About the Fragmentation Reagent

This Fragmentation Reagent is *extremely temperature sensitive* and rapidly loses activity at higher temperatures. To avoid loss of activity:

- Handle the tube only by the cap. Do not touch the sides of the tube because the heat from your fingers raises the reagent temperature.
- Keep at –20°C until ready to use. Transport and hold in a –20°C cooler. Return to the cooler immediately after use.
- Centrifuge so that the contents of the tube are uniform.
- Perform all steps rapidly and without interruption.

Prepare the reagents, equipment, and consumables

1. Power on the thermal cycler to preheat the lid. **Leave the block at room temperature**.
2. Power on the plate centrifuge, then turn down to 4°C at least 15—20 minutes before proceeding into the fragmentation step. Close the centrifuge lid to facilitate effective cooling.

   IMPORTANT! Always centrifuge the fragmentation plate in a centrifuge that has been cooled to 4°C.
3. Ensure that the plate centrifuge is at 4°C.
4. Leave 2 plate racks in the centrifuge buckets to cool.
   The racks are to be used for centrifuging the plate.

Thaw and prepare the reagents

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

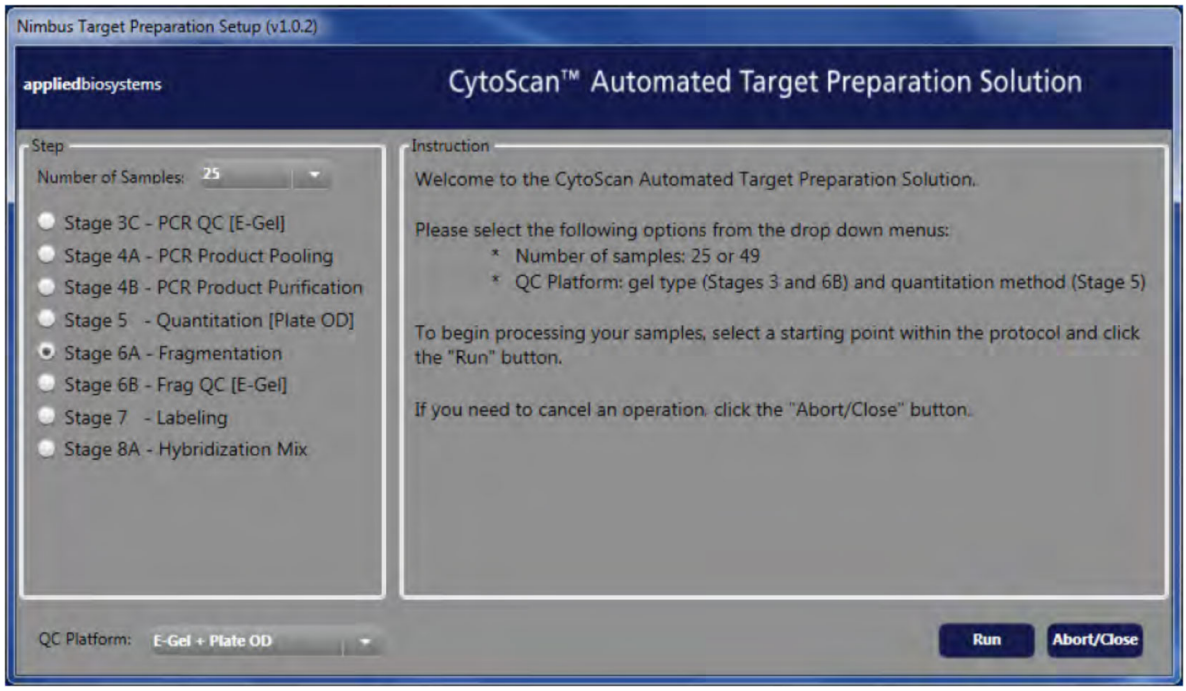
1. If the samples are frozen, remove the plate of purified, quantitated samples from the –20°C freezer, then thaw at room temperature. When thawed completely, ensure that the plate is sealed tightly, vortex, then centrifuge the plate.
2. Place the plate on the cooling block on ice, then chill for 10 minutes before use.
3. Thaw the Fragmentation Buffer (10X) at room temperature. When thawed, immediately place on ice.
4. Prepare the Fragmentation Buffer.
   a. Vortex 3 times, 1 second each time.
   b. Briefly centrifuge for 3 seconds.
   c. Place on ice.
5. Remove the Fragmentation Reagent from the freezer, then immediately place it in the cooler, chilled to –20°C.
   a. Vortex the Fragmentation Reagent at high speed 1 time for 1 second.
   b. Immediately briefly centrifuge for 3 seconds to bring down any reagent clinging to the top of the tube.
   c. Immediately place in the –20°C cooler.

Confirm these steps before the Fragmentation step run on the NIMBUS™ Instrument

- Cool the plate centrifuge to 4°C at least 15–20 minutes before proceeding with the fragmentation step. Place a plate holder rack in the centrifuge bucket so that the rack is already chilled when the fragmentation plate is ready to centrifuge.
- Pre-chill the reagents, empty the tube for master mix, and empty the strip tube before starting the fragmentation step.
- Leave the fragmentation reagent at –20°C until ready to load the NIMBUS™ Instrument deck.
- Always carry the sample plate to the centrifuge or the thermal cycler on the cooling block on ice.

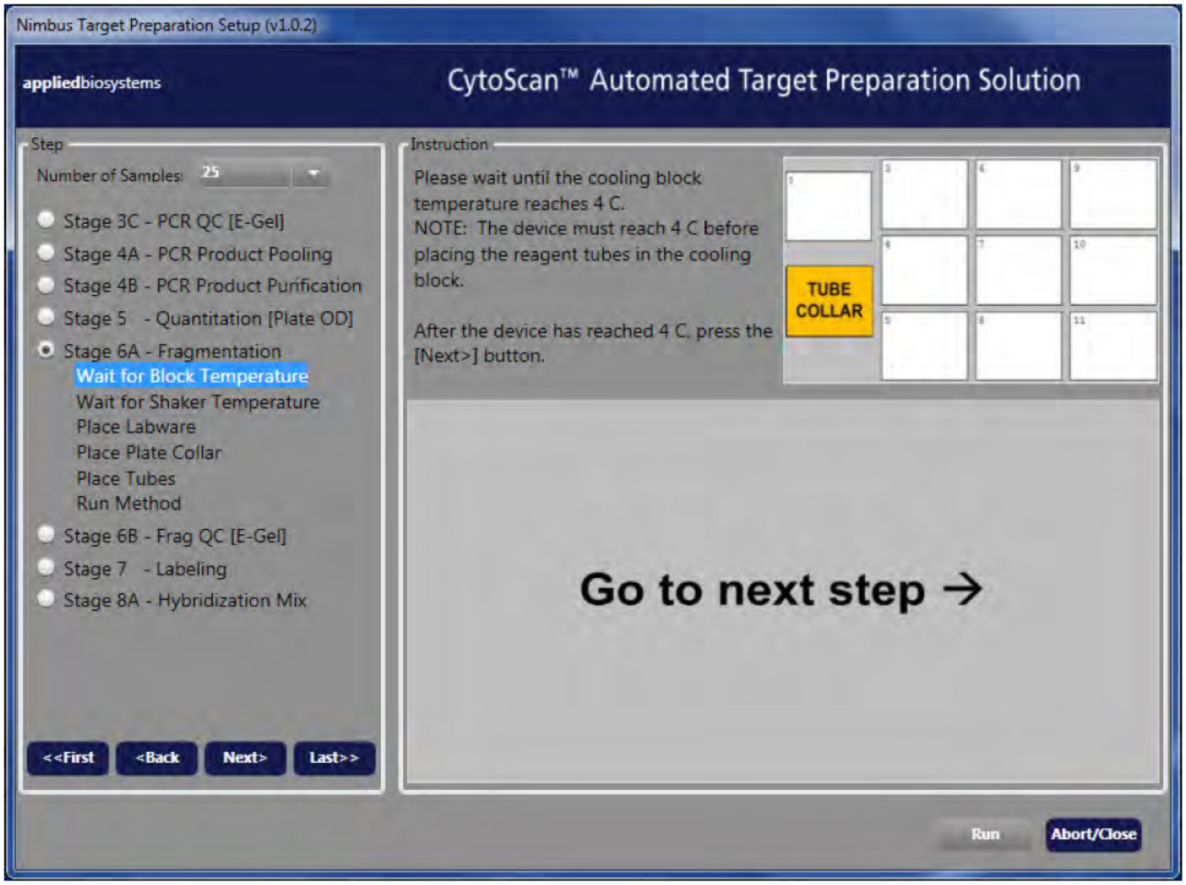
Add the Fragmentation Master Mix to the samples

1. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 6A–Fragmentation**
   - **QC Platform**—For example, “E-Gel + Plate OD”.

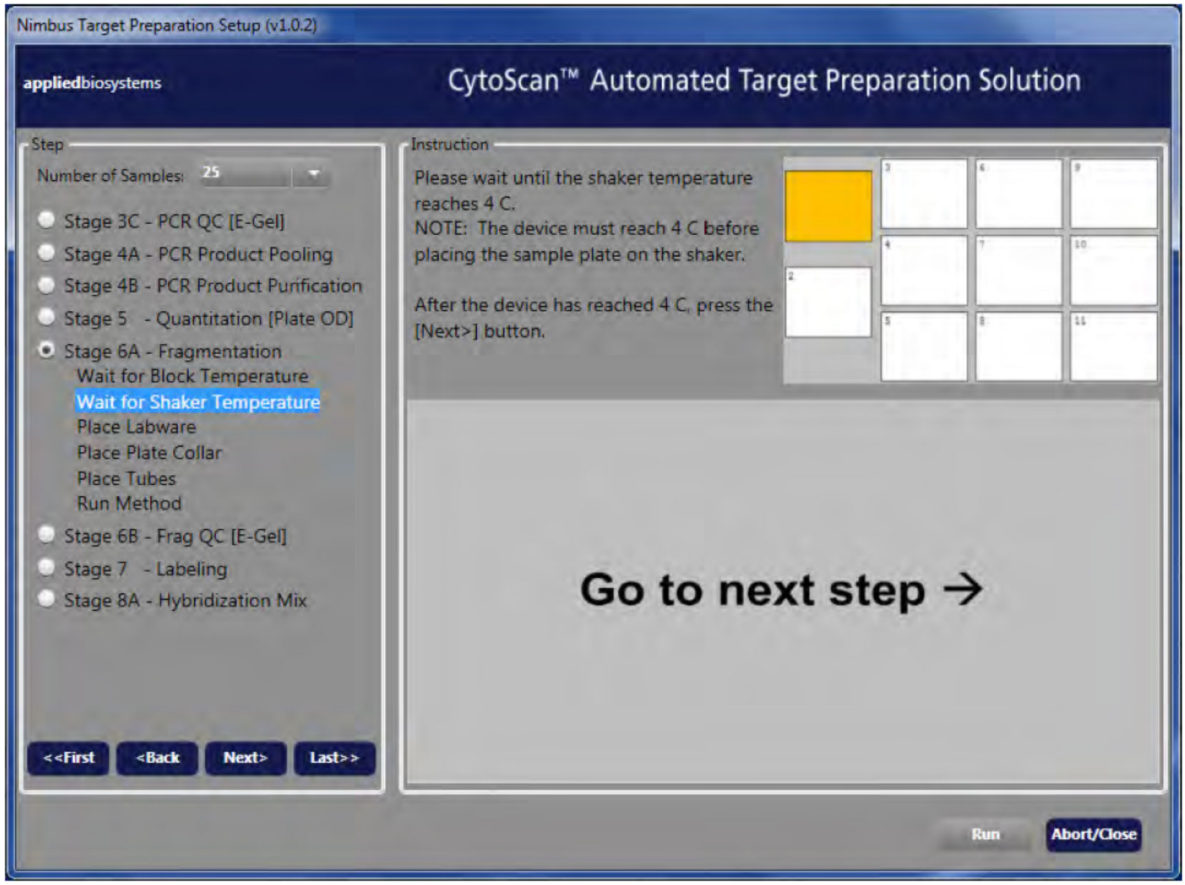


2. Click **Run** to start the method, then click **Yes** to confirm.

3. Wait until the block temperature reaches 4°C, then click **Next**.



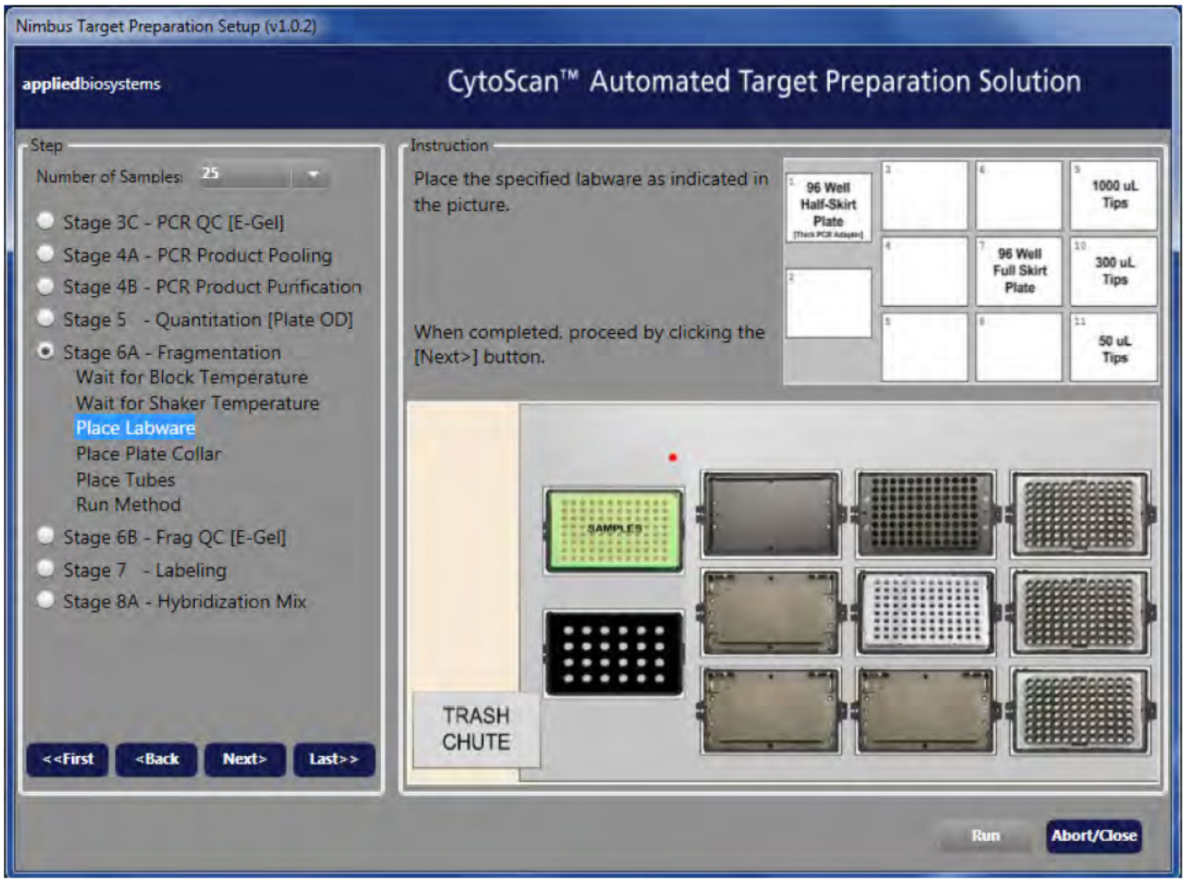
4. Wait until the shaker temperature reaches 4°C, then click **Next**.



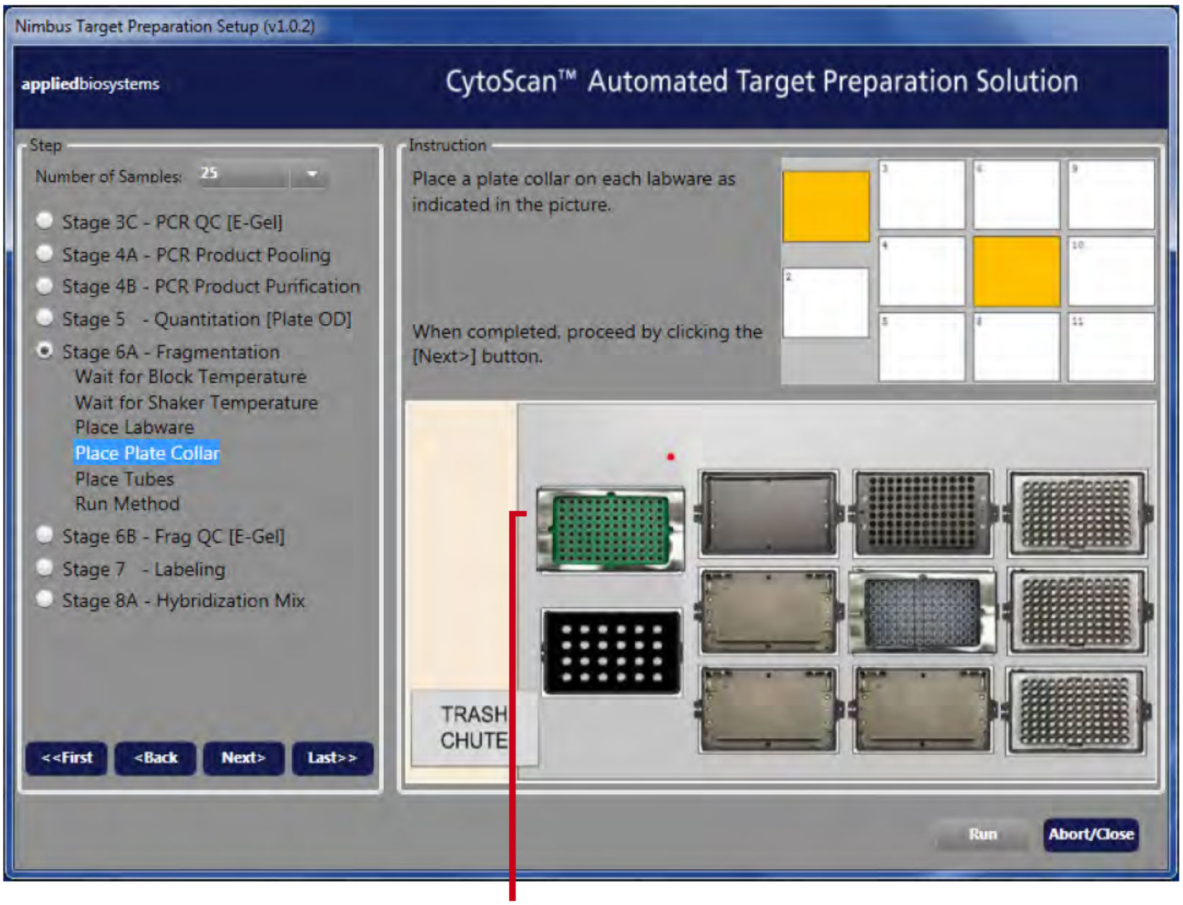
5. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   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.



6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1 and 7 require a plate collar.



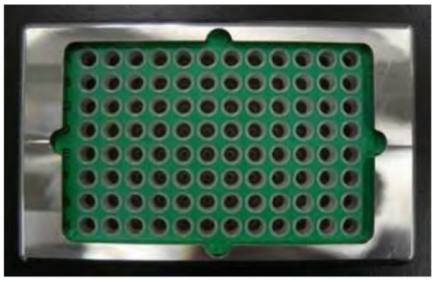
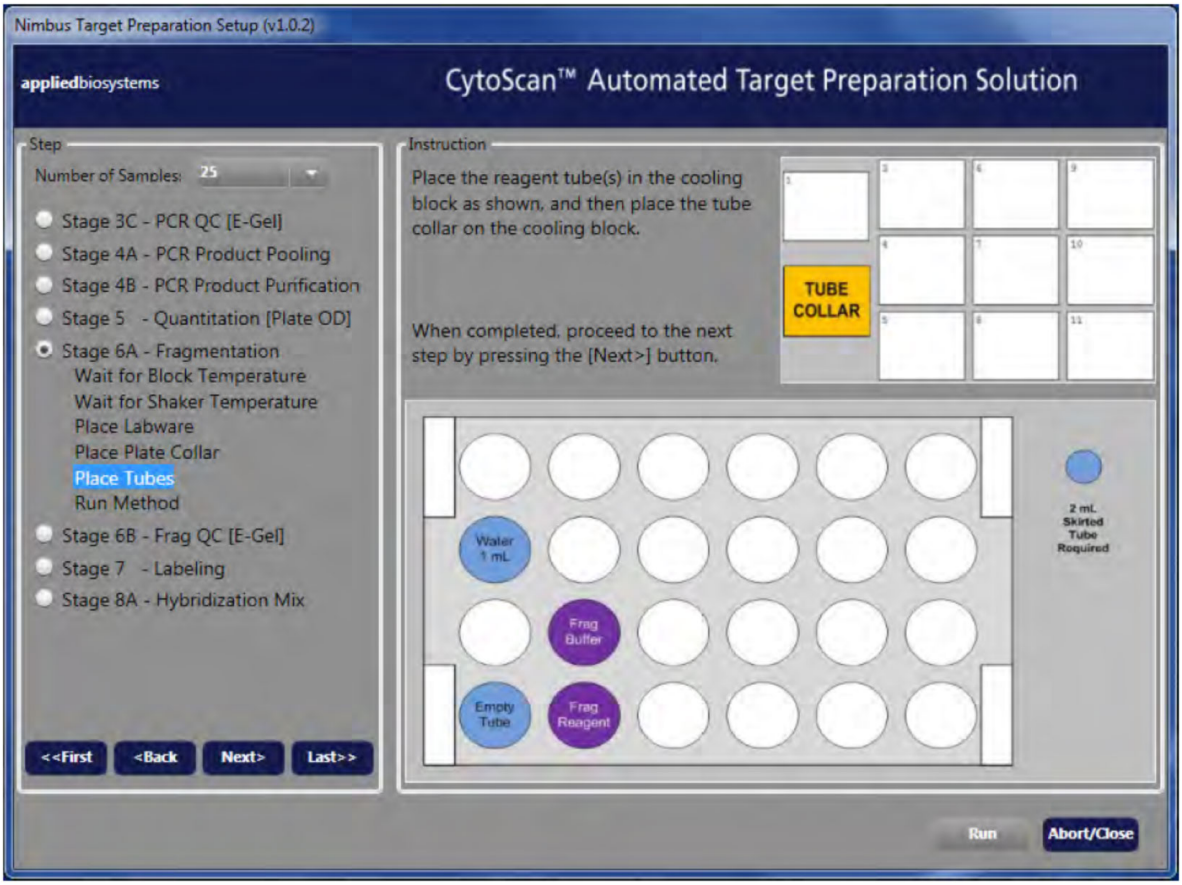
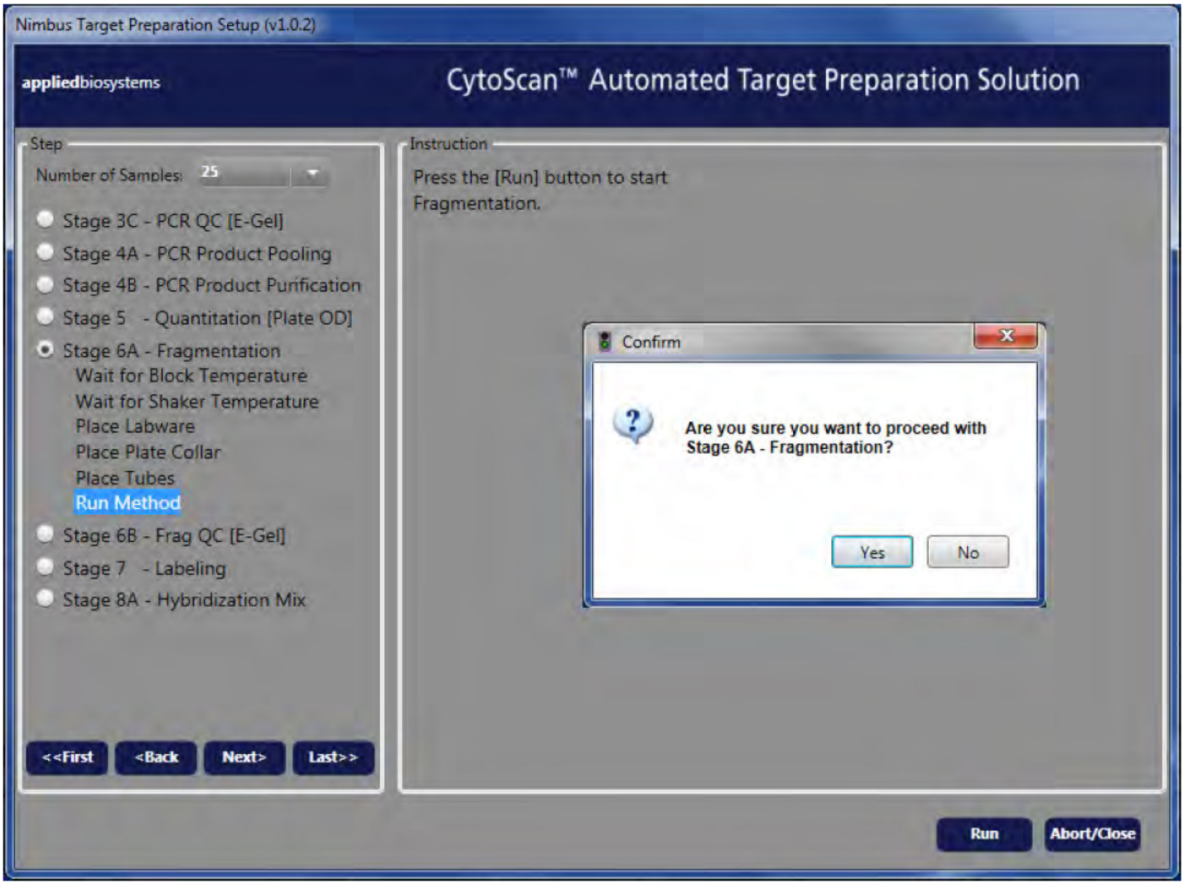


Plate collar on a sample plate

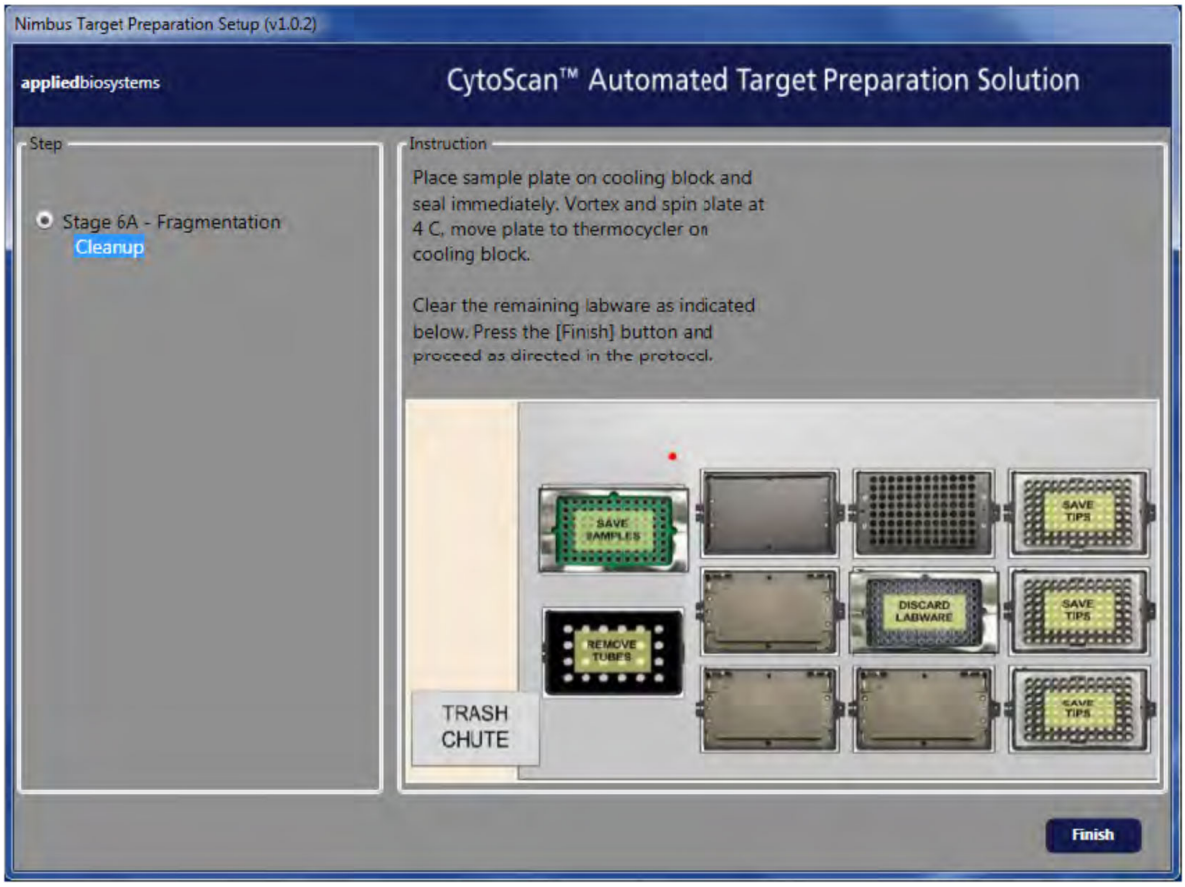
7. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Immediately after the method is complete, 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.

10. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40.

11. Bring the sample plate to the centrifuge on the cooling block in the ice box. Centrifuge the plate on chilled racks in the prechilled centrifuge at 2,000 rpm for 1 minute. Quickly remove the plate from the centrifuge and place in the cooling block in the ice box.

12. Carry the sample plate on the cooling block in the ice box, then immediately load the Fragmentation Plate onto the thermal cycler with preheated lid.

13. Run the **CytoScan Fragment** thermal cycler protocol. Total volume for Fragmentation = 55 µL.

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	Hold

14. Remove and discard any remaining Fragmentation Master Mix.

IMPORTANT! Never reuse Fragmentation Master Mix.

Note: At this point the plate centrifuge can be turned back to room temperature.

15. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

16. Click **Finish**, then click **Yes** to confirm.
    The method closes.

17. Verify the fragmentation reaction by running gels as described in “Stage 6B—Fragmentation QC” on page 142.

Stage 6B—Fragmentation QC

This stage verifies the fragmentation by running a TBE gel or an E-Gel™ Agarose Gel using the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration.

Perform fragmentation QC using one of these methods:

- “Fragmentation QC procedure using TBE gels” on page 143 .
- “Fragmentation QC procedure using E-Gel™ Agarose Gels” on page 152.

Note: Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 6B—Fragmentation QC

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in “Thermal cyclers” on page 41.

Table 26 Gels and related materials required for Fragmentation QC using TBE gels.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration (Cat. No. 00-0401)
As required	MicroAmp™ Clear Adhesive Film for 96-well plates (Cat. No. 4306311)
1 (Optional)	MicroAmp™ Adhesive Film Applicator (Cat. No. 4333183)
1	Mini centrifuge
1	Plate centrifuge
1	Electrophoresis gel box
1	Electrophoresis power supply
1	Gel, 4% TBE (precast or house-made)
1 liter	1X TBE Buffer
Few drops	Ethidium bromide solution
1	5X RapidRun™ Loading Dye
1 rack	Conductive 50 µL filter tips in frames
1 rack	Conductive 300 µL filter tips in frames
1	Reservoir 4 ROW PYR PP 73 mL
1	Tube, screw cap, no ribs, 2 mL

Table 26 Gels and related materials required for Fragmentation QC using TBE gels. *(continued)*

Quantity	Item
2	96-well Full Skirt PCR Plate, clear
1	25 bp DNA Ladder (25–300 bp)

Table 27 Gels and related materials required for Fragmentation QC using E-Gel™ Agarose Gels.

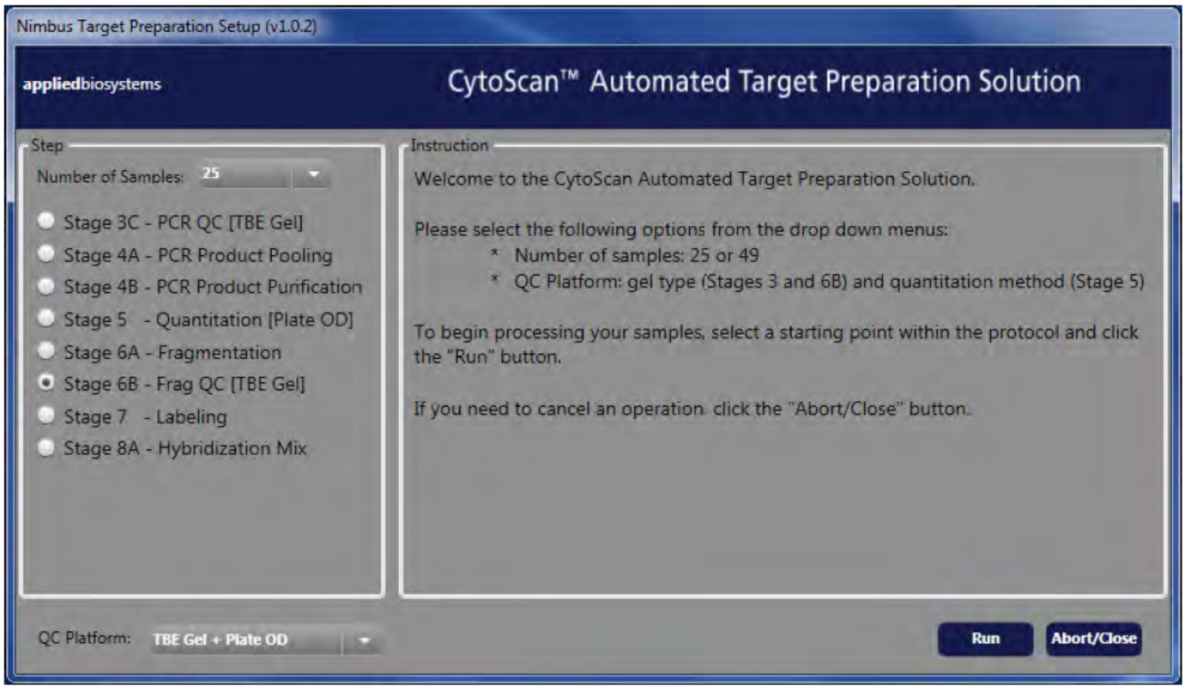
Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Mini centrifuge
1	Plate centrifuge
1	Mother E-Base™ Device
1	Daughter E-Base™ Device (optional for running multiple gels simultaneously)
2	96-well Full Skirt PCR Plate, clear
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1	Reservoir 4 ROW PYR PP 73 mL
1	Tube, screw cap, no ribs, 2 mL
2	E-Gel™ *48* Agarose Gels, 4%, Cat. No. G800804
1	TrackIt™ Cyan/Orange Loading Buffer
1	25 bp DNA Ladder (25–300 bp)

Fragmentation QC procedure using TBE gels

The controls can be placed in any microplate well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

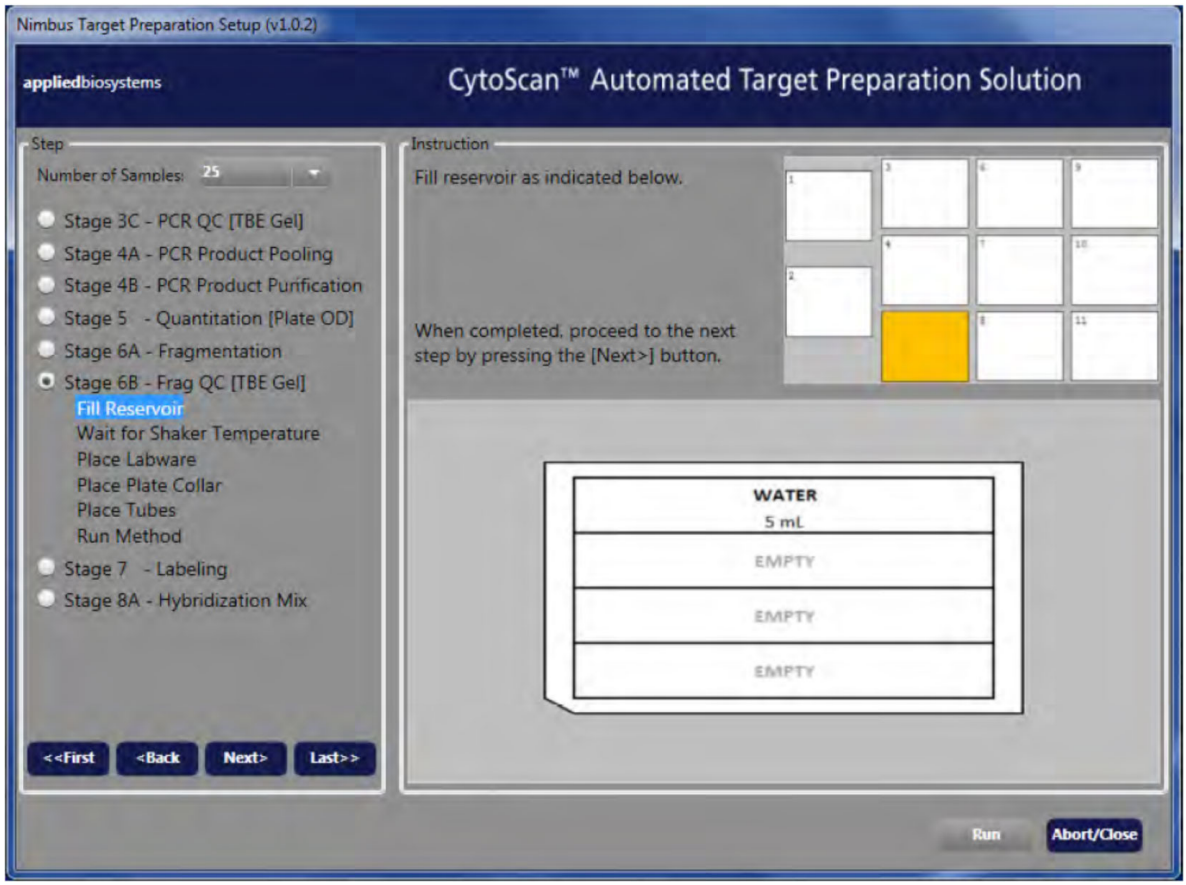
1. When the **CytoScan Fragment** thermal cycler protocol is finished:
   a. Remove the sample plate from the thermal cycler.
   b. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute. Place on a cooling block on ice.

2. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 6B–Frag QC (TBE Gel)**
   - **QC Platform**—For example, “TBE Gel + Plate OD”.

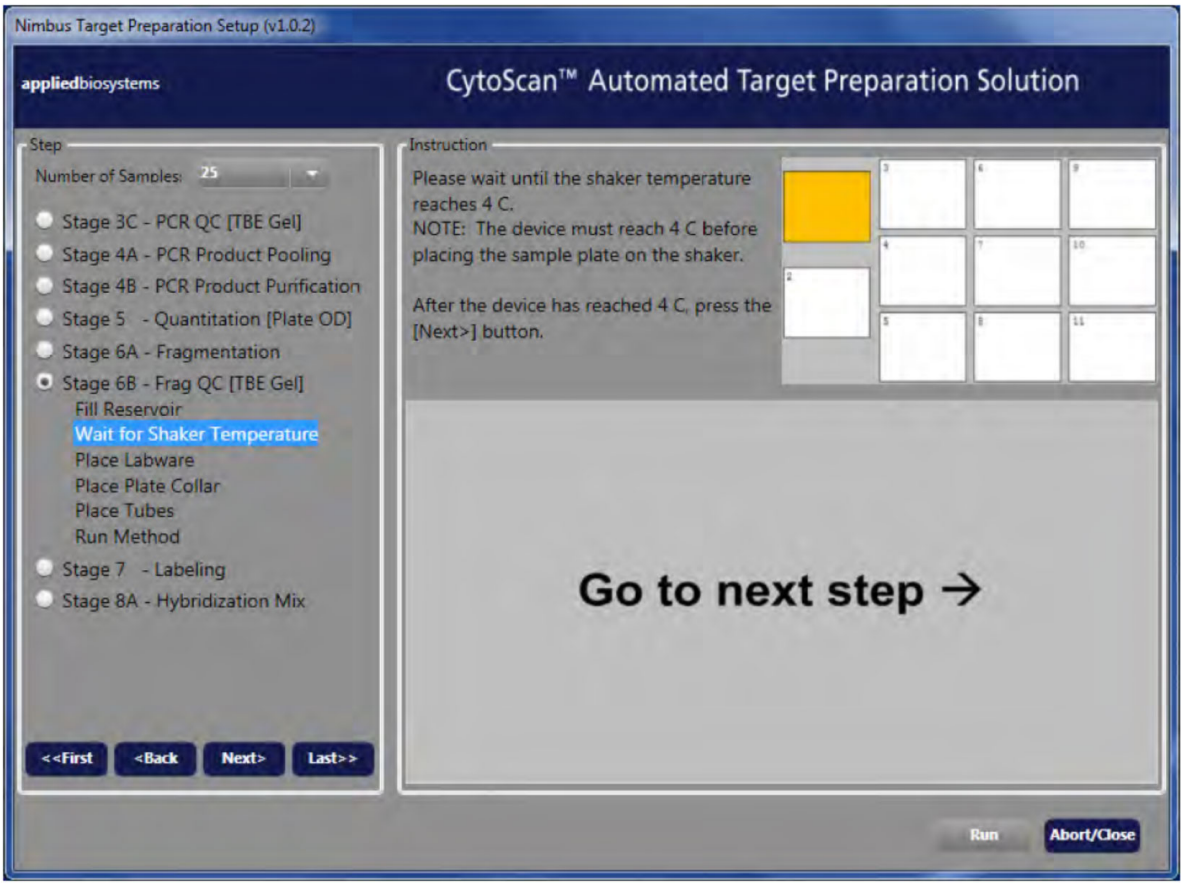


3. Click **Run** to start the method, then click **Yes** to confirm.

4. Fill the reservoir as shown in the on-screen instructions, then click **Next**.



5. Wait until the shaker temperature reaches 4°C, then click **Next**.

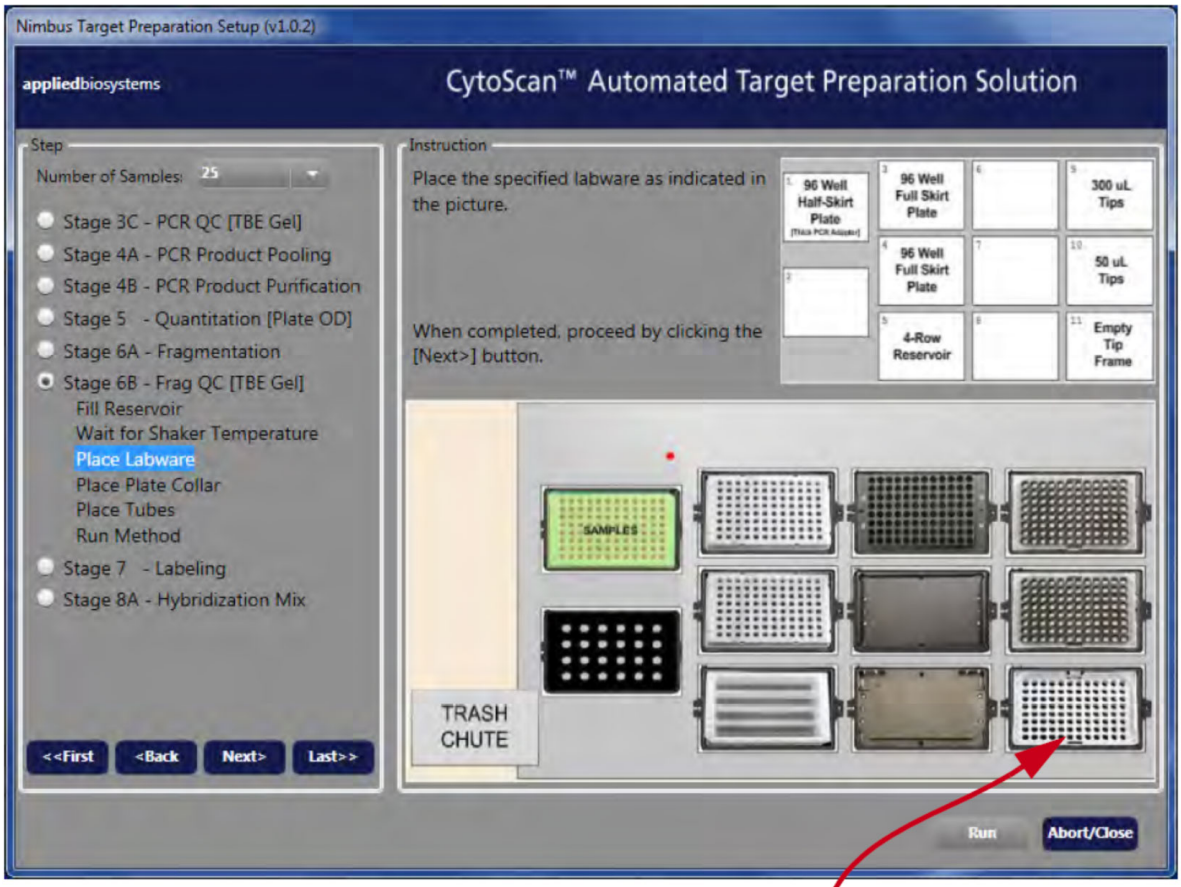


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

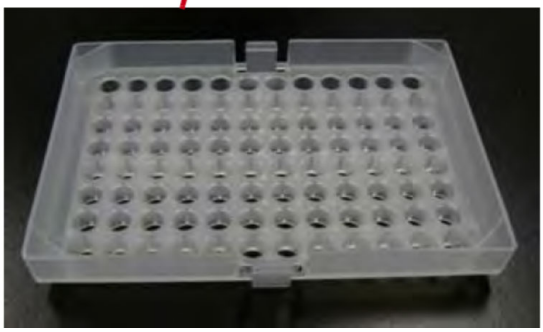
Note: Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

Place an empty tip frame on deck position 11.

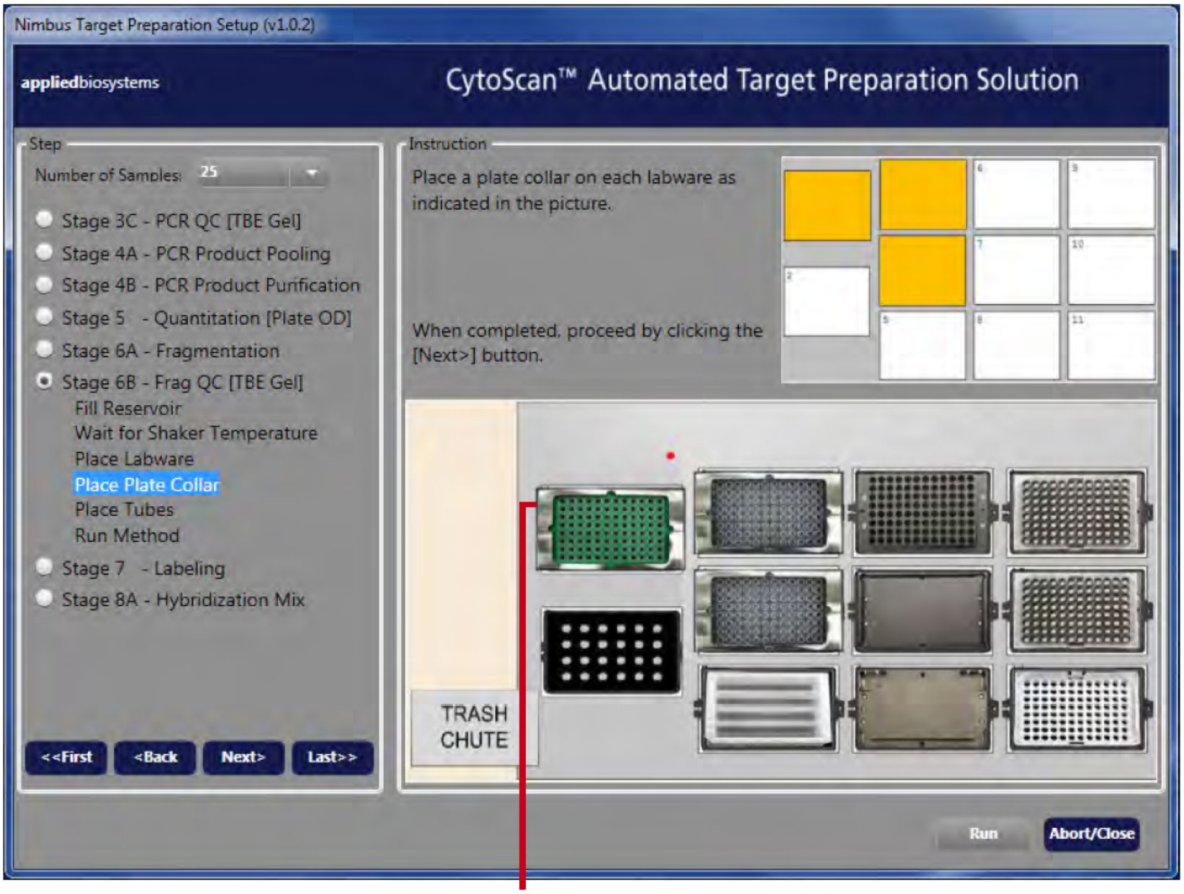
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 3, 4, and 5 require a plate collar.



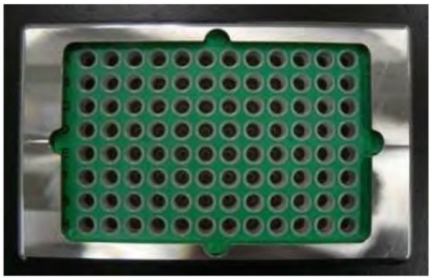
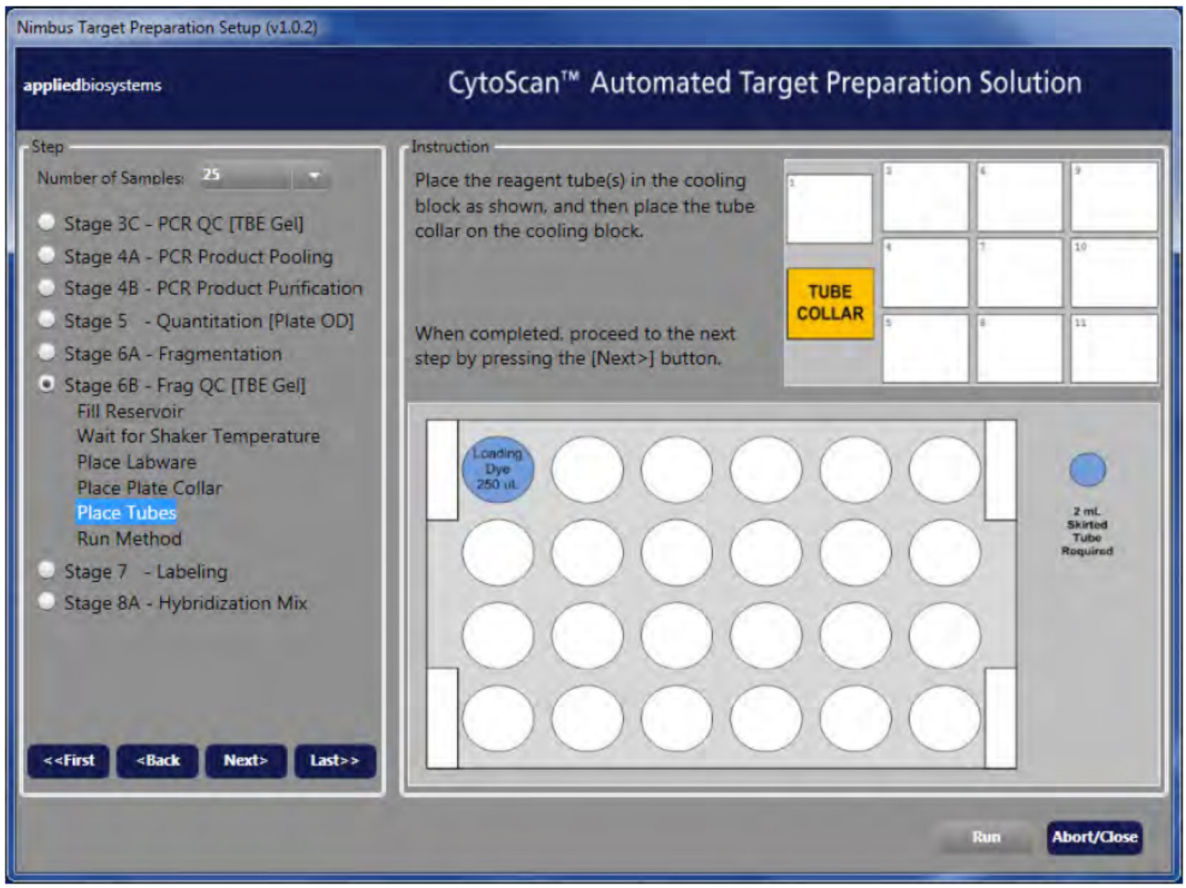
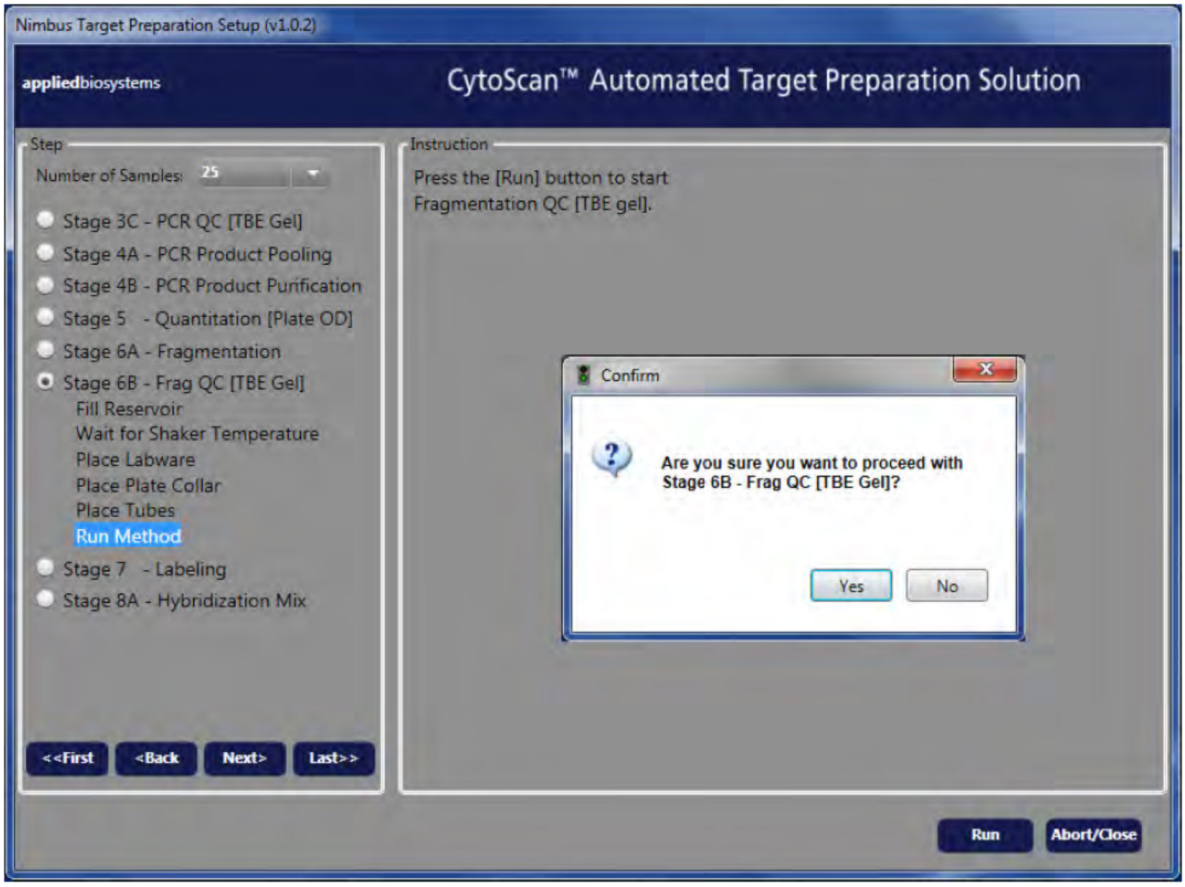


Plate collar on a sample plate

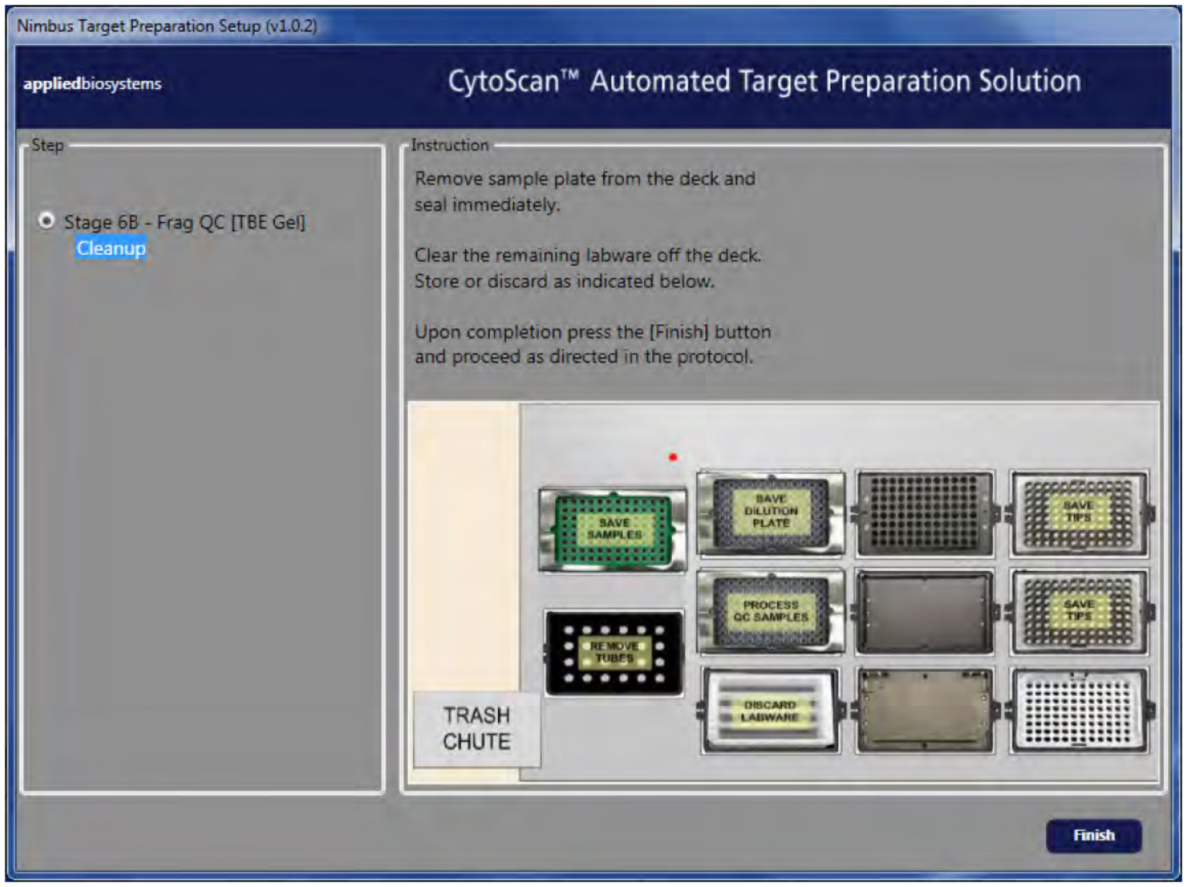
8. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen and click **Next**.



9. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



10. Remove the sample plate from the deck, then tightly seal the plate.
    The plate of fragmented DNA can be stored at –20°C for up to 10 days.

11. Seal and store the Fragmentation QC Sample Dilution plate at –20°C for further analysis using the Agilent™ 2100 Bioanalyzer™.
    See Appendix C, “Analyzing sample fragmentation using the Agilent™ 2100 Bioanalyzer™ Instrument”.

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

13. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

14. Click **Finish**, then click **Yes** to confirm.
    The method closes.

Run the TBE gel

1. Load 8 µL of the samples from the QC sample plate onto the gel.

2. Load 2 µL of 25 bp DNA Ladder to the first and last lanes.

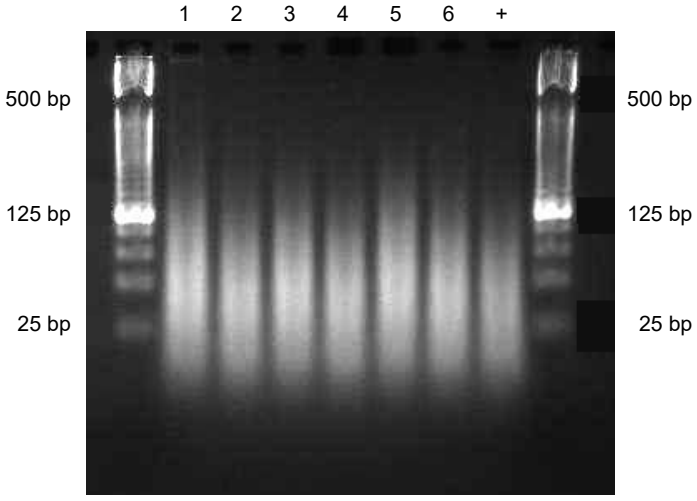
3. Run the samples on a 4% TBE gel at 5 V/cm for 45 minutes or until the dye front reaches at least 75% of distance down the gel.

   Run gels at 5 V/cm (5 volts X distance in cm between electrodes). For example, run a 33-cm electrophoresis box at 165 V; run a 16-cm electrophoresis box at 80 V.

4. Inspect the gel, then compare it against the example shown in the following example.

   The majority of fragment distribution must be between 25 and 125 bp.

Figure 22 Example of fragmented PCR products run on 4% TBE Gel at 5 V/cm for 45 minutes. Fragmentation is verified by the majority of distribution between 25 and 125 bp.



Fragmentation QC procedure using E-Gel™ Agarose Gels

The controls can be placed in any microplate well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

Dilute the TrackIt™ Cyan/Orange Loading Buffer

The following instructions prepare a 1,000-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer.

1. Add 50 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.95 mL Nuclease-Free Water (total volume is 50 mL).

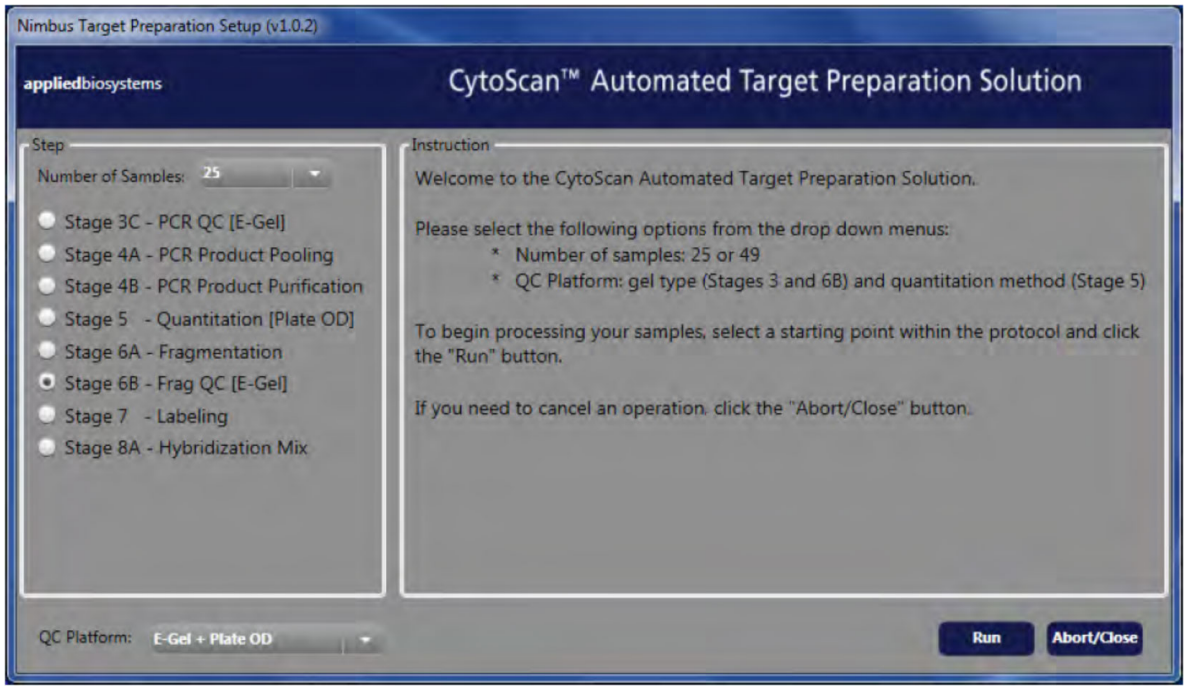
2. Mix well, then store at room temperature.

Prepare the QC gel plate

1. When the **CytoScan Fragment** thermal cycler protocol is finished:
   a. Remove the sample plate from the thermal cycler.

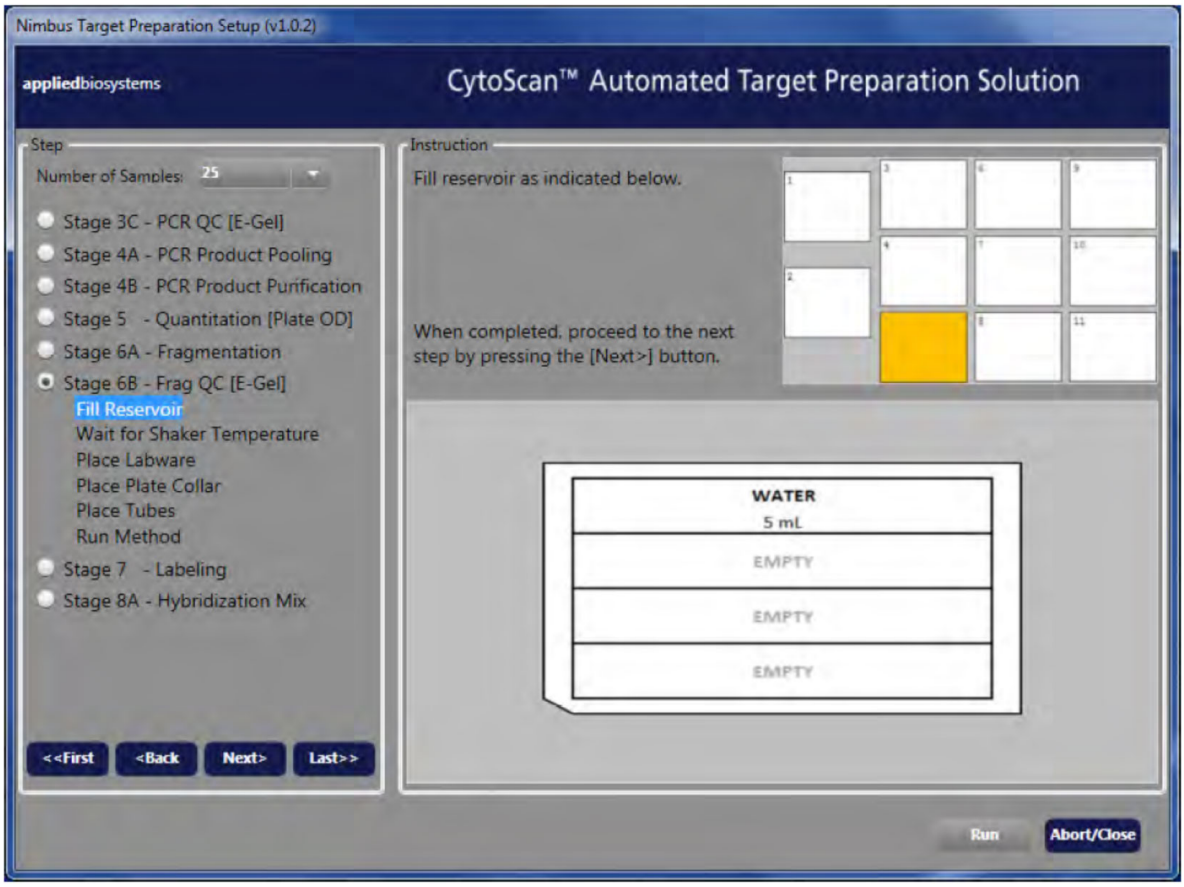
   b. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute. Place on a cooling block on ice.

2. On the **Welcome** screen, select the following options.
   - **Number of samples**—25
   - **Stage 6A–Fragmentation**
   - **QC Platform**—For example, “E-Gel + Plate OD”.

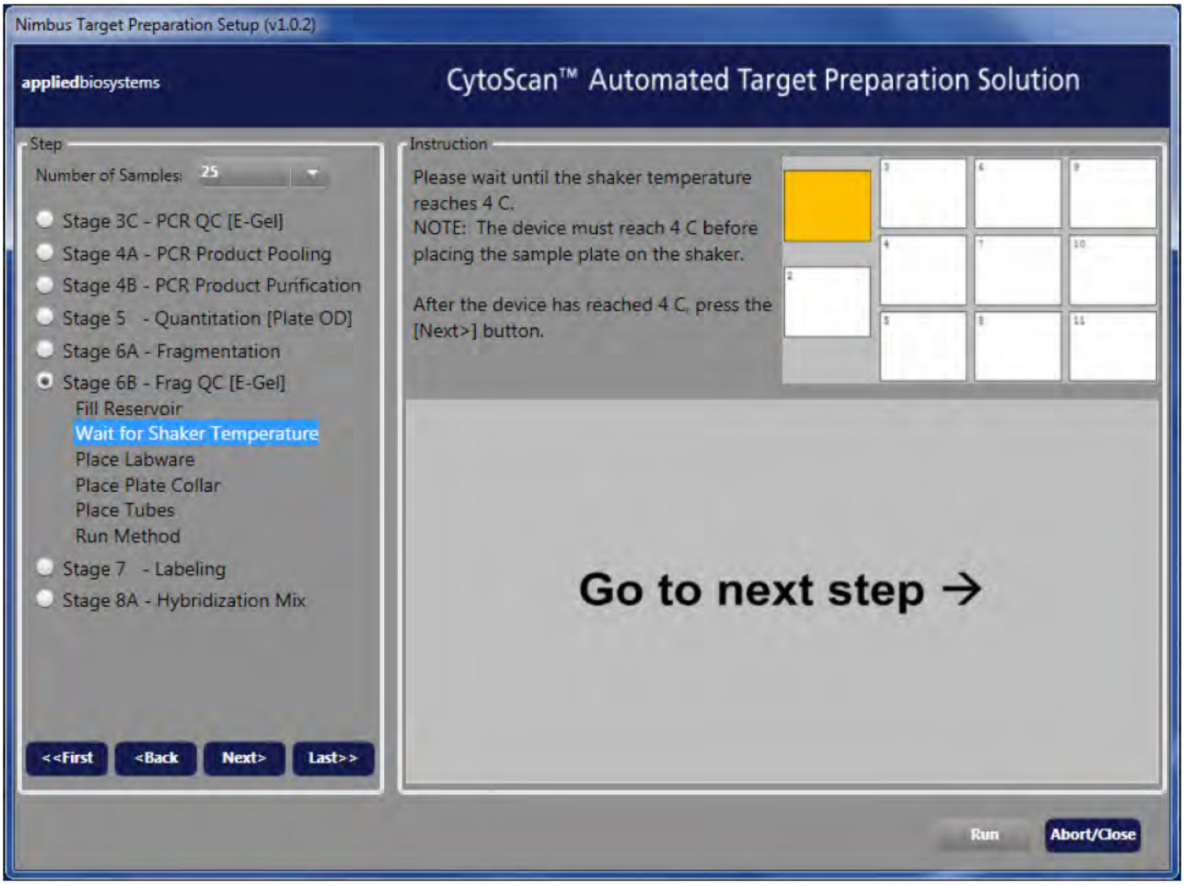


3. Click **Run** to start the method, then click **Yes** to confirm.

4. Fill the reservoir as shown in the on-screen instructions, then click **Next**.



5. Wait until the shaker temperature reaches 4°C, then click **Next**.

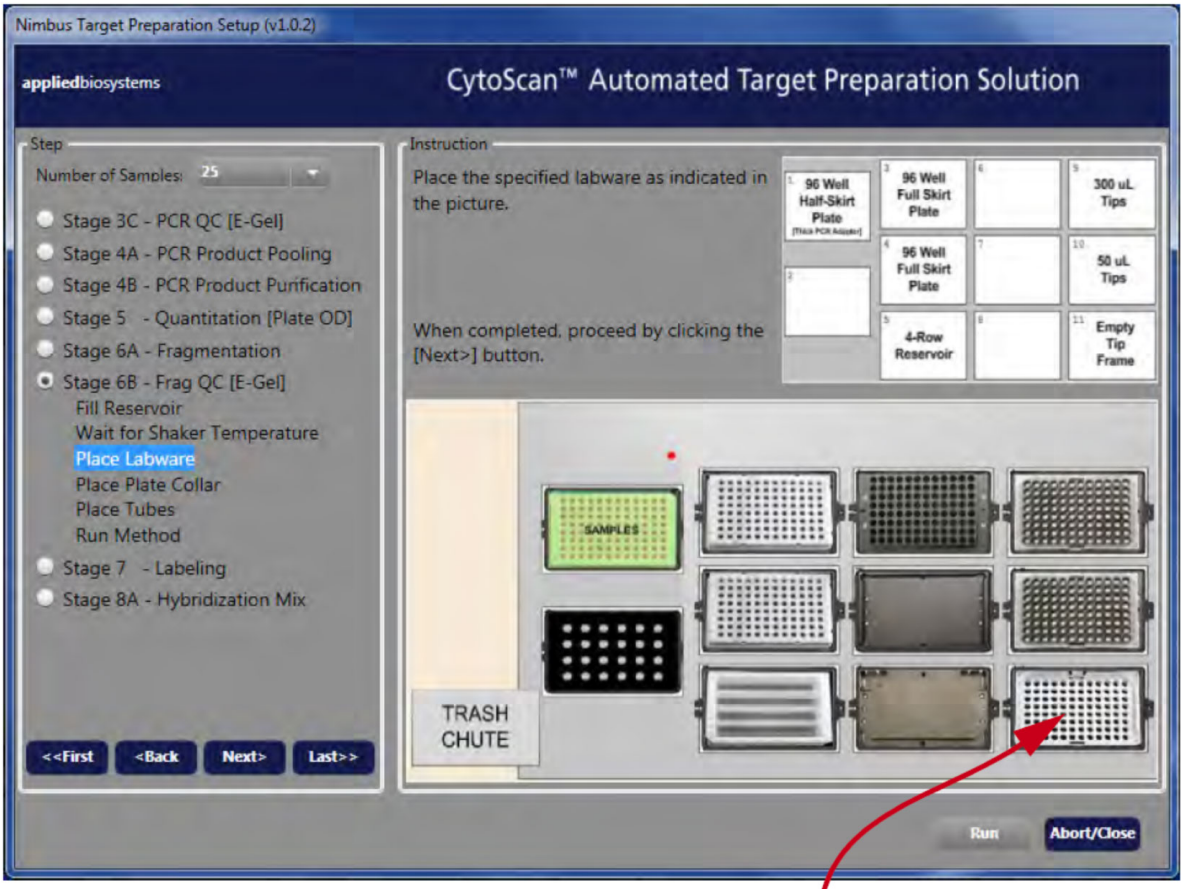


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

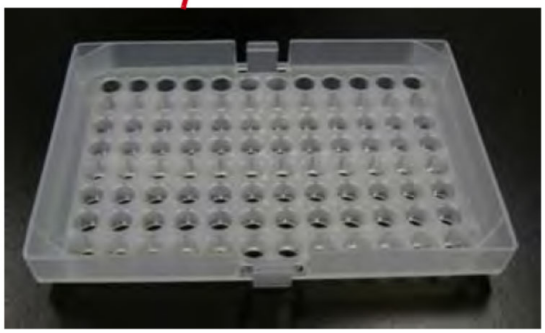
   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   Place an empty tip frame on deck position 11.

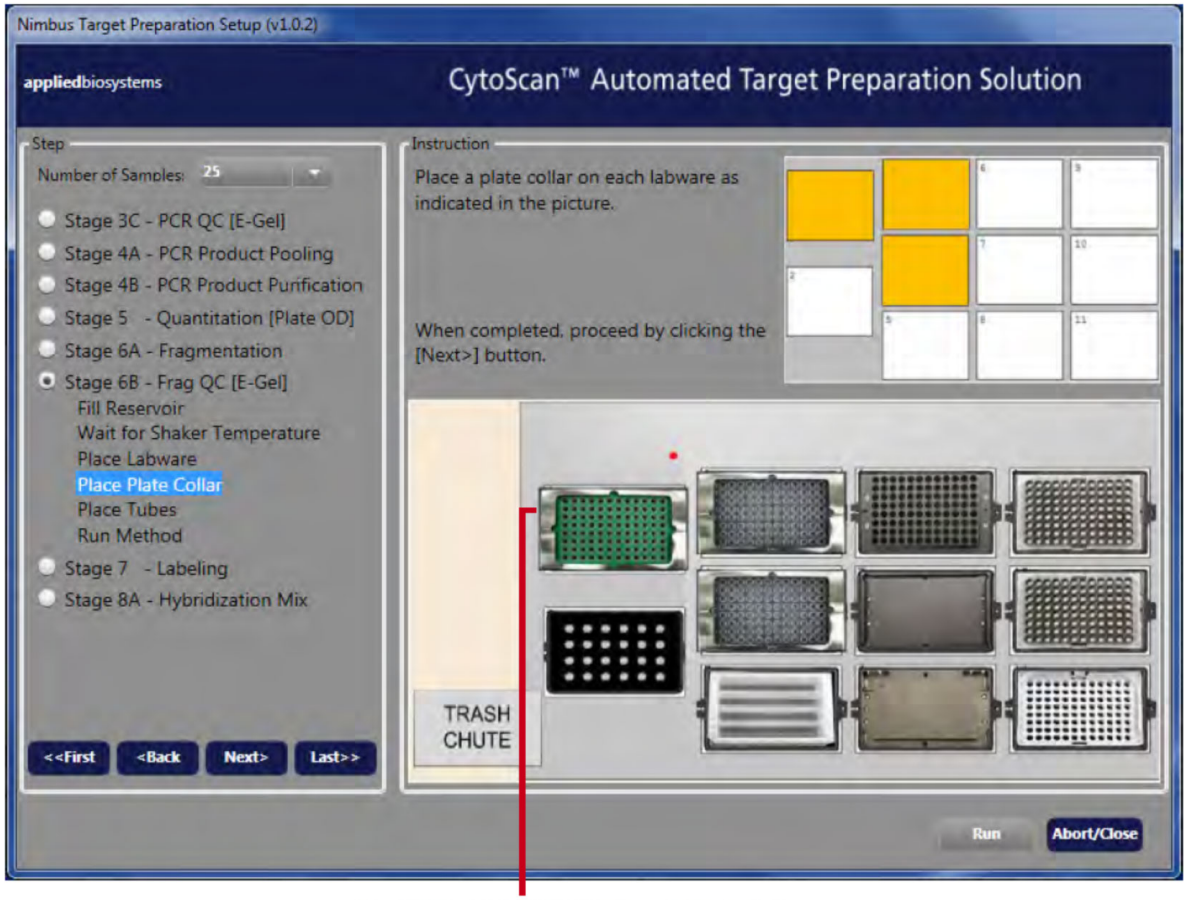
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 3, and 4 require a plate collar.



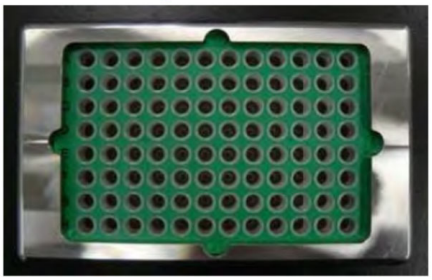
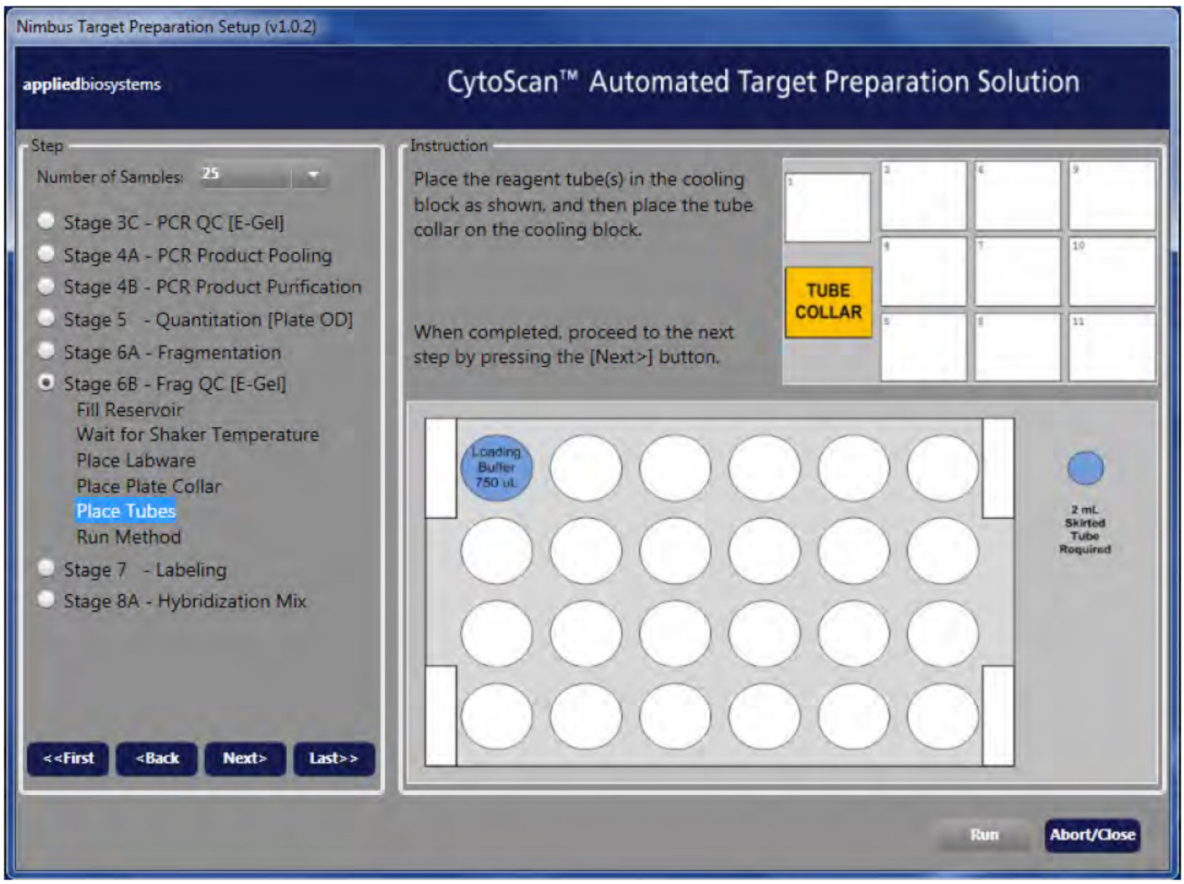
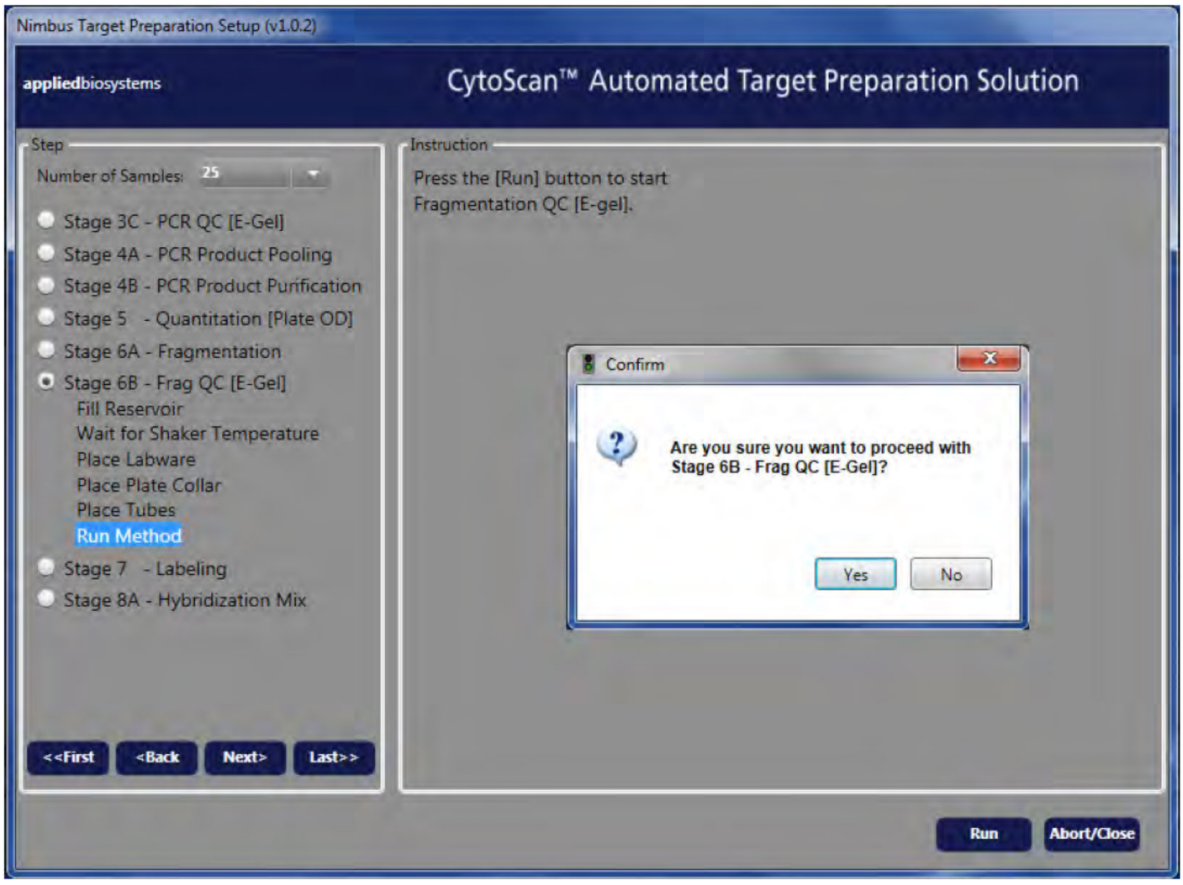


Plate collar on a sample plate

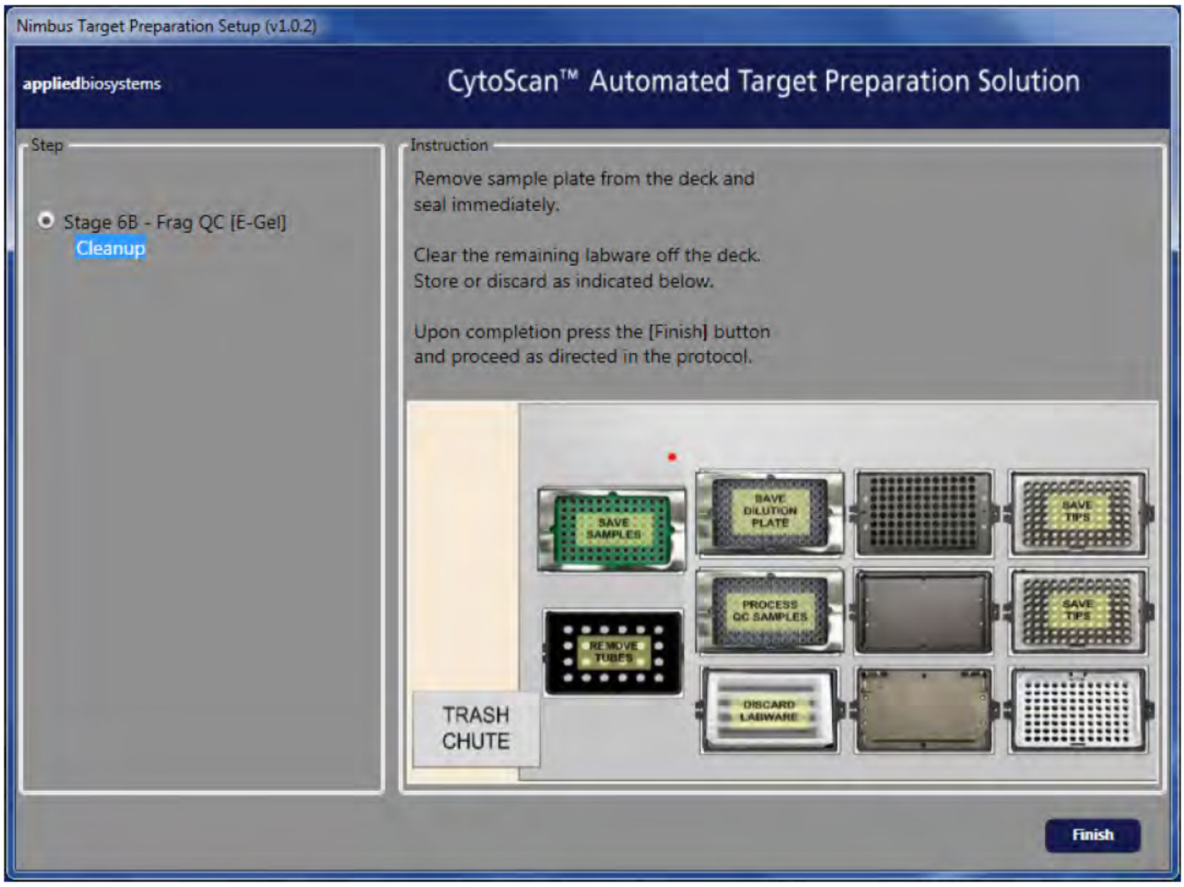
8. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



9. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



10. Remove the sample plate from the deck, then tightly seal the plate.
    The plate of fragmented DNA can be stored at –20°C for up to 10 days.

11. Seal and store the Fragmentation QC Sample Dilution plate at –20°C for further analysis using the Agilent™ 2100 Bioanalyzer™ Instrument.
    See Appendix C, “Analyzing sample fragmentation using the Agilent™ 2100 Bioanalyzer™ Instrument”.

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

13. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

14. Click **Finish**, then click **Yes** to confirm.
    The method closes.

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 .

   A 12-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 of the marker wells (as needed). Fill empty wells with 20 µL water.
7. Set the run time to 19 minutes.
8. Push the Power/Prg button again (the light 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.

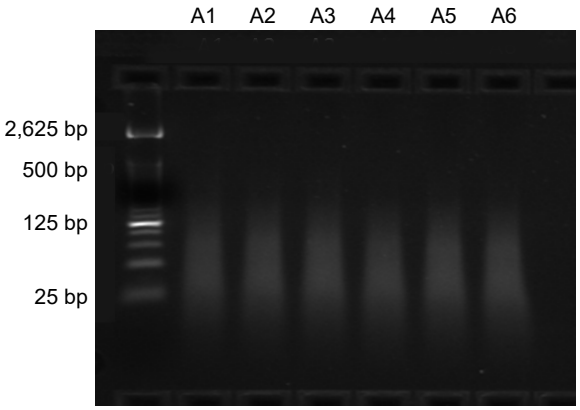


Figure 23 Gel image of fragmented product from REF DNA 103 (50 ng/µL) on E-Gel™ *48* Agarose Gels, 4%.

9. Proceed to “Stage 7—Labeling” on page 162.

Stage 7—Labeling

This stage uses the NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration. The instrument:

- Prepares the Labeling Master Mix.
- Aliquots the Labeling Master Mix into a distribution plate.
- Adds the Labeling Master Mix to samples and performs mixing.

Note:

- Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.
- To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..
- Controls can be placed in any well from A1 through C1 because the NIMBUS™ Instrument processes all samples.

Materials required for Stage 7—Labeling

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 28 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini centrifuge
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	Thermal cycler

Table 28 Equipment and consumables required. *(continued)*

Quantity	Item
1	Tube, screw cap, no ribs, 2 mL
1	96-well Full Skirt PCR Plate, clear
1	Vortexer
1	PCR Tube Rack

Table 29 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	TdT Enzyme	Module 3	902675
1	TdT Buffer		902676
1	DNA Labeling Reagent		902677

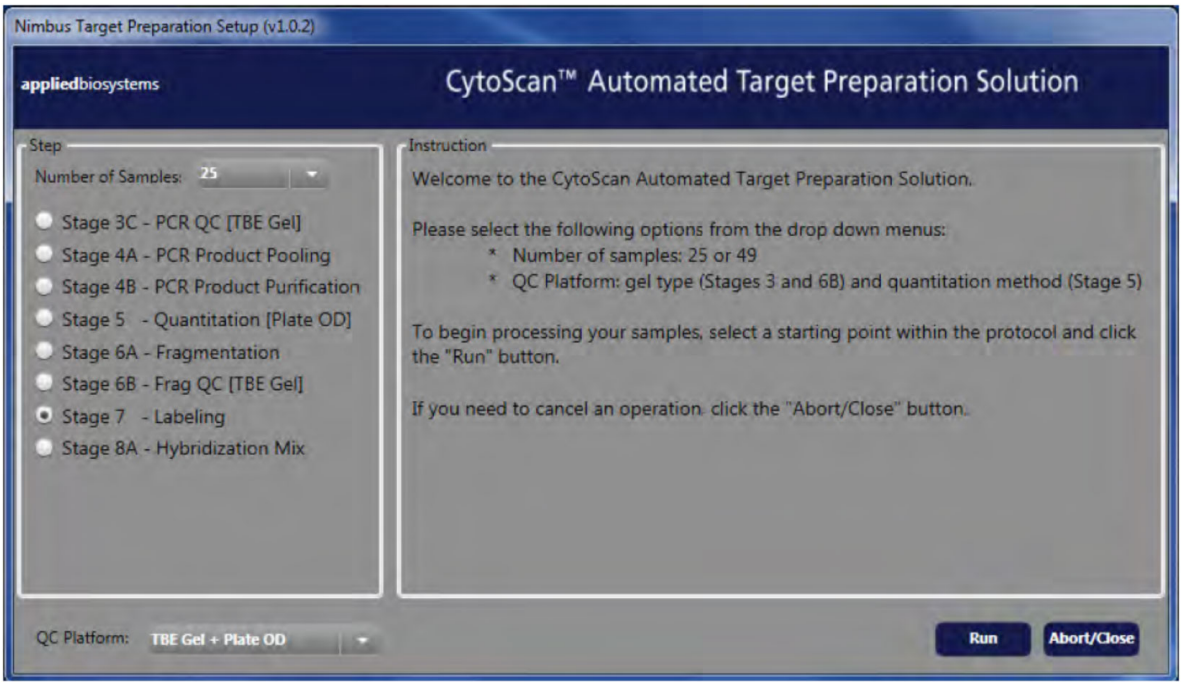
Prepare the reagents, equipment, and consumables

1. Power on the thermal cycler to preheat the lid. **Leave the block at room temperature.**
2. Place a double cooling block on ice.
3. Thaw the following reagents at room temperature. Immediately place on cooling block on ice when thawed.
   - TdT Buffer
   - DNA Labeling Reagent

   IMPORTANT! Leave the TdT Enzyme at –20°C until ready to use.
4. Prepare the TdT Buffer and DNA Labeling Reagent.
   a. Vortex each reagent at high speed 3 times, 1 second each time.
   b. Briefly centrifuge for 3 seconds, then place in the cooling block.
5. If the fragmented samples are frozen, allow them to thaw at room temperature.
6. When thawed, immediately centrifuge the plate at 2,000 rpm for 1 minute, then place on the cooling block on ice.
7. Remove the TdT Enzyme from the freezer, then immediately place in the cooler, chilled to –20°C.
8. Vortex the enzyme at high speed 1 time for 1 second.
9. Briefly centrifuge the enzyme for 3 seconds, then immediately place back in the – 20°C cooler.

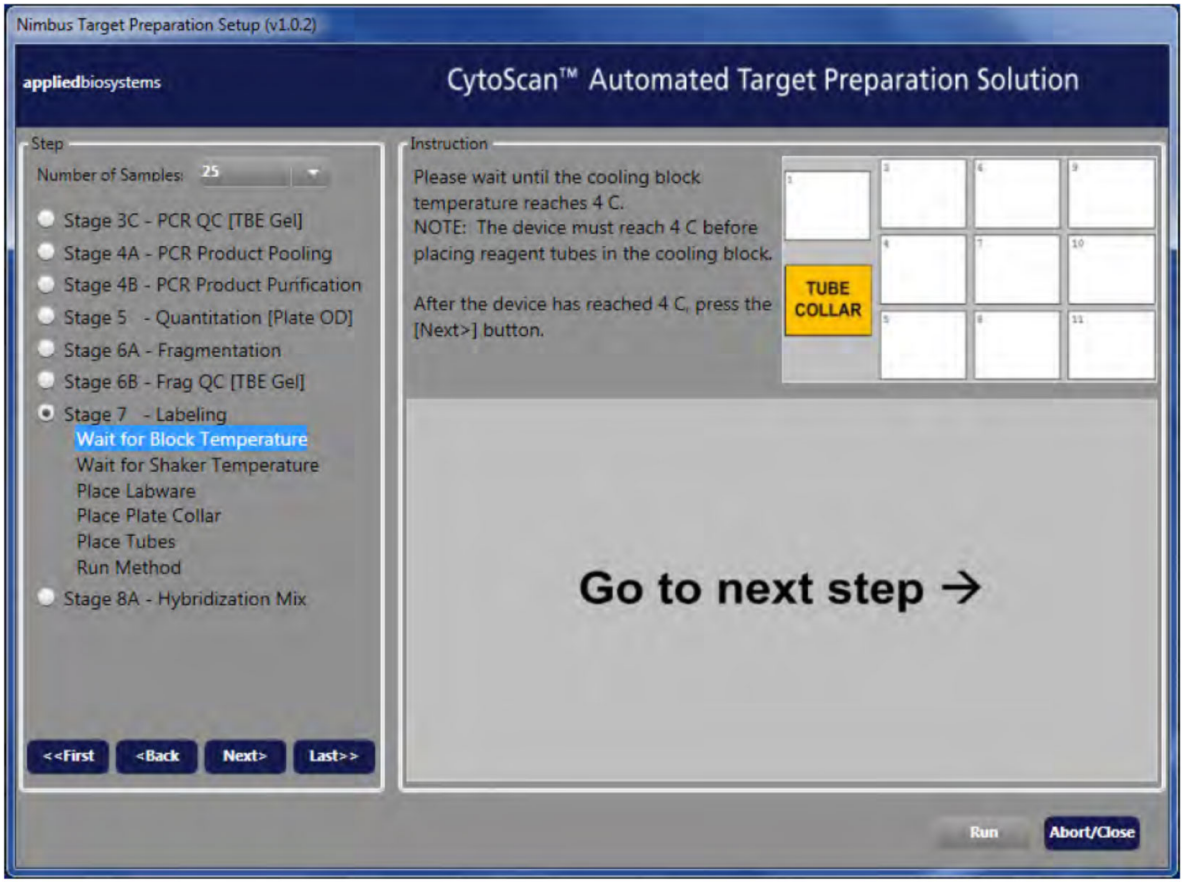
Run the Labeling method

1. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 7–Labeling**

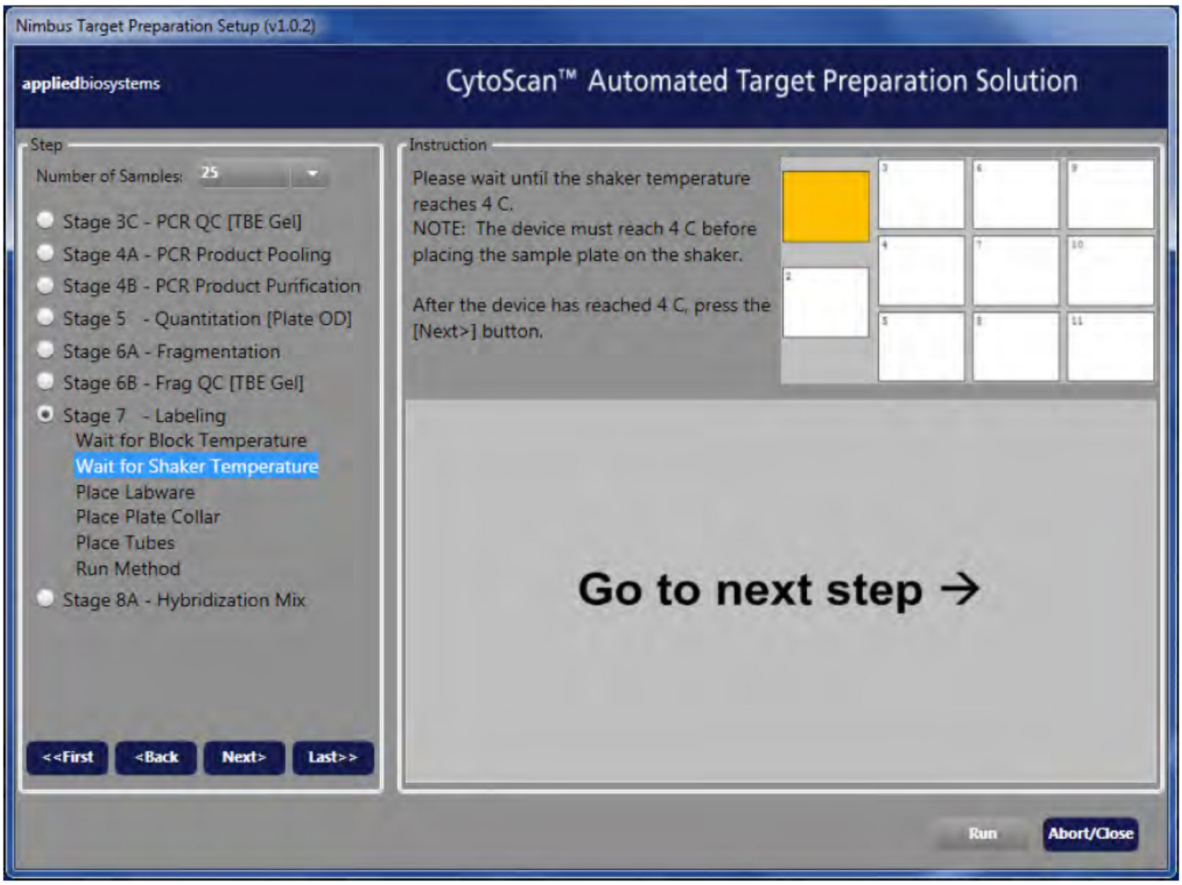


2. Click **Run** to start the method, then click **Yes** to confirm.

3. Wait until the cooling block reaches 4°C, then click **Next**.



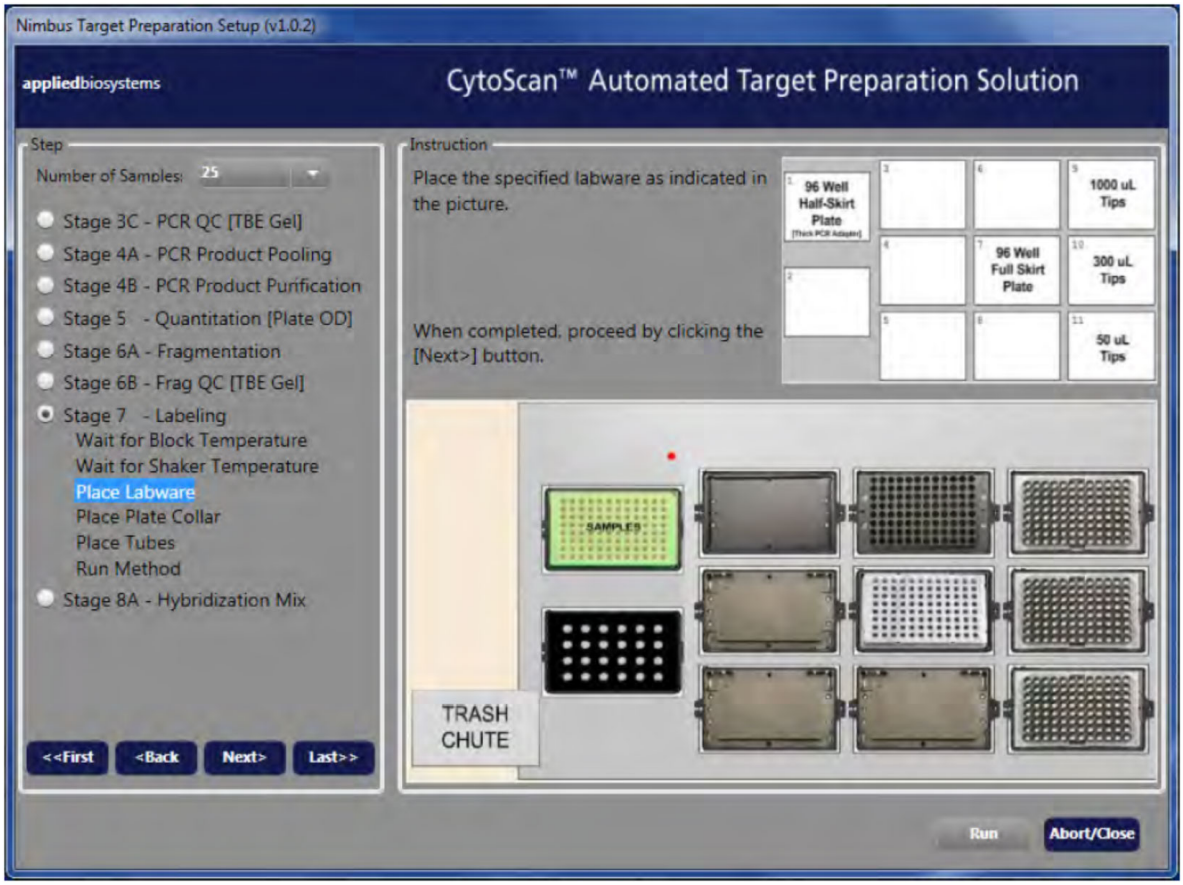
4. Wait until the shaker temperature reaches 4°C, then click **Next**.



5. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

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.



6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1 and 7 require a plate collar.

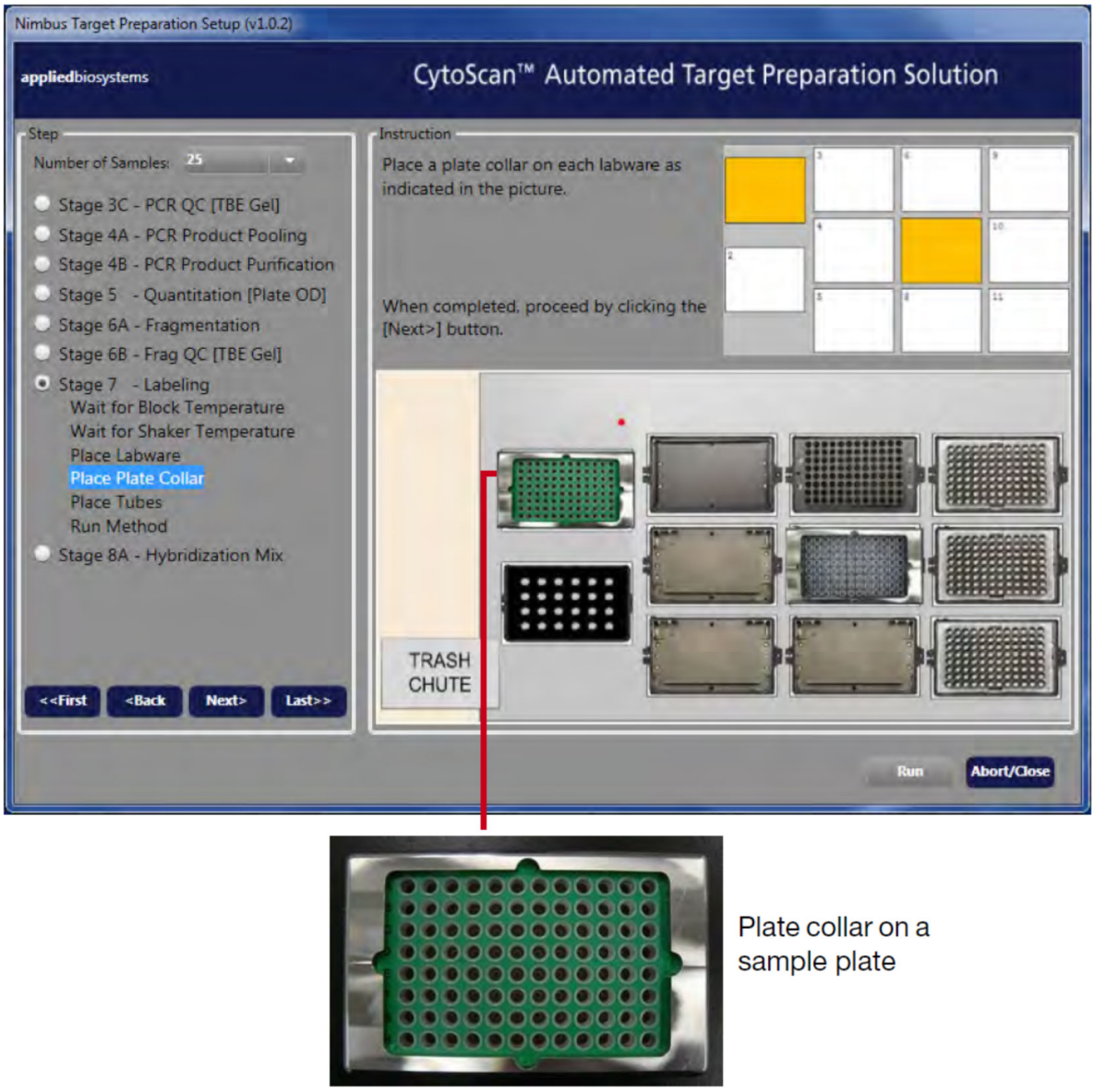
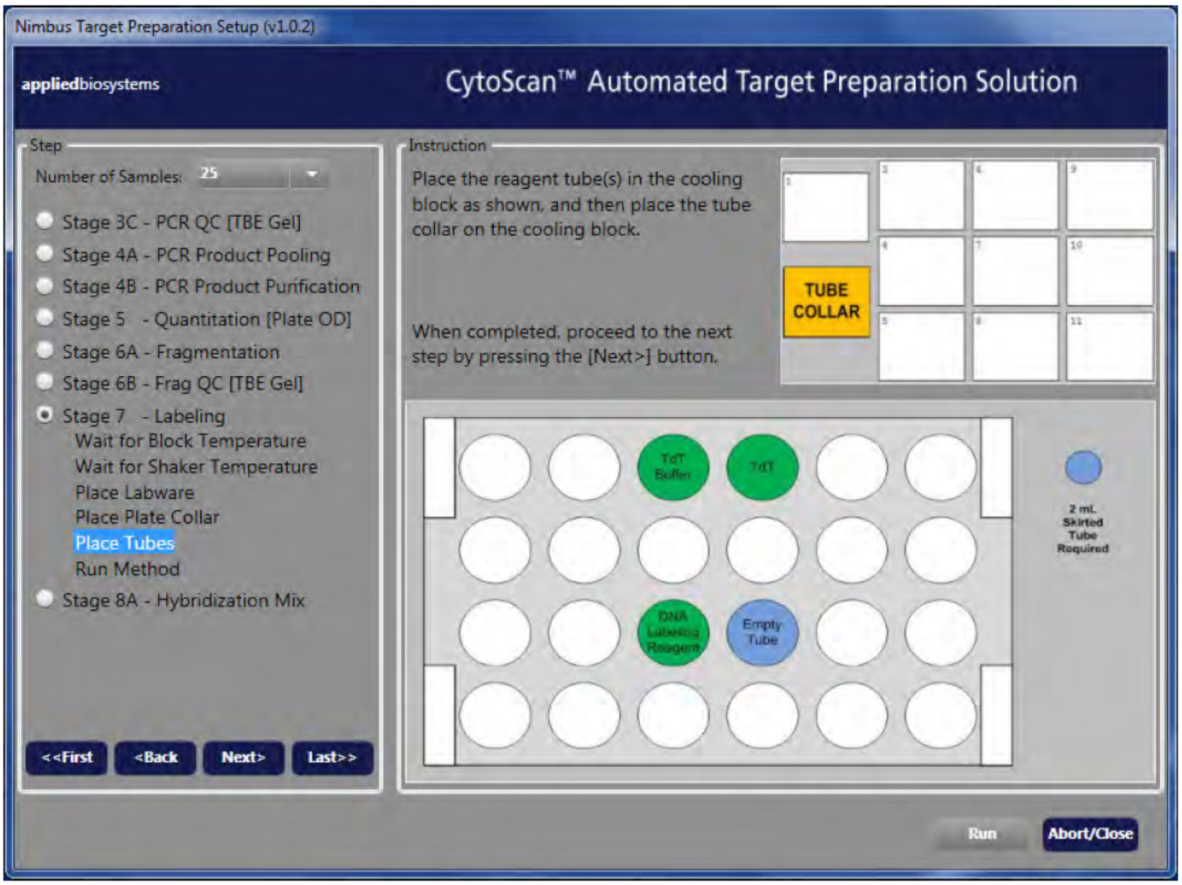
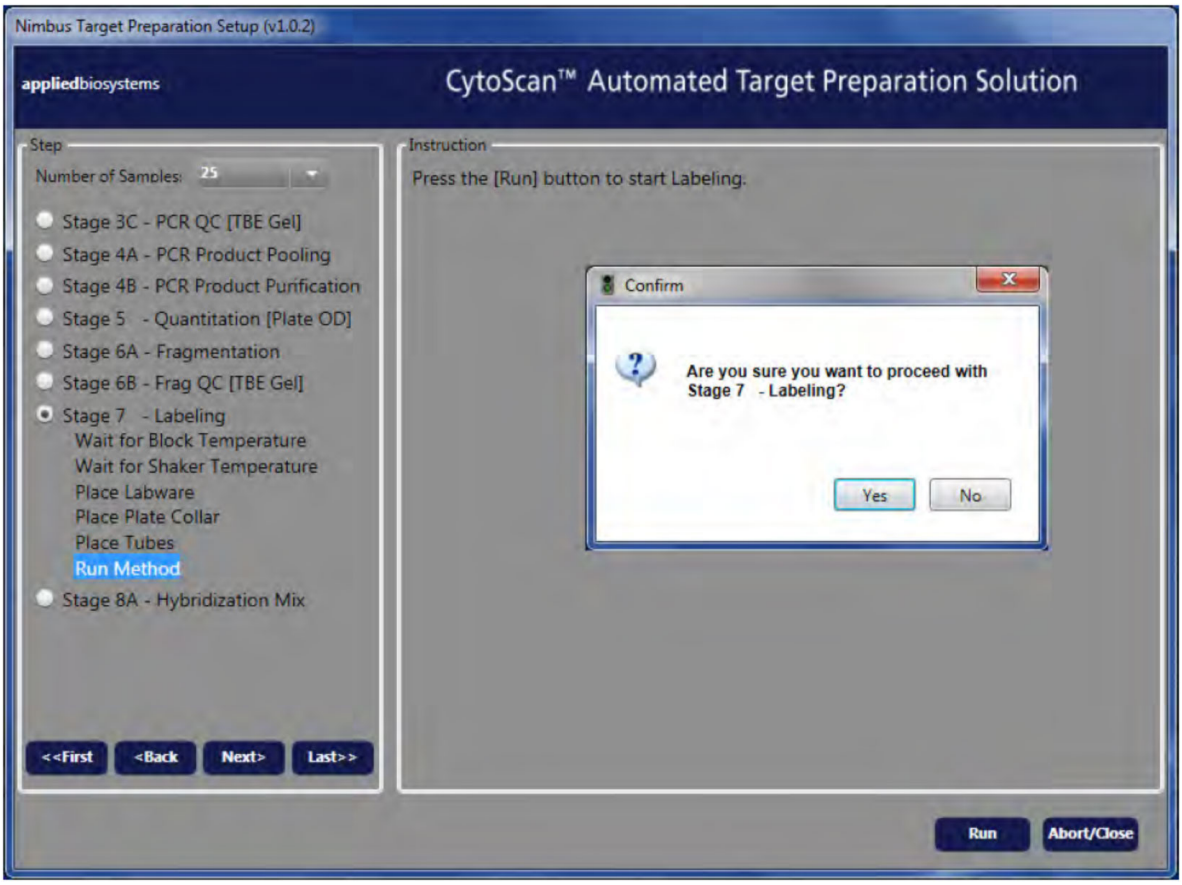


Plate collar on a sample plate

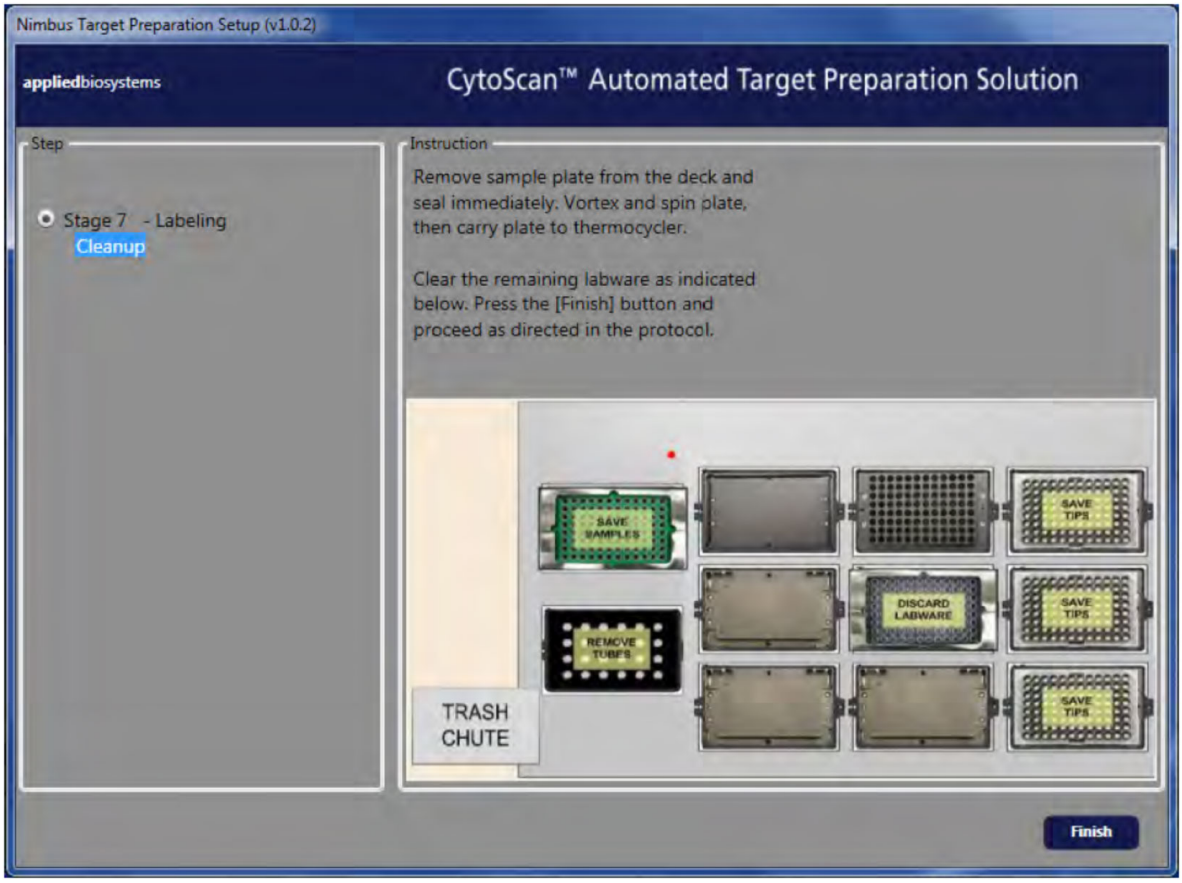
7. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Seal the sample plate tightly with a new seal.

10. Vortex at high speed for 1 second each in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge for 1 minute at 2,000 rpm.

11. Place the labeling plate in the preheated thermal cycler block, then run the **CytoScan Label** thermal cycler protocol. Total volume for Labeling = 70.5 µL

Temperature	Time
37°C	4 hours
95°C	15 minutes
4°C	Hold (OK to hold overnight)

12. When the **CytoScan Label** thermal cycler protocoll is finished, leave the sample plate in the thermal cycler overnight or transfer to a chilled cooling block on ice.

13. Ensure that the plate is tightly sealed, then centrifuge at 2,000 rpm for 1 minute.

14. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

15. Do one of the following:
    - Proceed to "Stage 8A—Hybridization Mix" on page 173.
    - If not proceeding directly to the next stage, do one of the following:
      - Hold at 4°C on the thermal cycler overnight.
      - Freeze the samples at –20°C. The plate can be stored at –20°C for up to 10 days.
16. Click **Finish**, then click **Yes** to confirm.
    The method closes.

Stage 8A—Hybridization Mix

This stage uses NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration. The instrument:

- Prepares the Hybridization Master Mix.
- Aliquots the Hybridization Master Mix into a distribution plate.
- Adds the Hybridization Master Mix to samples and performs mixing.

Note:

- Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.
- To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 8A—Hybridization Mix

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in “Thermal cyclers” on page 41.

Table 30 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Mini centrifuge
1 rack	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	Plates, 96-well, semi-skirted
1	PCR tube rack
1	Reservoir 4 ROW PYR PP 73 mL
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Sample plate with labeled samples from Stage 7
1	Vortexer

Table 31 Reagents required.

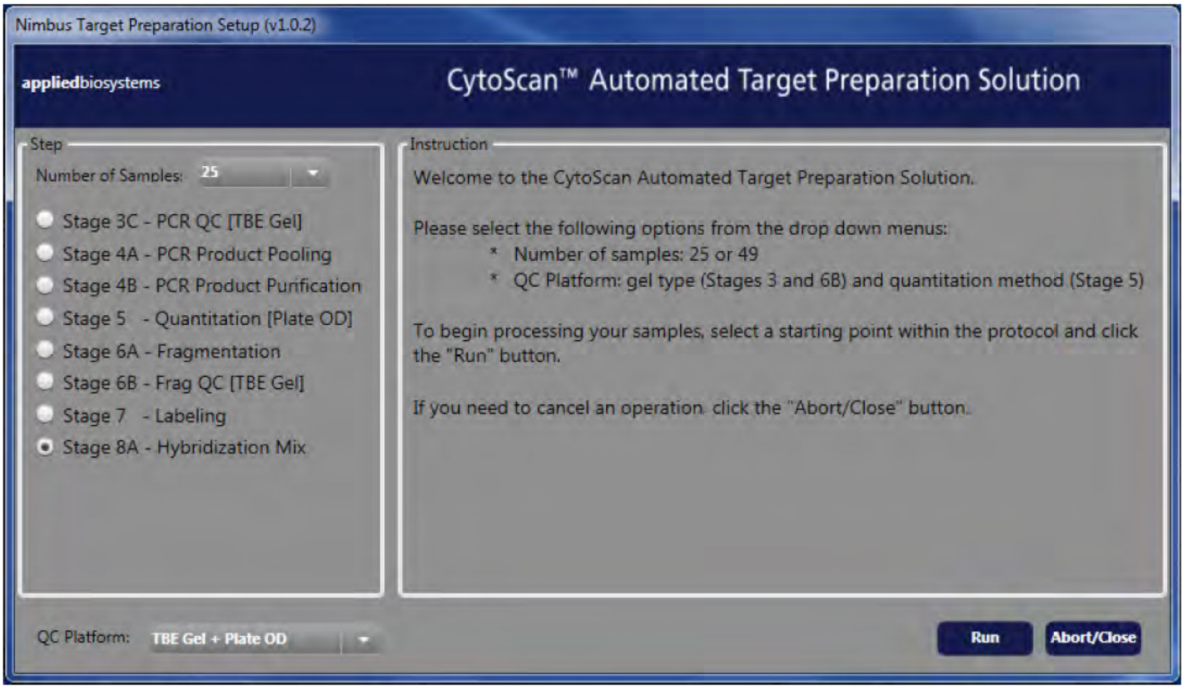
Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	Oligo Control Reagent	Module 3	902678
1	Hyb Buffer Part 1		901725
1	Hyb Buffer Part 2		901726
1	Hyb Buffer Part 3		901727
1	Hyb Buffer Part 4		901728

Prepare the reagents and consumables

1. Place a double cooling block on ice.
2. Prepare the samples.
   a. If the labeled samples from the previous stage are 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.
3. Thaw the following reagents at room temperature. When thawed, immediately place on the cooling block on ice.
   - Hyb Buffer Part 1
   - Hyb Buffer Part 2
   - Hyb Buffer Part 3
   - Hyb Buffer Part 4
   - Oligo Control Reagent
4. Vortex each reagent at high speed 3 times, 1 second each time.
5. Briefly centrifuge for 3 seconds, then place in the cooling block.

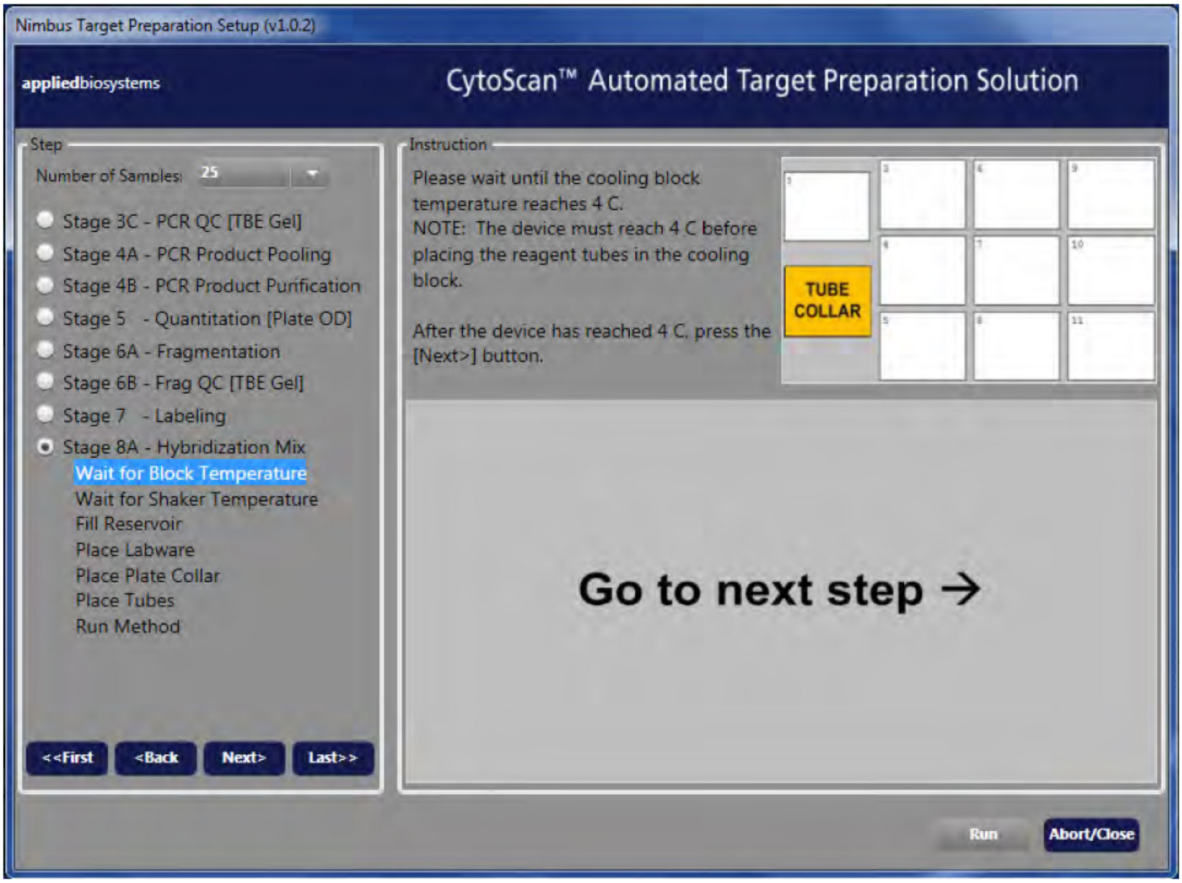
Run the Hybridization Mix method

1. On the **Welcome** screen, select the following options.
   - **Number of Samples**—25
   - **Stage 8A–Hybridization Mix**

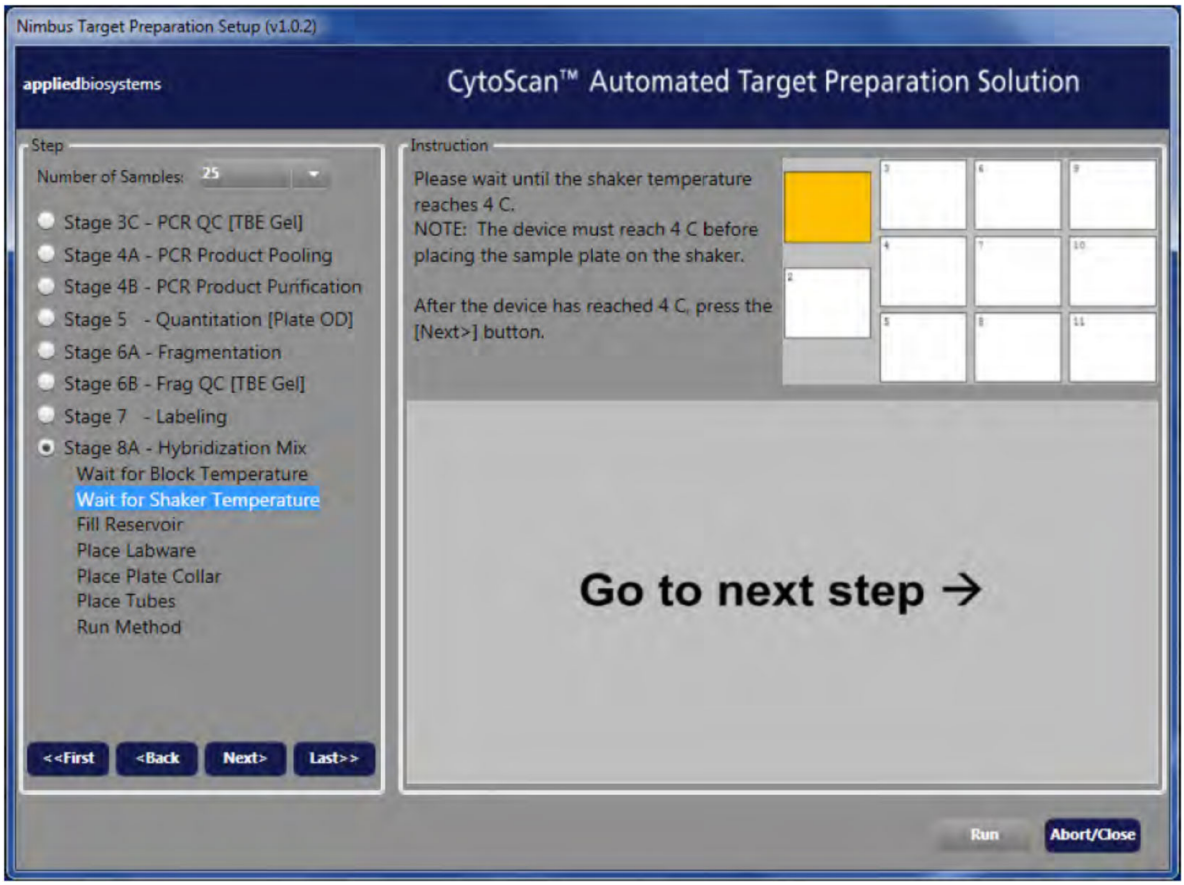


2. Click **Run** to start the method, then click **Yes** to confirm.

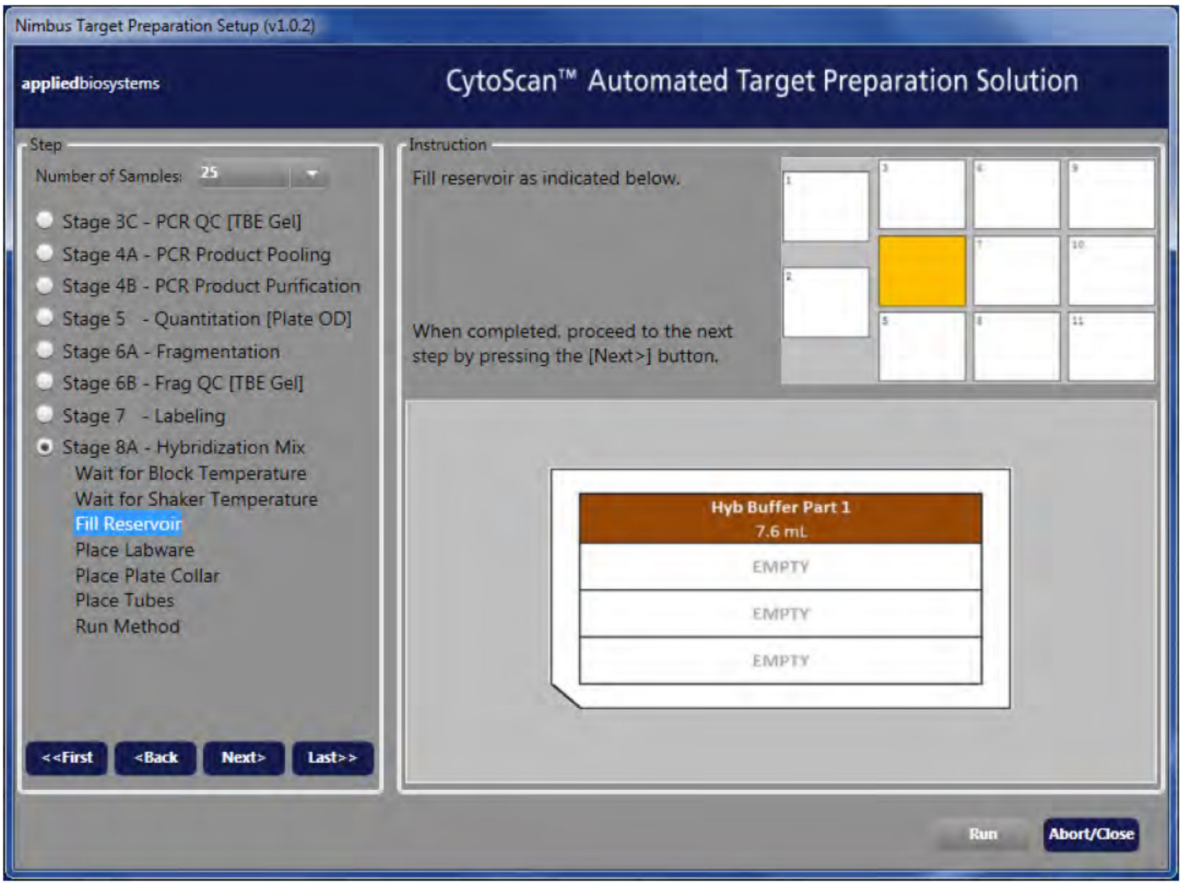
3. Wait until the cooling block reaches 4°C, then click **Next**.



4. Wait until the shaker temperature reaches 4°C, then click **Next**.



5. Fill the reservoir as shown in the on-screen instructions, then click **Next**).

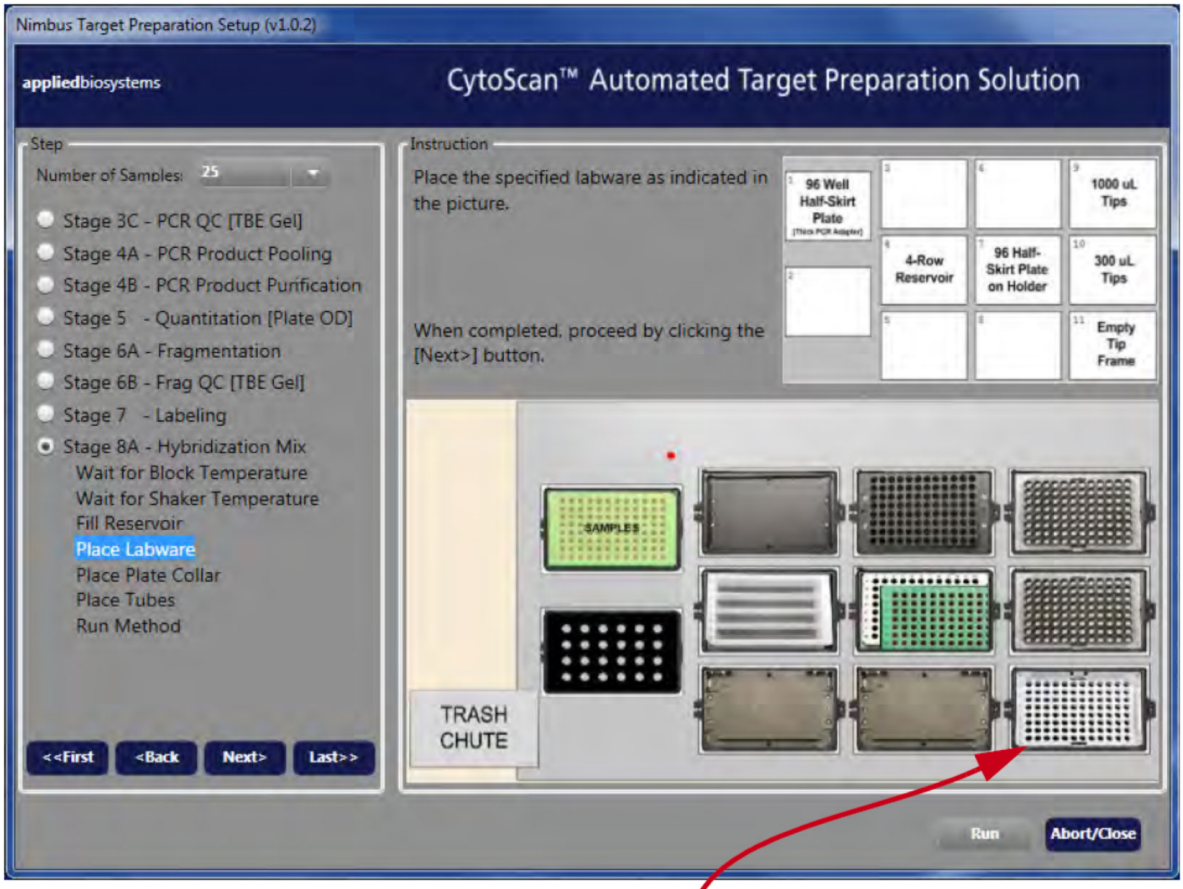


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

Note: Place an empty tip frame on deck position 11.

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.



Empty tip frame for position 11

7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 4, and 7 require a plate collar.

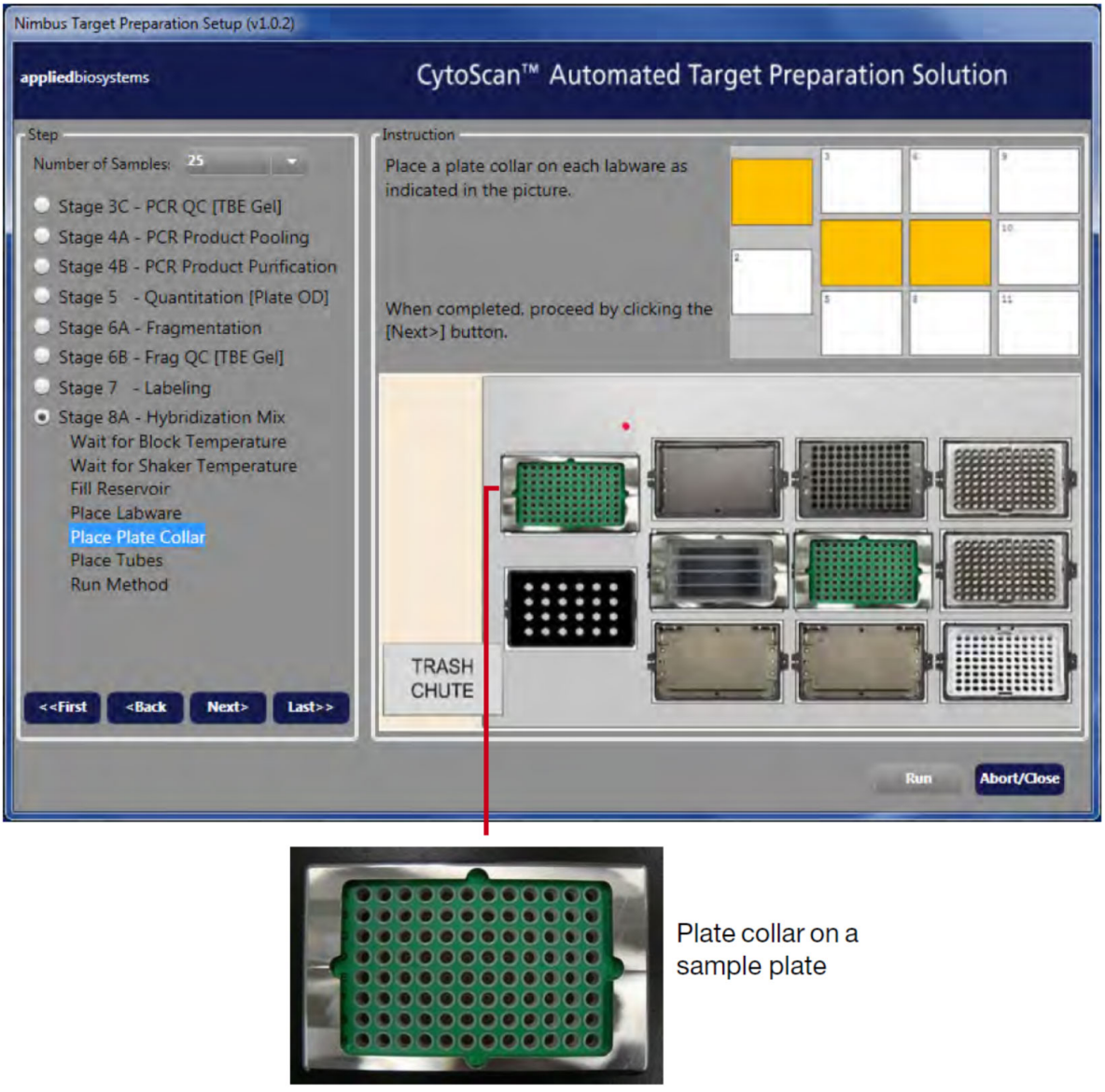
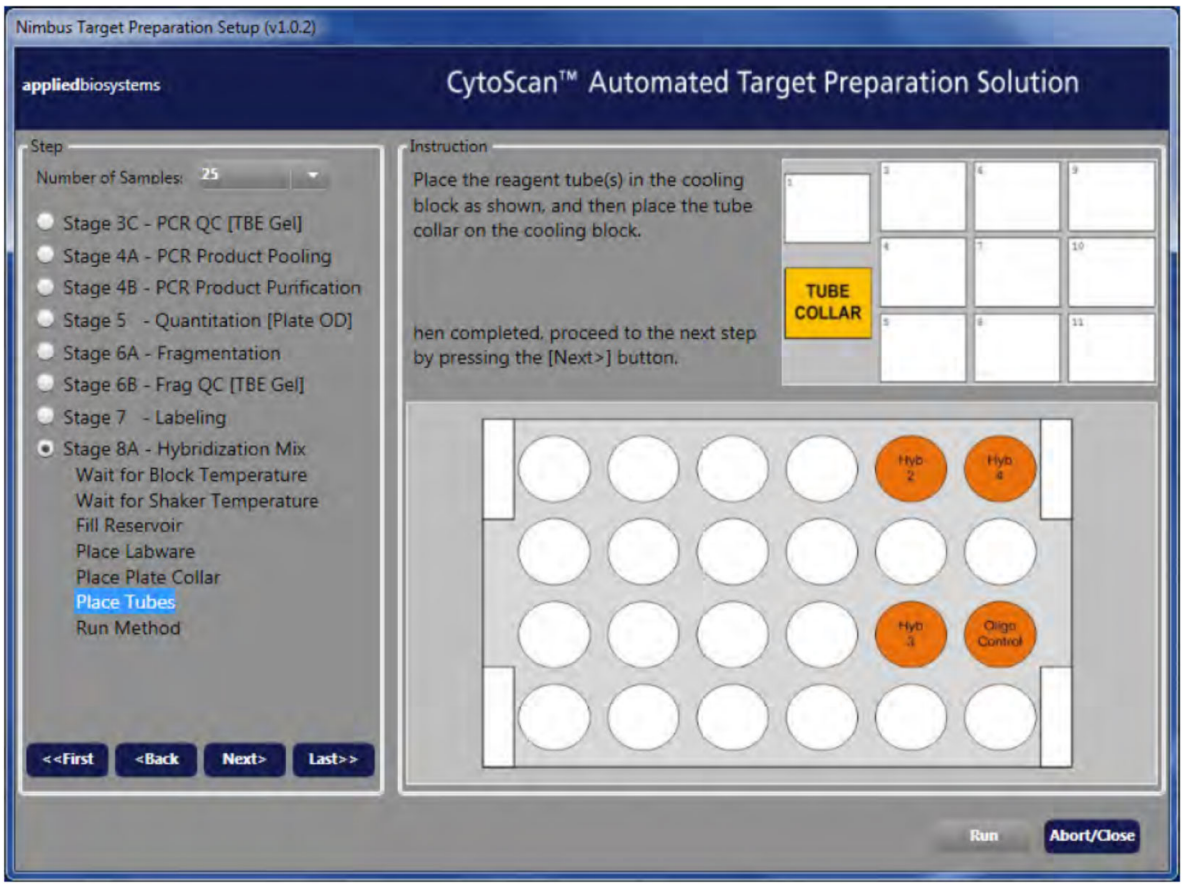
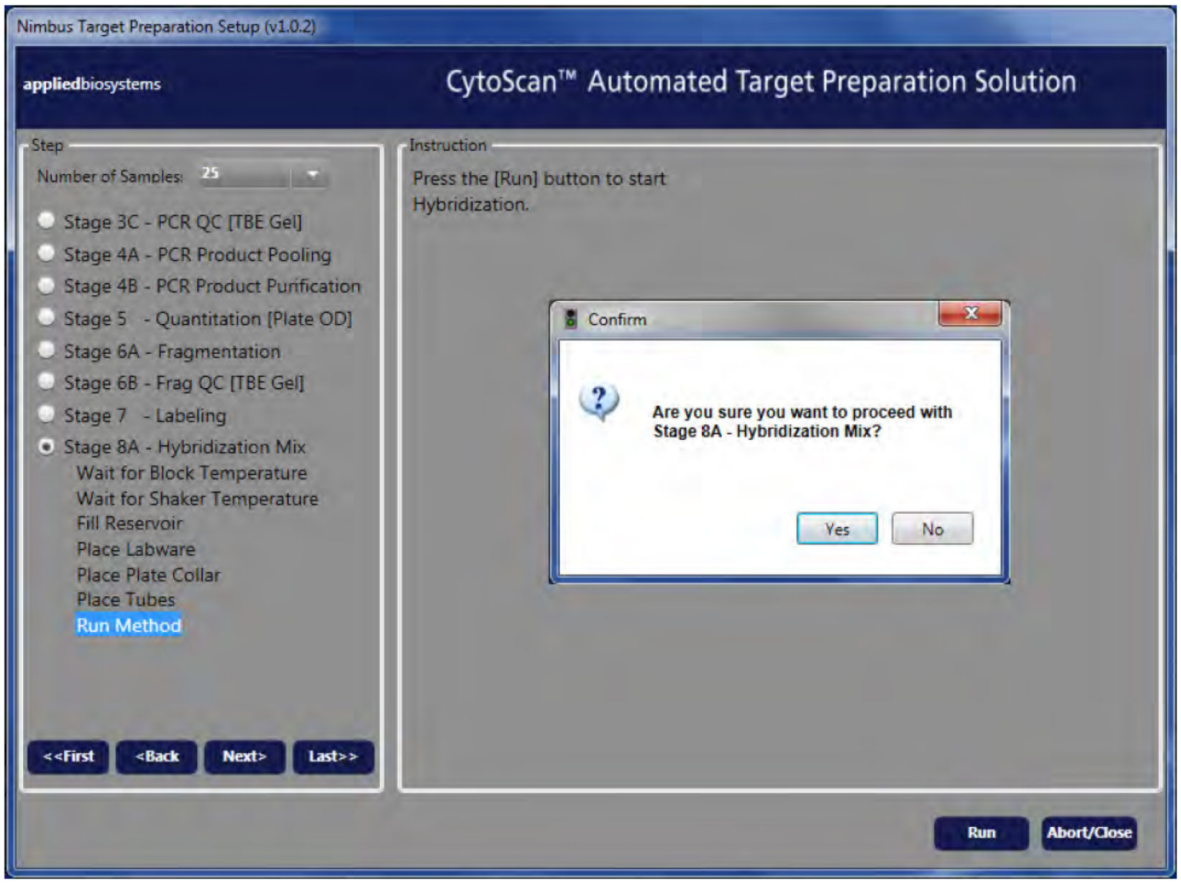


Plate collar on a sample plate

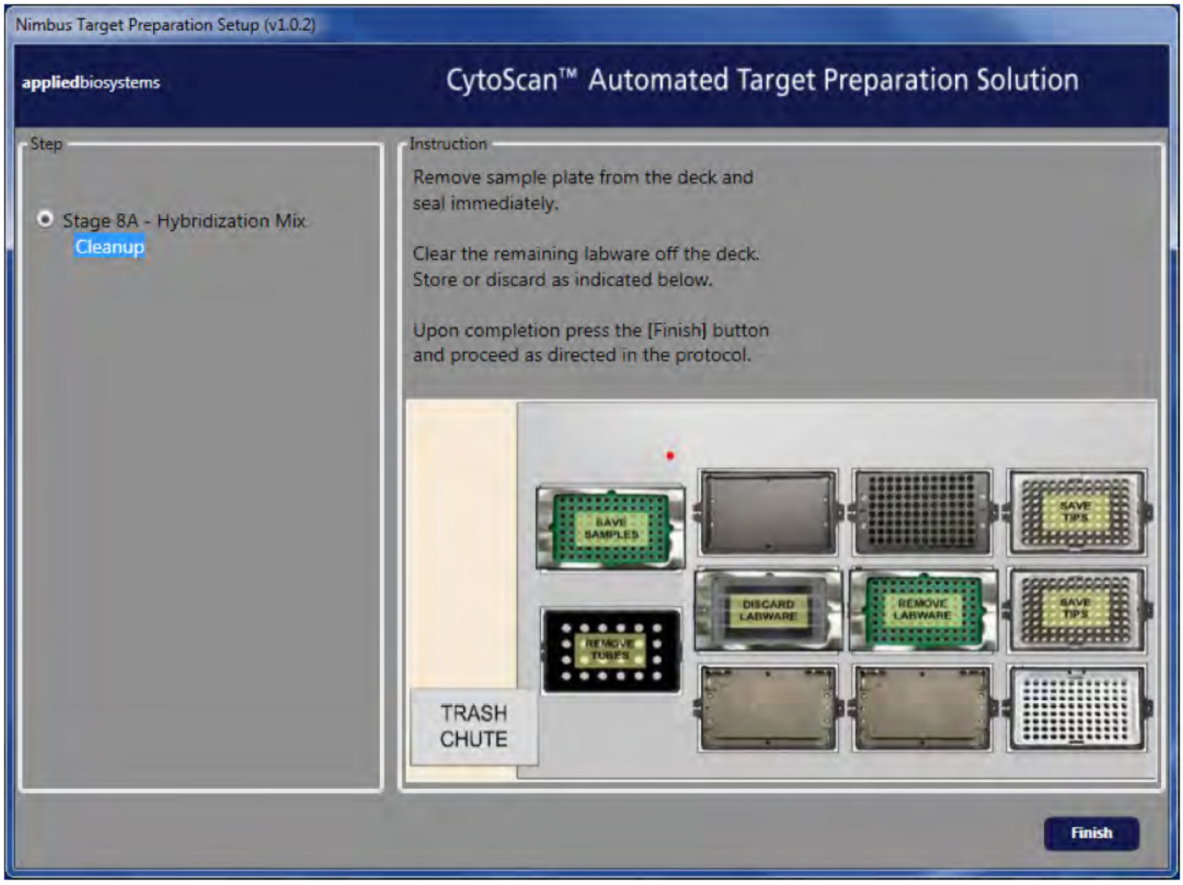
8. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



9. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



10. Tightly seal the sample plate with a new seal. Verify that the plate is well sealed.
11. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.
12. Click **Finish**, then click **Yes** to confirm.
    The method closes.
13. Do one of the following:
    - Hold the plate on ice and proceed to “Stage 8B—Hybridization” on page 184.
    - Freeze the samples at –20°C. The plate can be stored at –20°C for up to 10 days.

Stage 8B—Hybridization

Materials required for Stage 8B—Hybridization

When you prepare the hybridization setup, leave the samples on the cooling block on ice.

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 32 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1 (Optional)	Plate centrifuge
1	Mini microcentrifuge
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1 array per sample	CytoScan™ Array
1	GeneChip™ Hybridization Oven 645
1	Ice bucket, filled with ice
1	Sample plate with Hybridization Mix from Stage 8A
1	Pipette, single-channel P200
As needed	Pipette tips for P200 single-channel pipette
1	Thermal cycler
2 per array	Tough-Spots™ labels, 1/2" diameter
1	Vortexer
2	PCR Tube Racks

Important information about this stage

To ensure the best results, carefully read the following information before you start this stage of the assay. Becase this user guide is intended as an assay protocol document, there is no specific section on all of the various features and workflows available in the GeneChip™ Command Console™ software. To learn more about Command Console™, see the *GeneChip™ Command Console™ User Guide*.

Prepare the equipment

Verify that the GeneChip™ Hybridization Oven 645 is calibrated.

Service the hybridization oven at least once per year to ensure that operation is within specification.

1. Power on the thermal cycler to preheat the lid. Leave the block at room temperature.
2. Preheat the GeneChip™ Hybridization Oven 645.
   a. Power on the oven at least 1 hour before hybridization with the temperature set to 50°C.
   b. Set the rpm to 60.
   c. Turn the rotation on, then allow the oven to preheat for 1 hour before loading arrays.

Prepare the arrays and create a batch registration file

1. Unwrap the arrays, then place the arrays on the benchtop, septa-side up.
2. Mark the front and back of each array with a designation that identifies which sample is loaded onto each array.
3. Allow the arrays to warm to room temperature on the benchtop for 10 to 15 minutes.
   During this time, scan the barcode to use for batch registration.

Create a batch registration file

Use the GeneChip™ Command Console™ (GCC) to create a batch registration file to register a new sample.

IMPORTANT! Verify that you are running GCC v.3.2.2 or higher. If not, update your version of GCC to v.3.2.2 or the latest available.

---

1. From the GCC, launch the GCC Portal.
2. On the **Samples** tab, select **Batch Registration**.
   The **Batch Sample Registration** window opens.

3. In Step 1:
   a. Under **Create a Spreadsheet for**, enter the number of samples for which a spreadsheet needs to be created.
   b. In the **Project Set to** list, select **Default**.
   c. In the **Probe Array type set to** list, select the appropriate array type.
      - If using the CytoScan™ HD Array, select **CytoScan™ HD_Array**.
      - If using the CytoScan™ 750K Array, select **CytoScan™ 750K_Array**.
   d. Click **Download**.
      An Excel™ spreadsheet opens.
4. In Step 2:
   a. Name the experiment file using the following convention: .
      SampleName_PlateCoordinate_ExperimentDescriptionString_ArrayType_OperatorInitials_yyyymmdd
      The sample file name and the 'Array name' would be identical.
   b. Scan the corresponding barcodes for each sample name.

c. Save the Excel™ file in “Excel 97-2003 workbook” format.

5. In Step 3:
   a. Browse to the location of the batch registration file that was saved.
   b. Upload the batch registration file by clicking the tab to create new sample (ARR) files.
6. In the new window, click **Save** to save the new sample files.

Prepare the arrays

1. Place the arrays on a clean benchtop area designated for hybridization.

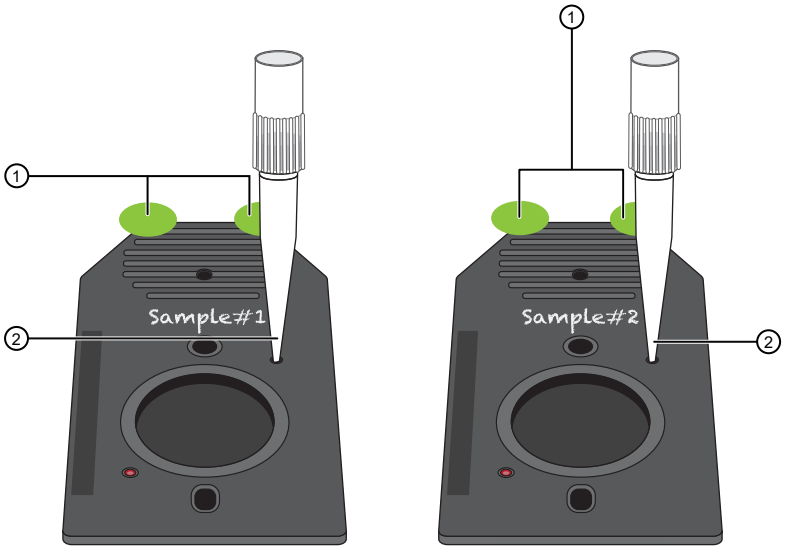
   IMPORTANT! To ensure that the data collected during scanning is associated with the correct sample, mark each array in a meaningful way. It is critical that you know which sample is loaded onto each array.

2. Unwrap the arrays and place on the benchtop, septa-side up.

   Allow the arrays to warm to room temperature on the benchtop for 10—15 minutes. During this time, scan the barcode for use in batch registration.

3. Mark the front and back of each array with a designation that identifies which sample is loaded onto each array.
4. Paste two 1/2” Tough-Spots™ labels on the top edge of the arrays for later use.
5. Insert a 200-µL pipette tip into the upper-right septum of each array.

   ① Tough-Spots™ label dots to cover the septa.
   ② 200-µL pipette tip to vent the array.



6. Vortex the plate at high speed for 1 second each in all corners and in the center. *Repeat* vortexing to ensure that the contents of the plate is well mixed, then centrifuge for 1 minute.

IMPORTANT! The volume in the hybridization plate is full. Vortex the plate to mix the sample and hybridization buffer well.

7. Place the plate onto the preheated thermal cycler, then run the **CytoScan Hybridization** thermal cycler protocol.

Temperature	Time
95°C	10 minutes
49°C	Hold

Load the samples onto arrays

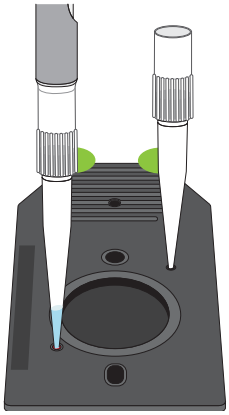
1. When the thermal cycler reaches 49°C, leave the samples at 49°C for at least 1 minute, then open the lid.

IMPORTANT! Load only 6–8 arrays at a time. Remove the seal from the hybridization plate for only 6–8 samples at a time.

2. If you are hybridizing more than 8 samples, cut and remove the seal from 6–8 samples at a time.

   Leave the remaining wells covered. Keeping these wells covered helps prevent cross-contamination and evaporation.

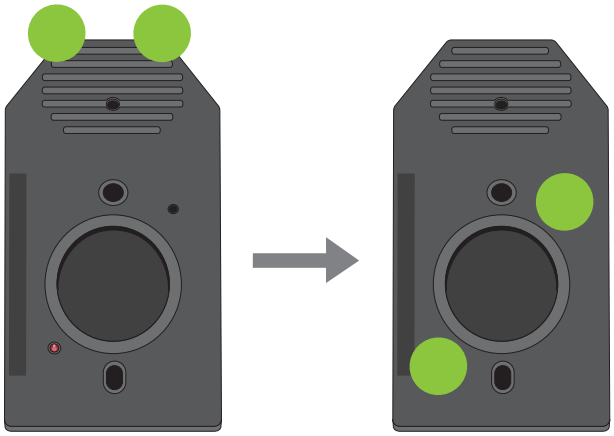
3. Using a P200 pipette, remove 200 µL of the first sample, then immediately inject it into an array.



IMPORTANT! The hybridization mix is viscous. Pipet slowly to ensure that all of the volume is loaded into the array.

4. Cover the septa on the array with the two 1/2” Tough-Spots™ labels that were previously placed on the top edge of the array.

   Press firmly on the labels so that they lay flat and to ensure a tight seal.



5. When 6–8 arrays are loaded and the septa are covered:
   a. Load the arrays into an oven tray evenly spaced.
   b. Immediately place the tray into the hybridization oven.

      Do not allow loaded arrays to sit at room temperature for more than about 1 minute. Ensure that the oven is balanced as the trays are loaded, and ensure that the trays are always rotating at 60 rpm.
6. Repeat this process until all samples are loaded onto arrays and are placed in the hybridization oven.

   Load all samples within 30 minutes.
7. Allow the arrays to rotate in the hybridization oven at 50°C and 60 rpm for 16–18 hours.

   IMPORTANT! This temperature is optimized for this product, and must be stringently followed.
8. For the next steps for processing the arrays, go to Chapter 7, “Wash, stain, and scan arrays”.

6 CytoScan™ Assay Automated Workflow for 49 samples

The Applied Biosystems™ CytoScan™ Assay Automated Workflow processes 25 or 49 samples (including controls) using the NIMBUS™ Target Preparation Instrument with CytoScan™ Assay-specific deck configuration.

This chapter provides instructions for processing 49 samples in parallel. The illustrations in this chapter are based on running 49 samples: 47 genomic DNA samples, plus 1 positive and 1 negative control. Use these illustrations as guidelines when processing 49 samples.

Stage 3C to Stage 8A are performed using the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument.

If processing 49 samples, see Chapter 5, “CytoScan™ Assay Automated Workflow for 25 samples”, which includes important guidelines for plate layouts.

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

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.

Stage 1—Restriction enzyme digestion

Materials required for Stage 1—Restriction enzyme digestion

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 33 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P10
1	Pipette, single-channel P100 or P200
1	Pipette, 12-channel, 2–20 µL
1	Pipette, 12-channel, 20–200 µL
As required	Pipette tips for pipettes
1	Thermal cycler
1	8-well or 12-well strip tubes, 0.2 mL
Optional	8-tube or 12-tube strip caps
1	Eppendorf™ Safe-Lock™ Tubes, 1.5 mL, natural)
1	Vortexer
2	GeneMate™ 96-Well PCR Tube Storage Rack

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

Table 34 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
2	Nsp I	Module 1	901718
2	Nsp I Buffer		901719
2	100X BSA		901720
As required	Chilled Nuclease-Free Water	Module 2	902976
1	Genomic DNA sample plate (49 samples normalized to 50 ng/uL, 1 positive control, and 1 negative control)	—	—

Prepare the equipment, consumables, and reagents

Power on the thermal cycler

Power on the thermal cycler to preheat the lid. Leave the block at room temperature.

Set up the work area for digestion

1. Place a double cooling block and the Nuclease-Free Water on ice.
2. Place an 8-well or 12-well strip tube on the upper half of the cooling block

3. Label a 1.5 mL Eppendorf™ tube as “Dig”, then place in the cooling block.

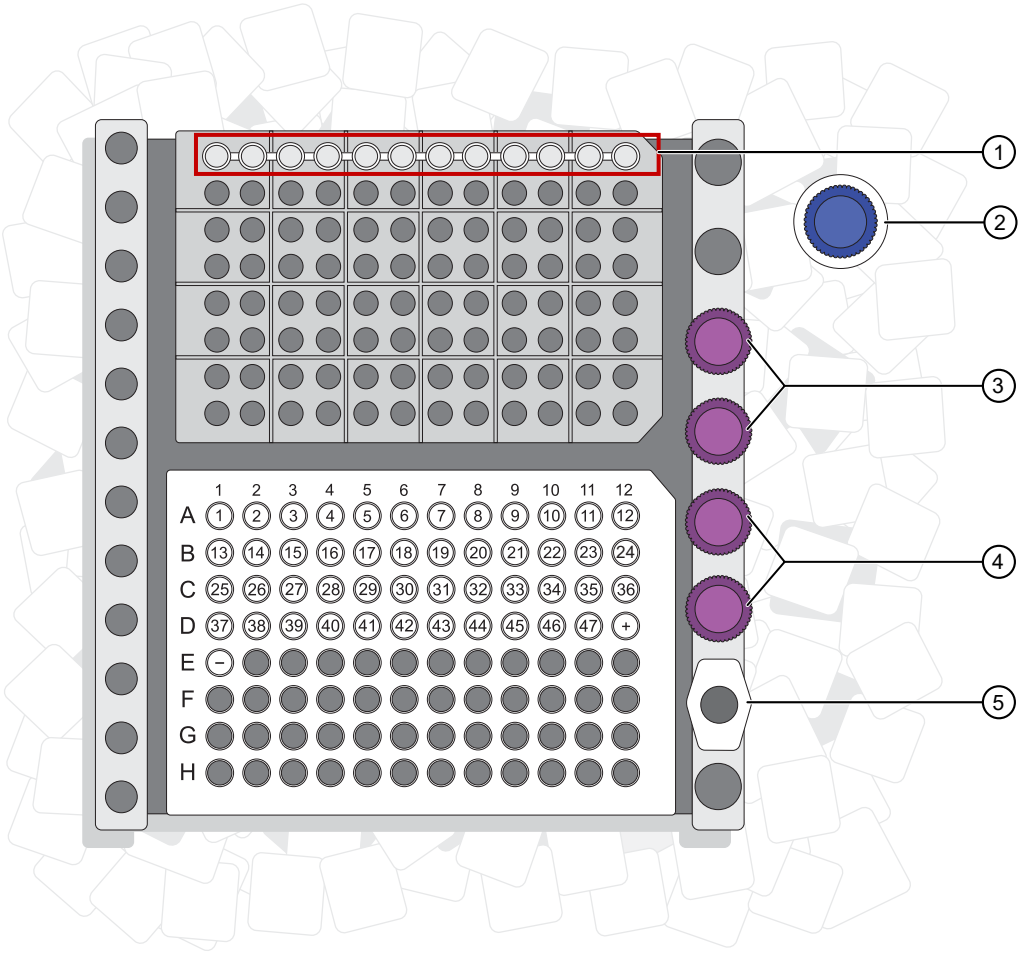


Figure 24 Setup for digestion (Nsp I enzyme not pictured; still at –20°C).

① 8-well or 12-well strip tube to aliquot Digestion Master Mix.
② Nuclease-Free Water.
③ Nsp I Buffer.
④ 100X BSA.
⑤ Digestion Master Mix tube labeled “Dig”.

4. Cut an adhesive seal into strips wide enough to seal 8-well or 12-well strip tubes.

Prepare the genomic DNA and the reagents

1. Prepare the genomic DNA and controls.
   a. Vortex at high speed 3 times, 1 second each time.
   b. Centrifuge at 2,000 rpm for 1 minute.
2. If the plate of genomic DNA and controls is frozen, allow it to thaw at room temperature. Immediately centrifuge the plate at 2,000 rpm for 1 minute and place on the cooling block on ice.

3. Allow the following reagents to thaw at room temperature. When the reagents are thawed, immediately place on the cooling block on ice.
   - Nsp I Buffer (2 tubes)
   - 100X BSA (2 tubes)

   IMPORTANT! Leave the Nsp I enzyme at –20°C until ready to use.

4. Prepare the Nsp I Buffer and 100X BSA.
   a. Vortex 3 times, 1 second each time.
   b. Briefly centrifuge for 3 seconds.
   c. Place in the cooling block on ice.
5. Place Nuclease-Free Water on ice.

Prepare the Digestion Master Mix

For this procedure, keep all reagents, tubes, and the cooling block on ice.

1. To the 1.5 mL Eppendorf™ tube labeled “Dig”, add the following reagents based on the volumes shown in Table 35.
   - Chilled Nuclease-Free Water
   - 10X Nsp I Buffer
   - 100X BSA
2. Place the master mix in the cooling block.
3. Remove the Nsp I enzyme from the freezer and immediately place in a cooler chilled to –20°C.
4. Vortex at high speed for 1 second.
5. Briefly centrifuge the enzyme for 3 seconds. Keep it in the –20°C cooler.
6. Immediately add the enzyme to the master mix.

Table 35 Digestion Master Mix.

Reagent	49 Samples (20% overage)
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. Return the enzyme to the –20°C cooler.
8. Vortex the master mix at high speed 3 times, 1 second each time.

9. Briefly centrifuge for 3 seconds.

10. Place in the cooling block.

Add the Digestion Master Mix to samples

Divide the master mix into strip tubes and dispense the master mix from the strip tubes into the samples using a multichannel pipette.

1. Divide the Digestion Master Mix equally into the 8-well or 12-well strip tubes on ice.

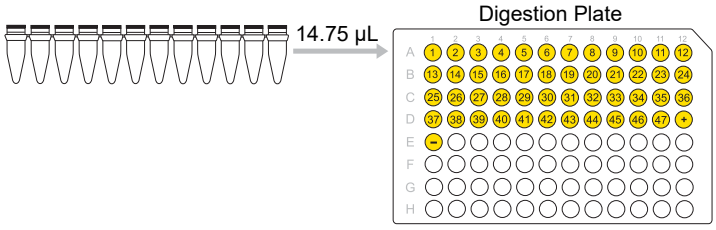
2. Seal the strip tube with an adhesive seal strip (or strip caps).

3. Centrifuge and place back on a cooling block on ice, then remove the seal and discard.

4. Unseal the sample plate, then discard the seal.

5. Using a multichannel P20 pipette, aliquot 14.75 µL of Digestion Master Mix to each sample and control in row A.

Reagents	Volume
Genomic DNA (50 ng/µL)	5.00 µL
Digestion Master Mix	14.75 µL
Total volume	**19.75 µL**



6. Seal the plate tightly with a new seal.

Load samples onto the thermal cycler

1. Vortex the plate at high speed for 1 second in all corners and in the center according to the guidelines in “Seal, vortex, and centrifuge” on page 40, then centrifuge at 2,000 rpm for 1 minute.

2. Ensure that the lid of thermal cycler is preheated.

3. Load the plates onto the thermal cyclers.

4. Run the **CytoScan Digest** thermal cycler protocol.

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

5. Return any remaining reagents to –20°C.

6. When the **CytoScan Digest** thermal cycler protocol is finished, remove the plate from the thermal cycler. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.

7. Do one of the following:

   - Place the plate in a cooling block on ice and proceed immediately to "Stage 2—Ligation" on page 197.
   - If not proceeding directly to Ligation, ensure that the plate is sealed tightly, then store the plate at –20°C.

Stage 2—Ligation

Materials required for Stage 2—Ligation

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 36 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P10
1	Pipette, single-channel P20
1	Pipette single channel P100 or P200
1	Pipette, 12-channel, 2–20 µL
1	Pipette, 12-channel, 20–200 µL
As needed	Pipette tips for pipettes listed above
1	Thermal cycler
1	8-well or 12-well strip tubes, 0.2 mL
As required	8-tube or 12-tube strip caps
1	Eppendorf™ Safe-Lock™ Tubes, 1.5 mL, natural)
1	Vortexer
2	GeneMate™ 96-Well PCR Tube Storage Rack

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

Table 37 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
2	Adaptor, Nsp I	Module 1	902702
2	DNA Ligase Buffer		901722
2	DNA Ligase		901723

Prepare the equipment, consumables, and reagents

Power on the thermal cycler

Power on the thermal cycler to preheat the lid. Leave the block at room temperature.

Set up the work area for ligation

1. Place a double cooling block on ice.
2. Label a 1.5 mL Eppendorf™ tube as *Lig* and place in the cooling block.
3. Place an 8-well or 12-well strip tube on the upper half of the cooling block.
4. Cut an adhesive seal into strips wide enough to seal 8-well or 12-well strip tubes.

Thaw the reagents and digested samples

IMPORTANT! Leave the DNA Ligase enzyme at –20°C until ready to use.

1. Allow the following reagents to thaw at room temperature. When the reagents are thawed, immediately place on the cooling block on ice.
   - Adaptor, Nsp I (2 tubes).
   - DNA Ligase Buffer (2 tubes, requires about 20 minutes to thaw).
2. If the digested samples are frozen, allow them to thaw at room temperature.

Prepare the digested samples and reagents

1. Prepare the digested samples.
   a. Centrifuge at 2,000 rpm for 1 minute.
   b. Place in the lower half of the cooling block on ice.
2. Prepare the reagents.
   a. Vortex the DNA Ligase Buffer and the Adaptor, Nsp I at high speed 3 times, 1 second each time.

   IMPORTANT! Vortex the buffer as long as necessary before use to ensure that any precipitate is resuspended and the buffer is clear.

b. Briefly centrifuge for 3 seconds.

c. Place in the cooling block.

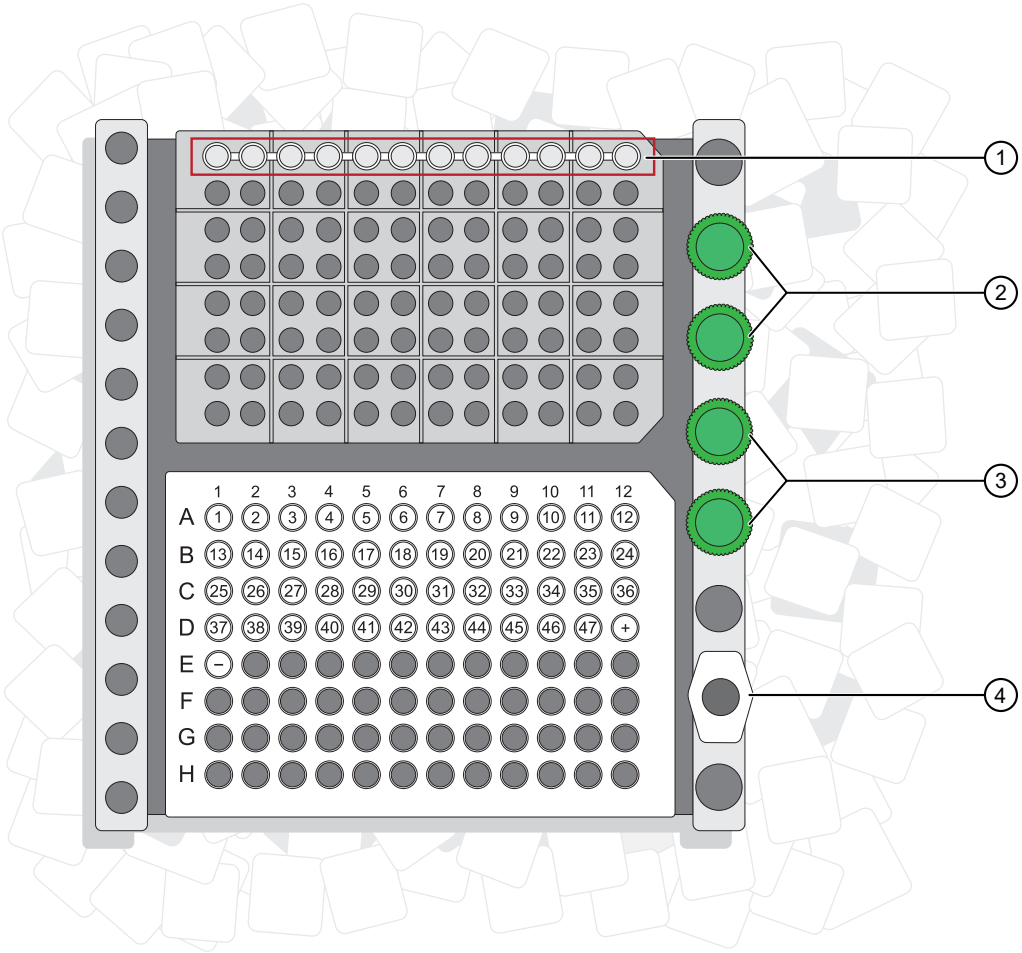


Figure 25 Setup for ligation (DNA Ligase enzyme not pictured; still at –20°C).

① 8-well or 12-well strip tubes to aliquot Ligation Master Mix.
② Adaptor, Nsp I.
③ DNA Ligase Buffer.
④ Ligation Master Mix tube labeled “Lig”.

Prepare the Ligation Master Mix

For this procedure, keep all reagents and tubes on ice.

1. To the 1.5 mL Eppendorf™ tube labeled “Lig”, add the following reagents based on the volumes shown in Table 38:
   - DNA Ligase Buffer
   - Adaptor, Nsp I
2. Remove the DNA Ligase from the freezer, then immediately place in the cooler chilled to –20°C.

3. Vortex at high speed for 1 second.
4. Briefly centrifuge the DNA Ligase for 3 seconds, then place it in the –20°C cooler.
5. Immediately add the DNA Ligase to the master mix, then place back in the –20°C cooler.

Table 38 Ligation Master Mix.

Reagent	49 Samples (25% overage)
DNA Ligase Buffer	150.0 µL
Adaptor, Nsp I	45.0 µL
DNA Ligase	120.0 µL
Total volume	**315.0 µL**

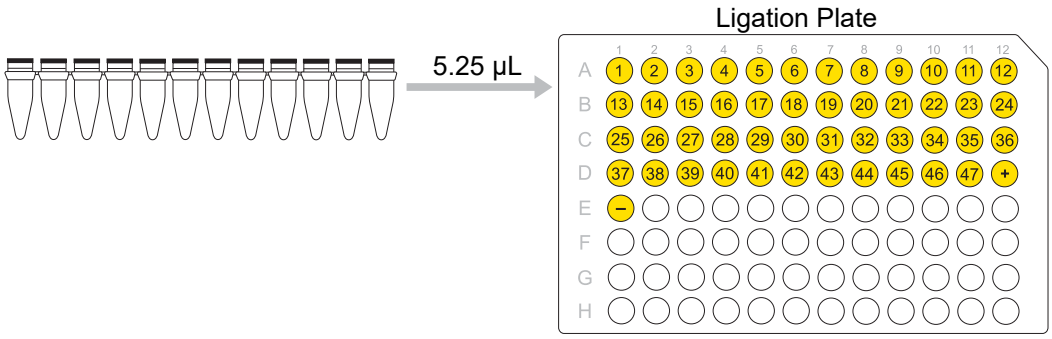
6. Vortex the master mix at high speed 3 times, 1 second each time.
7. Briefly centrifuge for 3 seconds.
8. Place the master mix in the cooling block on ice.
9. Proceed immediately to "Add the Ligation Master Mix to reactions" on page 200.

Add the Ligation Master Mix to reactions

When working with more than 8 samples, we strongly recommend dividing the master mix into strip tubes and dispensing the master mix from the strip tubes into the samples using a multichannel pipette.

1. Divide the Ligation Master Mix equally into the 8-well or 12-well strip tubes on ice.
2. Seal the strip tube with an adhesive seal strip (or strip caps), then briefly centrifuge. Place back in the cooling block on ice, then remove the seal and discard.
3. Unseal the digested sample plate and discard the seal.
4. Using a multichannel P20 pipette, aliquot 5.25 µL of Ligation Master Mix to each digested sample and control.

Reagent	Volume
Digested DNA	19.75 µL
Ligation Master Mix	5.25 µL
Total volume	**25.00 µL**



Load the samples onto the thermal cycler

1. Seal the plate tightly with a new seal.
2. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2000 rpm for 1 minute.
3. Ensure that the thermal cycler lid is preheated.
4. Load the plate onto the thermal cycler.
5. Run the **CytoScan Ligate** thermal cycler protocol.

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

6. Return the remaining reagents to the freezer and discard the remaining master mix.
7. When the **CytoScan Ligate** thermal cycler protocol is finished, remove the plate from the thermal cycler. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.
8. Do one of the following:
   - If following the recommended 3-day workflow, proceed immediately to "Stage 3A—PCR reaction setup" on page 202.
   - Samples can be stored in a cooling block on ice for up to 60 minutes.
   - The sample plate can also be left in the thermal cycler at 4°C hold overnight.
   - If not proceeding directly to the next step, ensure the plate is sealed tightly, then store the plate at –20°C.

Stage 3A—PCR reaction setup

Tip: Before starting Stage 3A, ask someone in the Post-PCR Area to queue up the **CytoScan PCR** protocol on 4 thermal cyclers in preparation for the PCR step.

Materials required for Stage 3A—PCR reaction setup

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 39 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
2	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge
1	Pipette, single-channel P20
1	Pipette, single-channel P100
1	Pipette, single-channel P200
1	Pipette, single channel P1000
1	Pipette, 12-channel, 2–20 µL
1	Pipette, 12-channel, 20–200 µL
As required	Pipette tips for pipettes listed above
4	Plates, 96-well, semi-skirted
2	GeneMate™ 96-Well PCR Rube Storage Rack
1	Reagent reservoir, 25 mL
1	Tube, centrifuge 15 or 50 mL
1	Vortexer
1	Electrophoresis gel box

Table 39 Equipment and consumables required. *(continued)*

Quantity	Item
1	Electrophoresis power supply
2	Thermal cyclers

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

Table 40 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
1	◯ PCR Primer	Module 1	902674
1	● Chilled Nuclease-Free Water	Module 2	902976
1	CytoScan™ Amplification Kit, Cat. No. 902975: • dNTP Mixture (2.5 mM each) • GC-Melt Reagent • 50X Titanium™ *Taq* DNA Polymerase • 10X Titanium™ *Taq* PCR Buffer	The CytoScan™ Amplification Kit is not included in the CytoScan™ Reagent Kit and must be ordered separately.	

Gels and related materials required

Verifying the PCR reaction is required for this stage.

About controls

To determine the presence of contamination, always include 1 PCR negative control with every set of samples in a run.

IMPORTANT! It is crucial to dilute the ligated DNA with chilled Nuclease-Free Water before PCR.

Dilute the ligated samples

If samples are frozen, thaw at room temperature (~30 minutes). Centrifuge 650 x *g* for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.

1. Place Nuclease-Free Water on ice 20 minutes before use.
2. Place a double cooling block on ice.
3. Place a reagent reservoir on the upper half of the cooling block on ice.
4. Pour chilled Nuclease-Free Water into the reagent reservoir.

5. When the **CytoScan Ligate** thermal cycler protocol is finished, take the plate out.
6. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.
7. Place the plate in the lower half of the cooling block on ice.
8. Unseal the ligated sample plate and discard the seal.
9. Using a P200 pipette, add 75 µL of Nuclease-Free Water to each reaction.

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

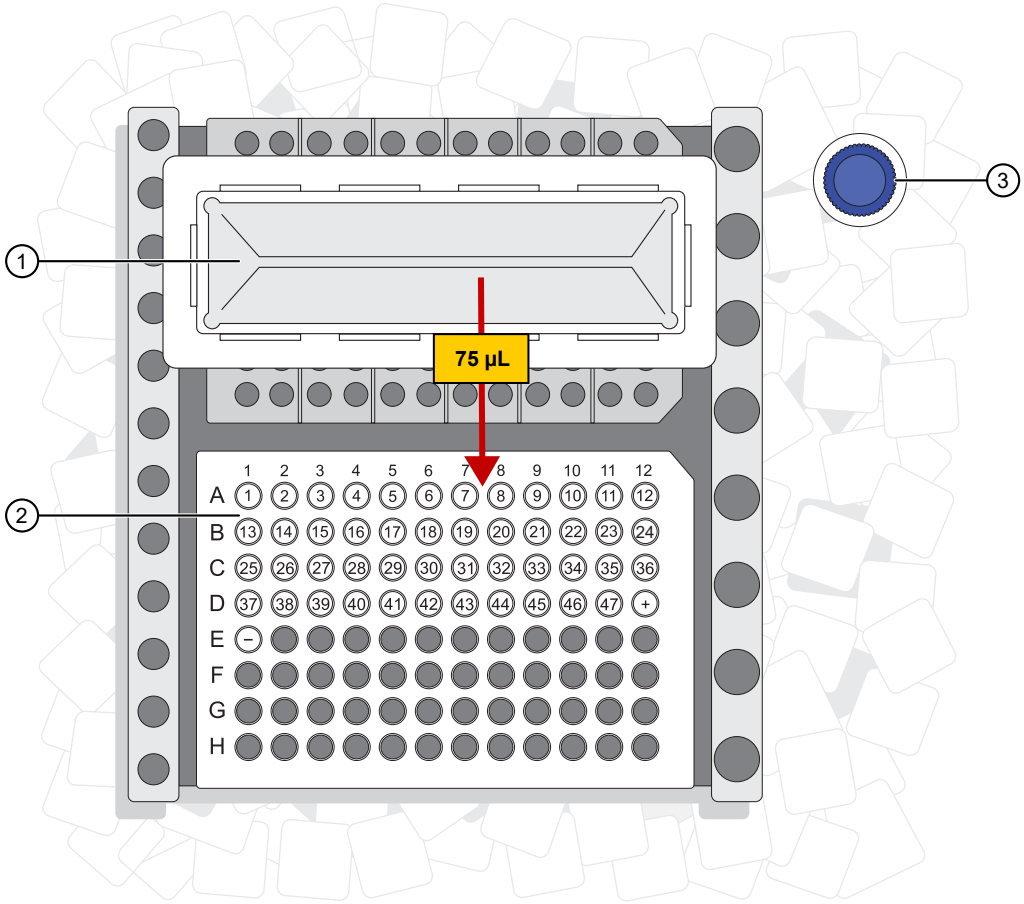


Figure 26 Dilute the ligated samples.

① Chilled Nuclease-Free Water in the reservoir.
② Ligated samples.
③ Nuclease-Free Water.

10. Tightly seal the plate with a new seal.

11. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2,000 rpm for 1 minute.

12. If not proceeding with PCR set up, store the plate at –20°C.

STOPPING POINT The plate can be frozen at –25°C to –15°C for up to 10 days.

Transfer diluted ligated samples to the PCR plate

1. Place 1 single and 2 double cooling blocks on ice.

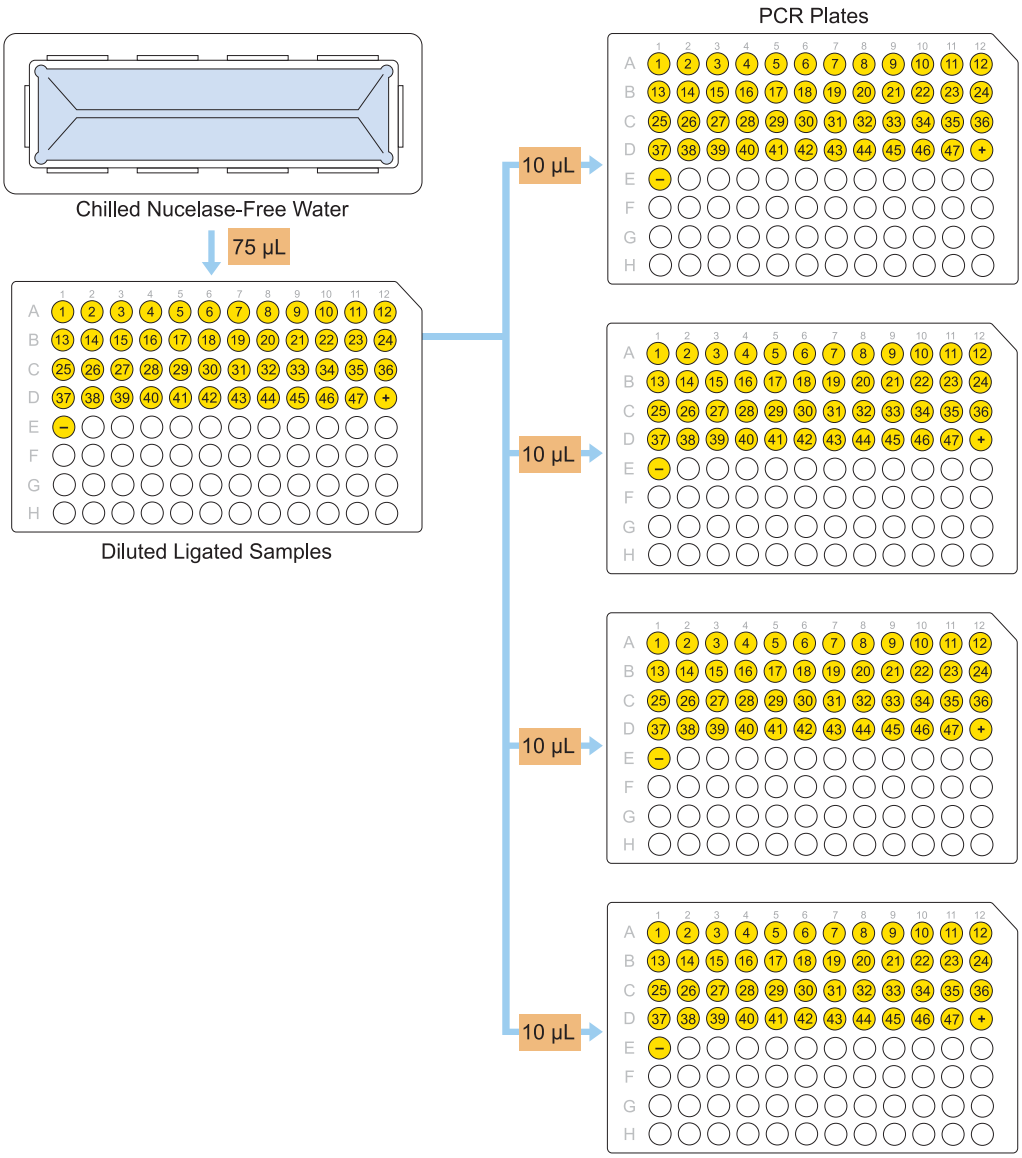
2. Keep the diluted ligated sample plate on the single cooling block.

   If the diluted ligated samples are frozen, thaw them at room temperature. Ensure that the plate is sealed tightly and vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2,000 rpm for 1 minute. Immediately place the plate on the upper half of the cooling block.

3. Label 4 new plates as "PCR" and place on the double cooling blocks, 2 on the upper halves and 2 on the lower halves of each cooling block.

4. Unseal the diluted ligated sample plate and discard the seal.

5. Using a multichannel P20 pipette, transfer 10 µL of each diluted ligated sample to the corresponding wells of a new PCR plate.



6. Seal the plates tightly with a new seal and centrifuge at 2,000 rpm for 1 minute.

7. If not proceeding immediately to PCR stage, store the plate with the remaining samples at –20°C. Left-over samples can be stored at –20°C for up to 10 days.

Thaw the reagents and samples

IMPORTANT! Leave the 50X Titanium™ *Taq* DNA Polymerase at –20°C until ready to use.

Allow the following reagents to thaw at room temperature. Immediately place on the cooling block on ice when reagents are thawed.

- 10X Titanium™ *Taq* PCR Buffer
- dNTP Mixture (2.5 mM each)
- PCR Primer (1 full tube is sufficient for master mix preparation of 49 samples.)

Prepare the samples and reagents

1. Label the 50-mL centrifuge tube “PCR”.
2. Place on ice:
   - Chilled Nuclease-Free Water
   - GC-Melt Reagent
   - Place the reagent reservoir on the bottom half of the cooling block on ice.
3. If the diluted ligated samples aliquoted into the PCR plate are frozen, thaw them at room temperature.
4. When thawed, ensure that the plate is sealed tightly, vortex at high speed for 1 second in all corners and in the center according to the guidelines in “Seal, vortex, and centrifuge” on page 40, then centrifuge at 2,000 rpm for 1 minute.

5. Immediately place on the chamber as shown in the following image.

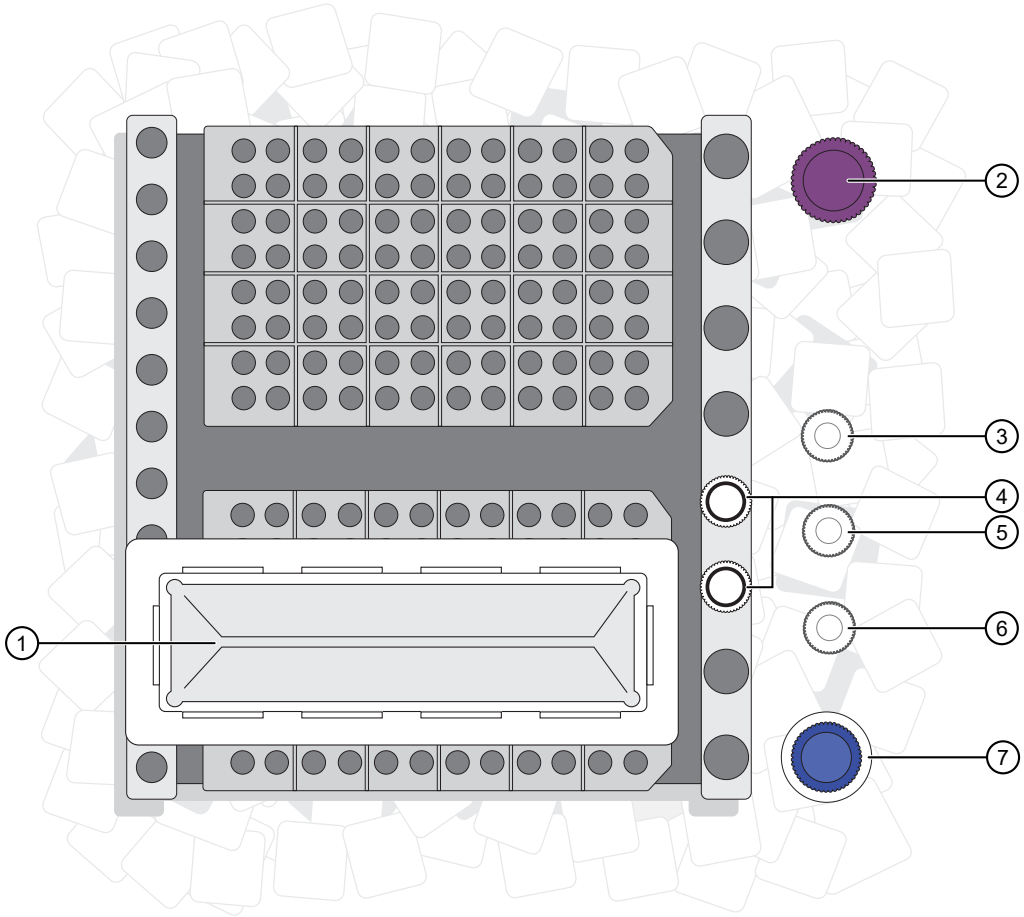


Figure 27 Setup for PCR (Titanium™ *Taq* DNA polymerase not shown; still at –20°C).

1. Reagent reservoir.
2. PCR Master Mix tube.
3. dNTP Mixture (2.5 mM each).
4. PCR Primer (1 full tube is sufficient for master mix preparation of 49 samples).
5. GC-Melt Reagent.
6. Titanium™ *Taq* PCR Buffer.
7. Nuclease-Free Water.

6. Prepare the reagents (except the enzyme):
   a. Vortex the reagents at high speed 3 times, 1 second each time. Briefly centrifuge for 3 seconds.
   b. Place in the cooling block.

Power on 4 thermal cyclers in the Post-PCR Area

Have someone in the Post-PCR Area power on 4 thermal cyclers to preheat the lid. Leave the block at room temperature.

To avoid contamination, do not go from the Pre-PCR Clean Area to the Post-PCR Area, and then back again.

Prepare the PCR Master Mix

IMPORTANT! Accurate pipetting of all components is critical for obtaining the correct size distribution of PCR products.

1. Keeping the 50-mL centrifuge tube labeled "PCR" on ice, add the reagents in the order shown in the following table, *except for the 50X Titanium™ Taq DNA polymeras*e.
2. Remove the 50X Titanium™ *Taq* DNA polymerase from the freezer, then immediately place in a cooler, chilled to –20°C.
3. Vortex at high speed for 1 second.
4. Briefly centrifuge the 50X Titanium™ *Taq* DNA polymerase for 3 seconds.
5. Immediately add the 50X Titanium™ *Taq* DNA polymerase to the master mix, then return the tube to the –20°C cooler.

Table 41 PCR Master Mix.

Reagent	49 Samples (15% overage)
Chilled Nuclease-Free Water	8,721.6 µL
10X Titanium™ *Taq* PCR Buffer	2,208.0 µL
GC-Melt Reagent	4,416.0 µL
dNTP Mixture (2.5 mM each)	3,091.2 µL
PCR Primer	993.6 µL
50X Titanium™ *Taq* DNA Polymerase Do not add until ready to aliquot master mix to ligated samples.	441.6 µL
Total volume	**19,872.0 µL**

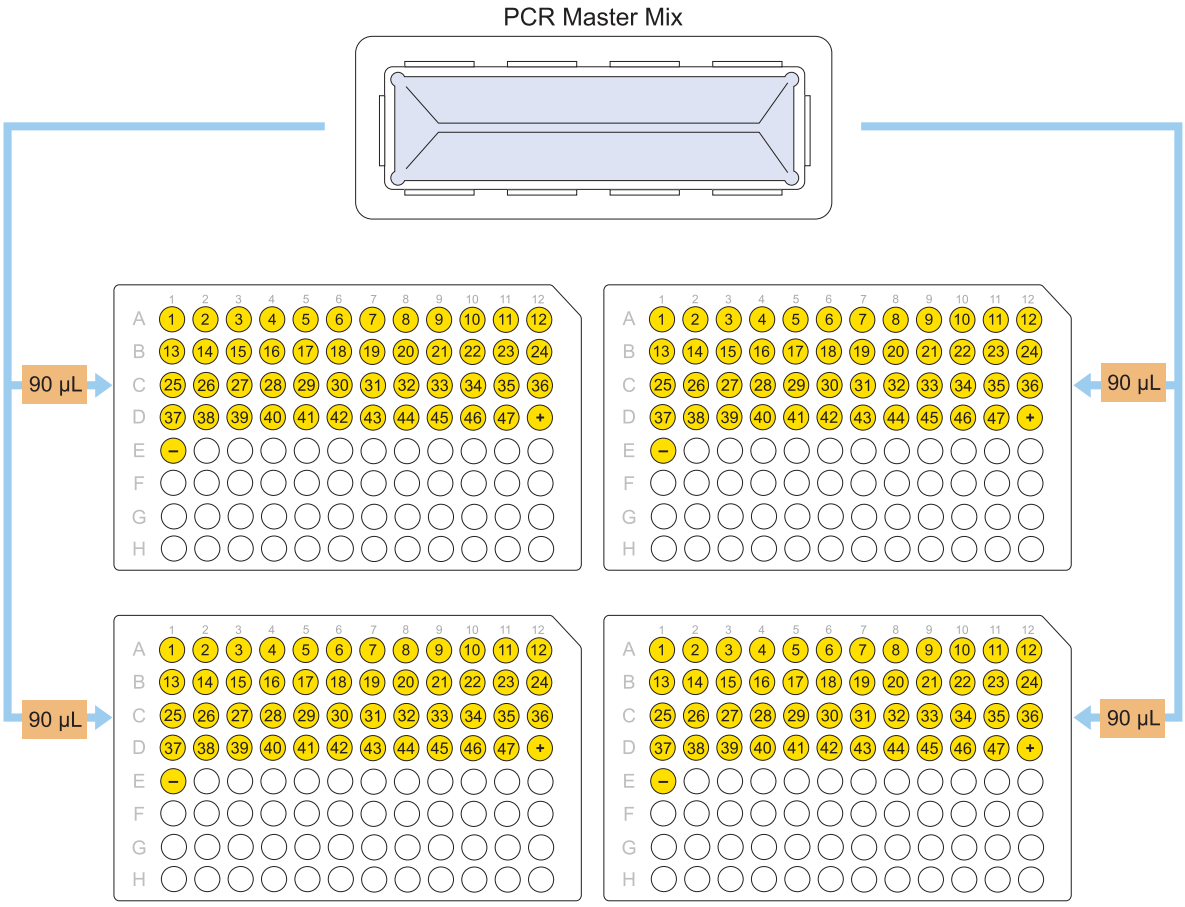
6. Vortex the master mix at high speed 3 times, 1 second each time.
7. Pour the master mix into the reagent reservoir, keeping the cooling block on ice.

Add the PCR Master Mix to each sample

1. Unseal the PCR sample plates and discard the seal.

2. Using a multichannel P200 pipette, aliquot 90 µL PCR Master Mix to each sample and control on the PCR plate.

   To avoid contamination, change pipette tips after each dispense. Ensure that each pipette tip picks up 90 µL.



After adding the master mix, the total volume in each well is 100 µL.

Reagent	Volue
Ligated and diluted DNA	10 µL
PCR Master Mix	90 µL
Total volume	**100 µL**

3. Tightly seal the plates with a new seal according to the guidelines in “Seal, vortex, and centrifuge” on page 40.

4. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in “Seal, vortex, and centrifuge” on page 40.

5. Repeat vortexing 1 more time, then centrifuge at 2,000 rpm for 1 minute.

6. Keep the plates in the cooling block on ice until ready to load onto the thermal cyclers.

Stage 3B—PCR

Equipment required and location

Stage 3B—PCR requires 4 preheated thermal cyclers located in the Post-PCR Area. Ensure that the thermal cycler lid is preheated. Ensure that the block is room temperature (15–30°C).

Load the plates and run the CytoScan PCR thermal cycler protocol

1. Transfer the plates on ice to the Post-PCR Area.
2. Ensure that the thermal cycler lid is preheated.
   Keep the block at room temperature.
3. Load the plates onto the thermal cyclers.
4. Run the **CytoScan PCR** thermal cycler protocol.

   IMPORTANT! Use only silver or gold-plated silver blocks.

Temperature	Time	Cycles
94°C	3 minutes	1X
94°C 60°C 68°C	30 seconds 45 seconds 15 seconds	30X
68°C	7 minutes	1X
4°C	Hold (Can be held overnight)	
Volume: 100 µL		

   After the **CytoScan PCR** thermal cycler protocol is finished, the plate can be left on hold at 4°C in the thermal cycler for up to 24 hours.
5. When done, leave the plate in the thermal cycler.
   If proceeding to purification, process the plate within 2.5 hours.
6. After removing the plate from the thermal cycler, keep the plate on a 96-well plate rack.
7. Ensure that the plate is tightly sealed, then centrifuge at 600 x *g* for 1 minute.
   If not proceeding immediately to the next step, the plate can be stored at –20°C for up to 10 days.

Stage 3C—PCR product check

This stage verifies the PCR reaction by using a TBE gel or an E-Gel™ Agarose Gel. This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration.

First, start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument, then follow the instructions for:

- "PCR product check using TBE gels" on page 215.

  or

- "PCR product check using E-Gel™ Agarose Gels" on page 222.

Materials required for Stage 3C—PCR product check

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 42 Materials required for running a TBE gel.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Electrophoresis gel box
1	Electrophoresis power supply
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1	Reservoir 4 ROW PYR PP 73 mL
1 tube	25 bp DNA Ladder
1	Gels, 2% TBE (precast or house-made)
1	1X TBE Buffer
1	Ethidium bromide solution
1	5X RapidRun™ Loading Dye
1	96-well Full Skirt PCR Plate, clear

Table 43 Materials required for running an E-Gel™ Agarose Gel.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1	MicroAmp™ Adhesive Film Applicator
1	Mother E-Base™ Device
1	Daughter E-Base™ Device (optional for running multiple gels simultaneously)
1 rack	Conductive 50 µL filter tips in frames
1 rack	Conductive 300 µL filter tips in frames
2	E-Gel™ *48* Agarose Gels, 2%
1	*Redi*Load™ Loading Buffer
1	TrackIt™ Cyan/Orange Loading Buffer
1	PCR marker (50–2,000 bp)

Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument

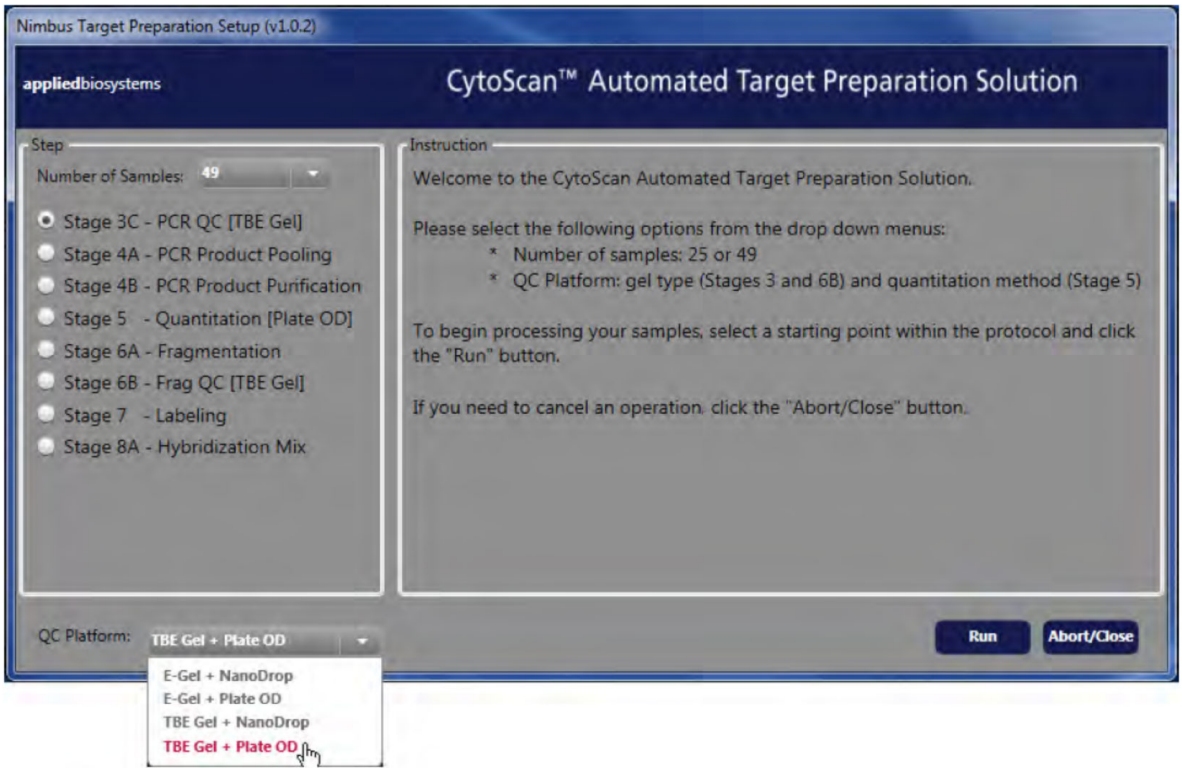
1. Power on the NIMBUS™ Instrument.
2. To open the **Welcome** screen, double-click the CytoScan™ icon on the desktop.

PCR product check using TBE gels

WARNING! E-Gel™ Agarose Gels and TBE gels contain ethidium bromide. Review the manufacturer's Safety Data Sheet for proper handling and disposal. Use good laboratory practices and always wear protective gloves when handling ethidium bromide. Dispose of the gel and gloves in accordance with national, state, and local regulations.

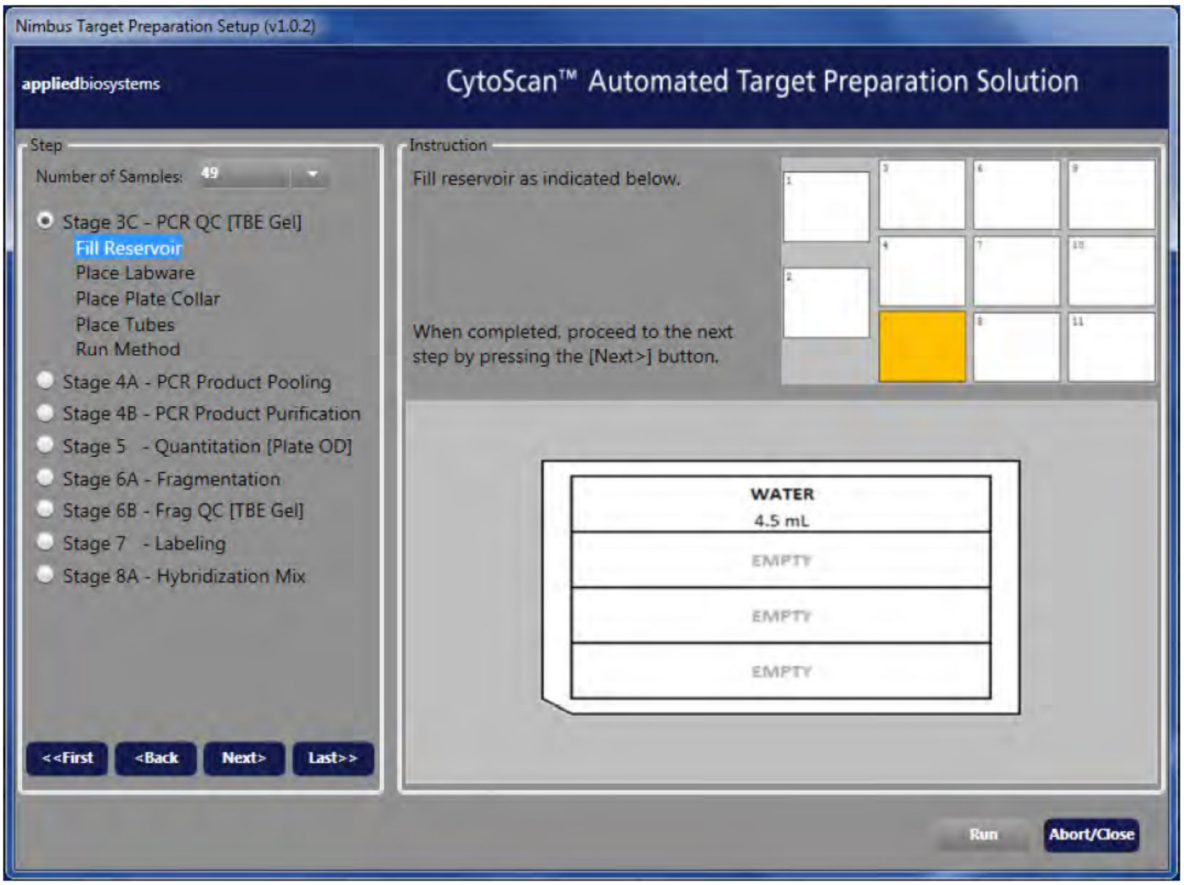
Note: If samples are frozen, thaw at room temperature (~30 minutes). Centrifuge 650 x *g* for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.

1. Remove the plate from the thermal cycler.
2. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute.
3. Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument.
   See "Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument" on page 214.
4. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 3C–PCR QC (TBE Gel)**
   - **QC Platform**—TBE Gel + a quantitation method (for example, "TBE + Plate OD")



5. Click **Run** to start the method, then click **Yes** to confirm.

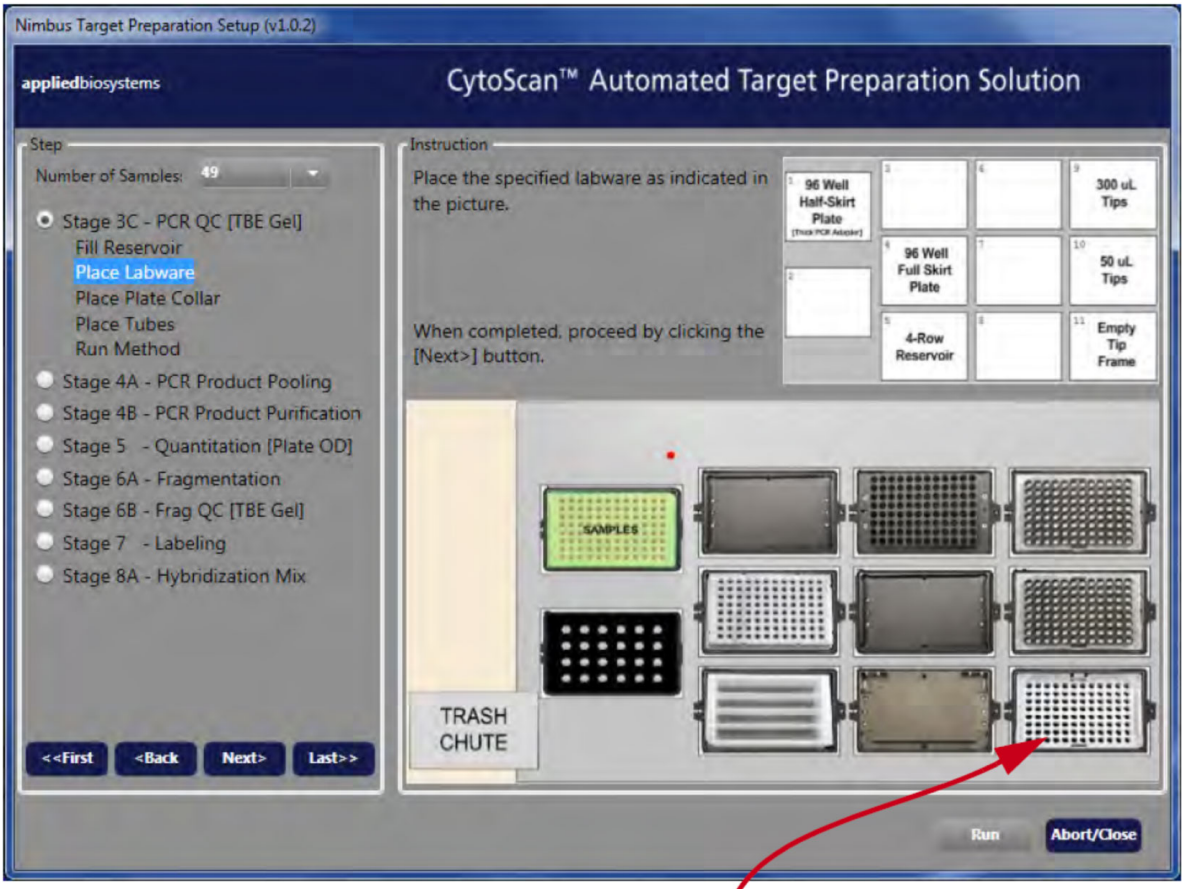
6. Fill the reservoir as shown in the on-screen instructions, then click **Next**.



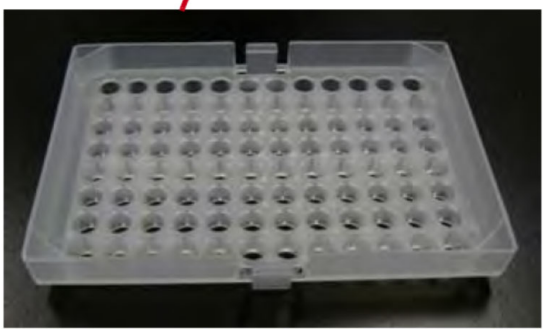
7. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions.

Note: Place an empty tip frame on position 11.

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.



Empty tip frame for position 11

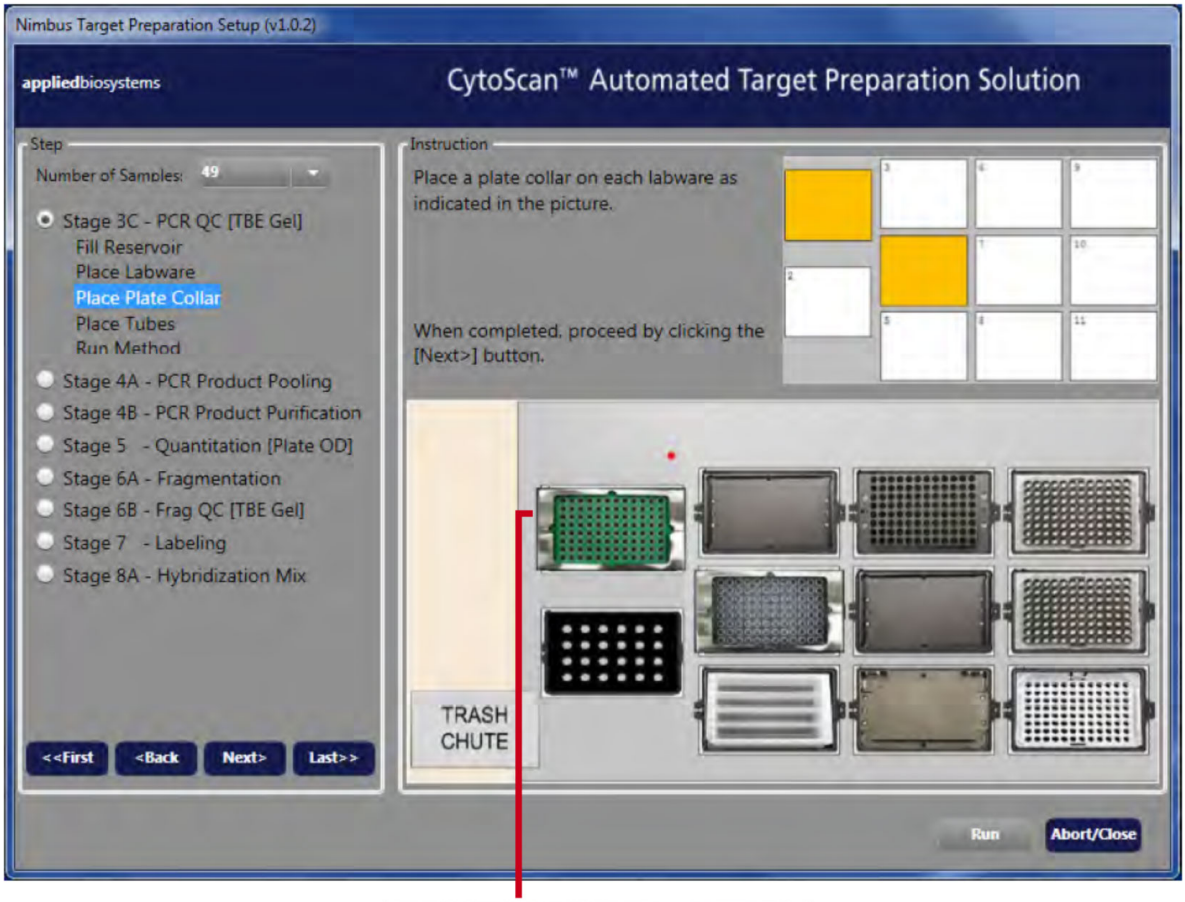


8. Unseal one of the PCR plates and discard the seal. Place the plate on the NIMBUS™ Instrument deck as shown in the previous image, then click **Next**.

   IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

9. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**.

   Labware at deck positions 1 and 4 require a plate collar.



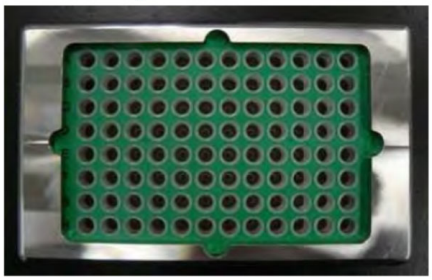
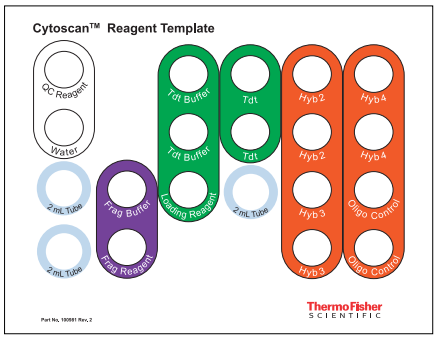
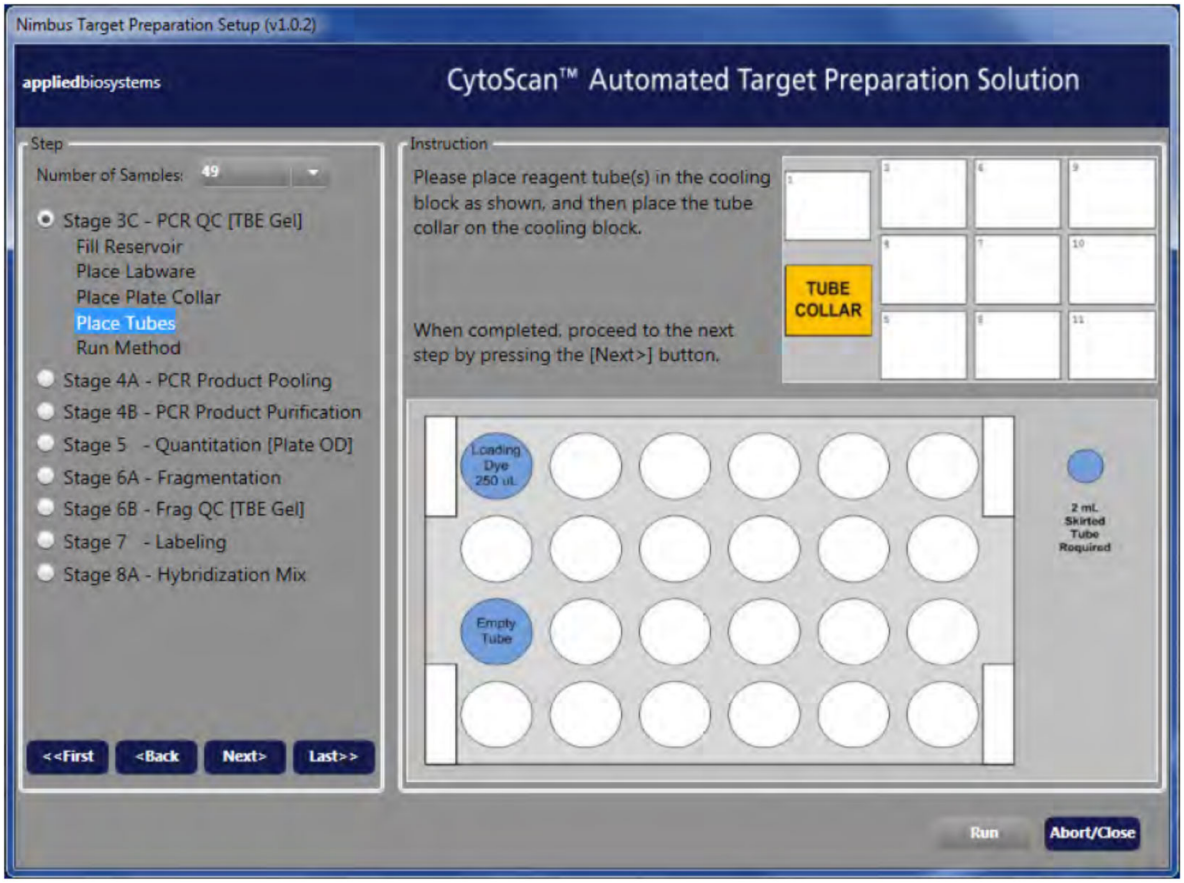


Plate collar on a sample plate

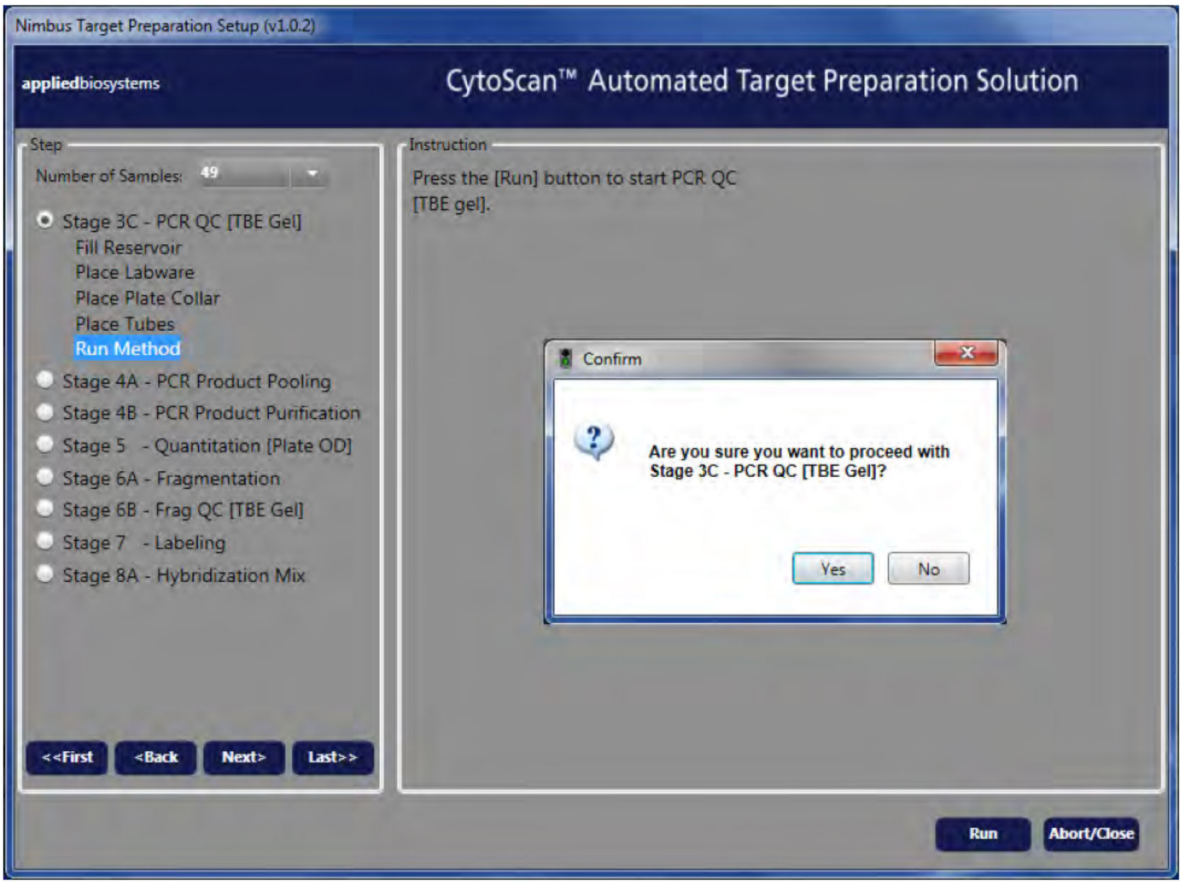
10. Place the tubes as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.

IMPORTANT! Use the CytoScan™ Reagent Template, Cat. No. 100981 Rev. 2. The template accommodates the fragmentation protocol and reagents.

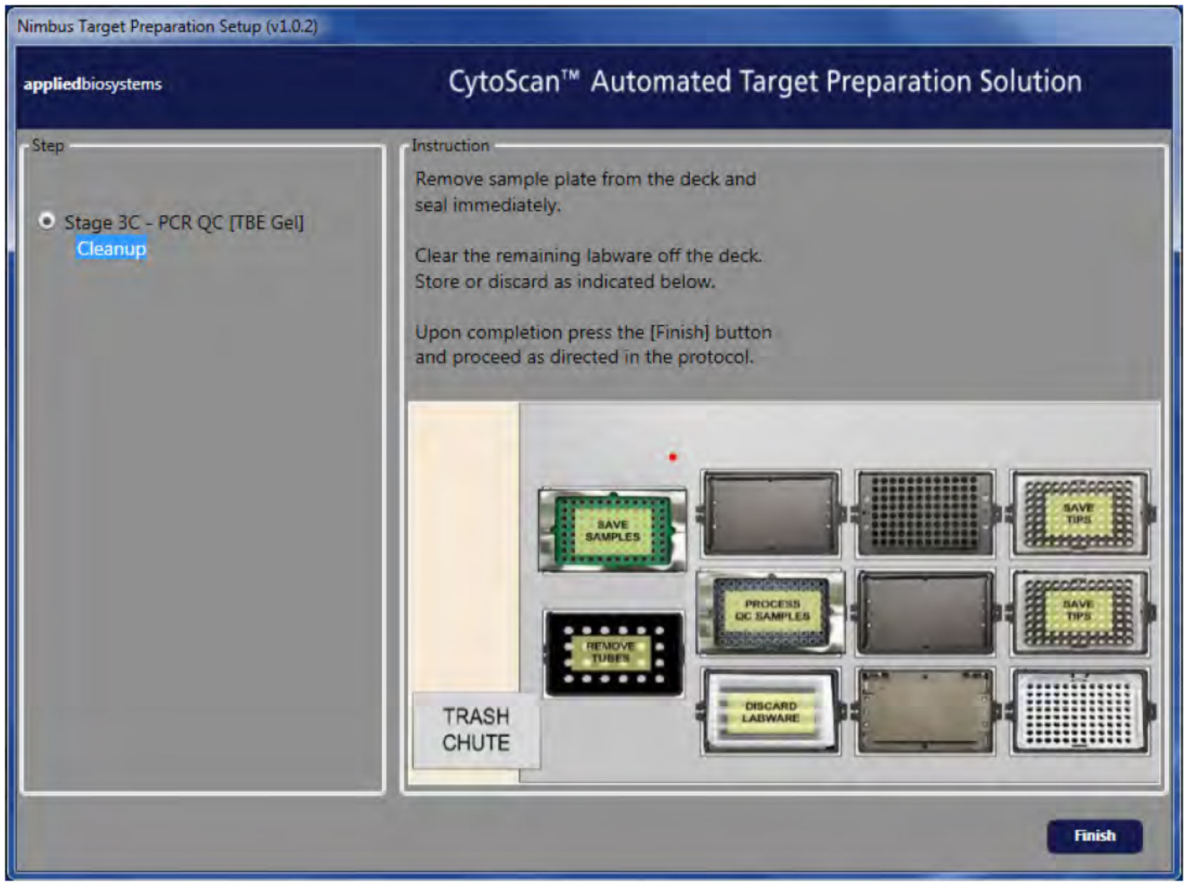




11. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



12. Remove the PCR plate from the NIMBUS™ Instrument deck, then seal the plate tightly with a new seal. If not proceeding to Stage 4B—PCR product purification, store the plate at –20°C.

13. Remove the gel QC plate from the NIMBUS™ Instrument deck, then seal the plate tightly with a new seal.

14. Remove labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

15. Click **Finish**, then click **Yes** to confirm.

    The method closes.

16. Vortex the gel QC plate, then centrifuge briefly in the plate centrifuge.

Note: Add ethidium bromide to the gel running buffer. Add 2 drops of ethidium bromide per liter of 1X TBE.

Run the TBE gel

1. Load 8 µL of the samples from the QC Sample Plate onto a 2% TBE gel.

2. Load 5 µL 25 bp DNA Ladder to the first and last wells of the gel.

3. Run the gel at 5 V/cm for 45 minutes or until the dye front reaches at least 75% of distance down the gel.

   Run gels at 5 V/cm (5 volts X distance in cm between electrodes). For example, run a 33-cm electrophoresis box at 165 V; run a 16-cm electrophoresis box at 80 V.

4. Verify that the PCR product distribution is between approximatey150 bp to 2,000 bp.

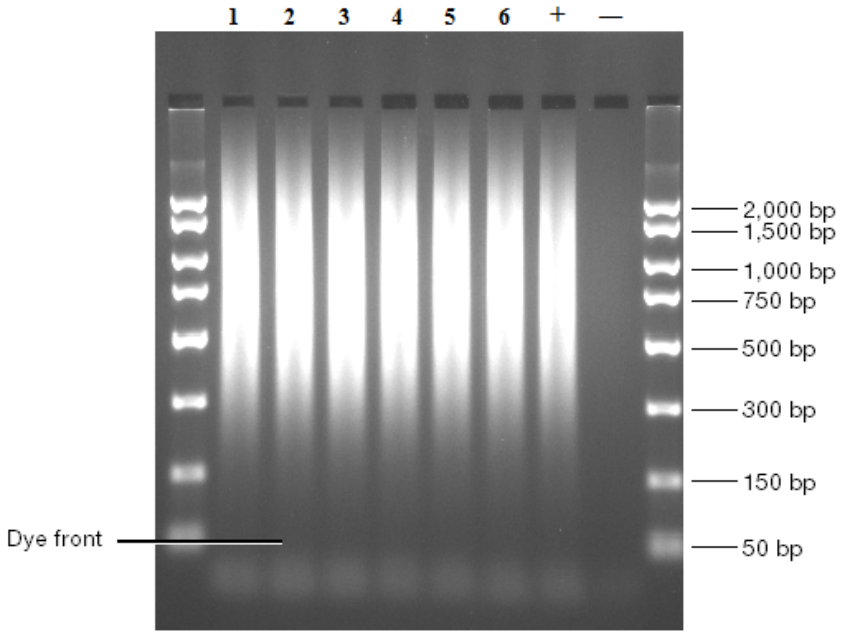


Figure 28 Example of PCR products run on 2% TBE gel at 5 V/cm for 45 minutes. Average product distribution is between about 150 to 2,000 bp.

5. Do one of the following:

   - If the PCR has been verified, proceed to "Stage 4A—PCR product pooling" on page 100.
   - If not proceeding directly to the next stage, seal the plate of PCR product, then store the plate at –20°C.

PCR product check using E-Gel™ Agarose Gels

WARNING! E-Gel™ Agarose Gels and TBE gels contain ethidium bromide. Review the manufacturer's Safety Data Sheet for proper handling and disposal. Use good laboratory practices and always wear protective gloves when handling ethidium bromide. Dispose of the gel and gloves in accordance with national, state, and local regulations.

Dilute the TrackIt™ Cyan/Orange Loading Buffer

1. Add 50 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.95 mL Nuclease-Free Water (total volume is 50 mL).

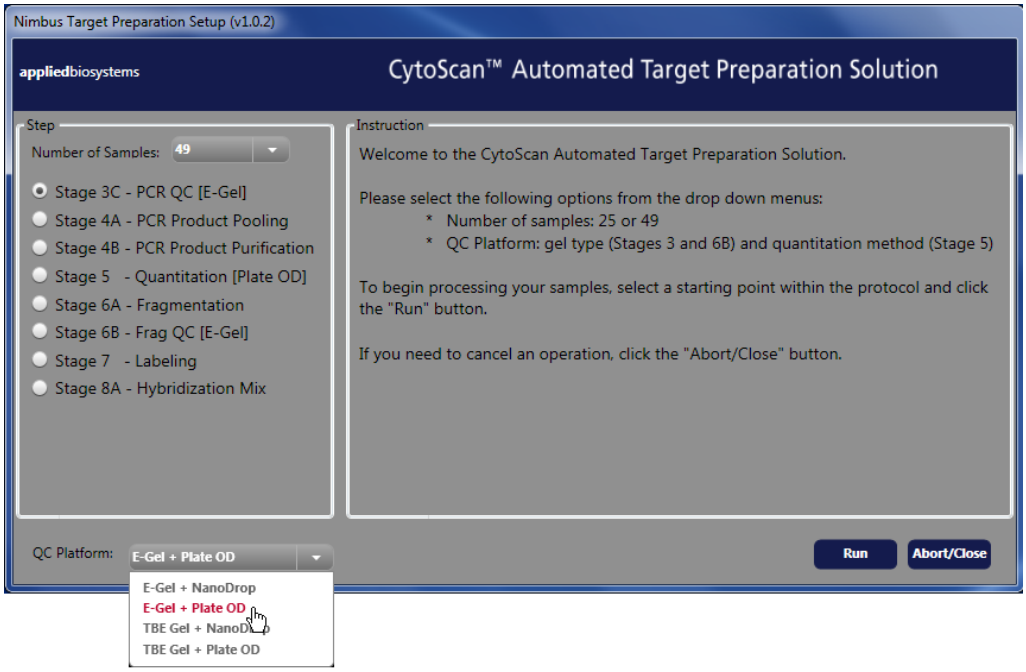
2. Mix well, then store at room temperature.

Prepare a QC gel plate for PCR product

Note: If samples are frozen, thaw at room temperature (~30 minutes). Centrifuge 650 x *g* for 1 minute. Immediately place plate on the lower half of the cooling block. Process within 1 hour.

1. Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument.
   See "Start the CytoScan™ post-PCR software methods for the NIMBUS™ Instrument" on page 214.

2. On the **Welcome** screen, select the following options.
   - **Number of samples**—49.
   - **Stage 3C–PCR QC**.
   - **QC Platform**—E-Gel and a quantitation method (for example, "E-Gel + Plate OD").

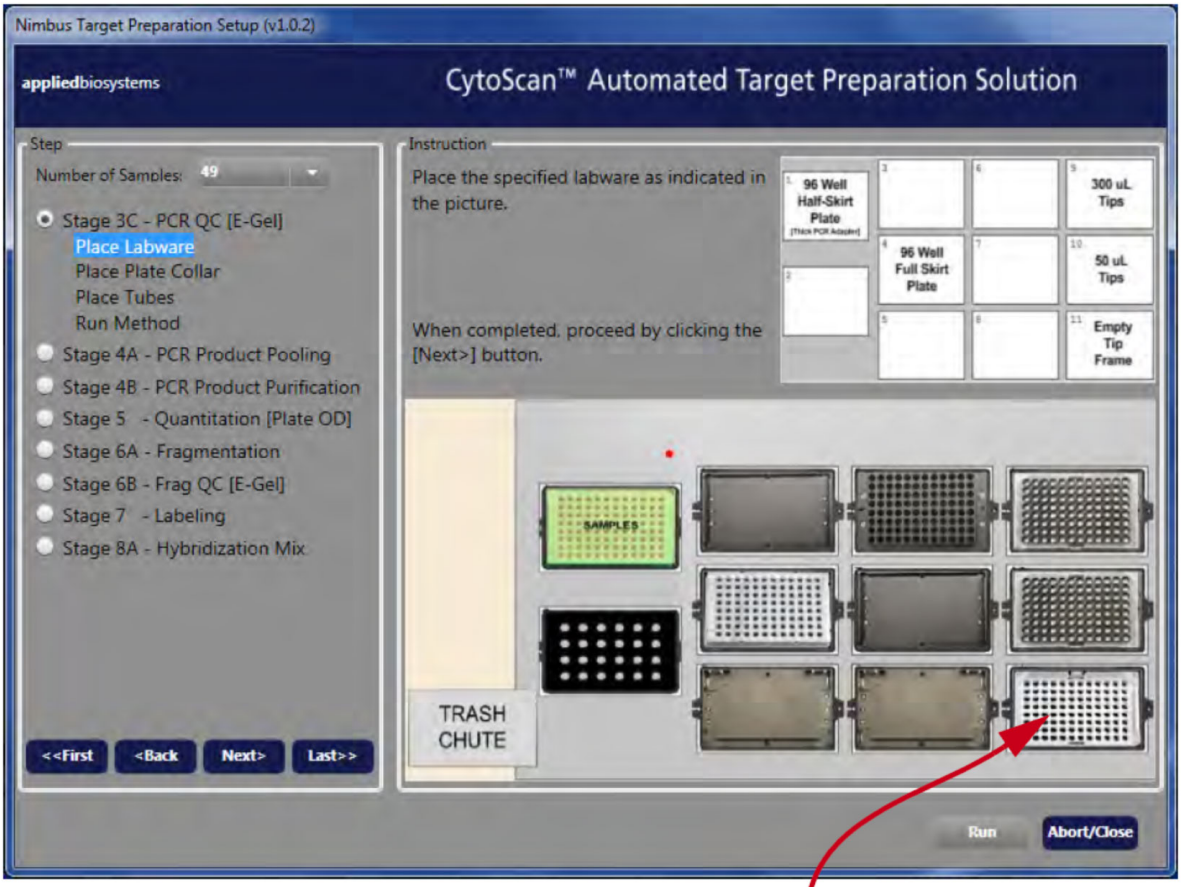


3. Click **Run** to start the method, then click **Yes** to confirm.

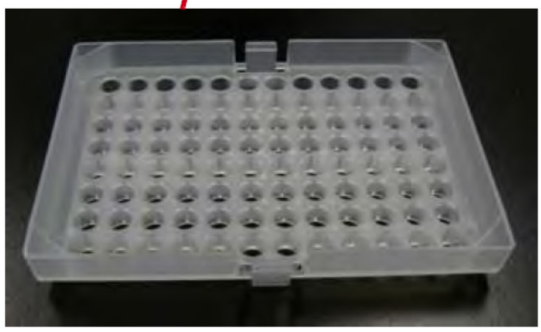
4. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions.

   Note: Place an empty tip frame on deck position 11.

   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.



Empty tip frame for position 11

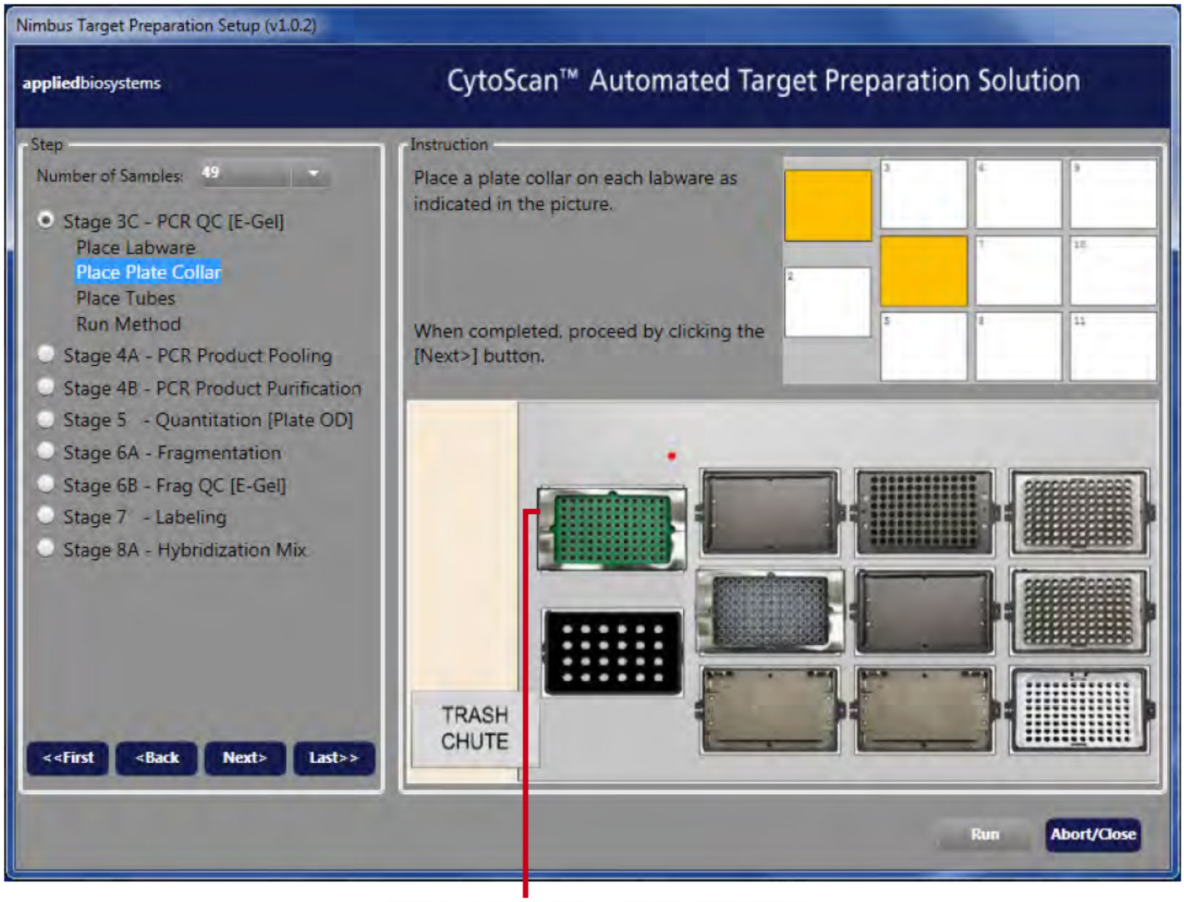


5. Unseal one of the PCR plates, then discard the seal. Place the plate on the NIMBUS™ Instrument deck as shown, then click **Next**.

   IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**.

   Labware at deck positions 1 and 4 require a plate collar.



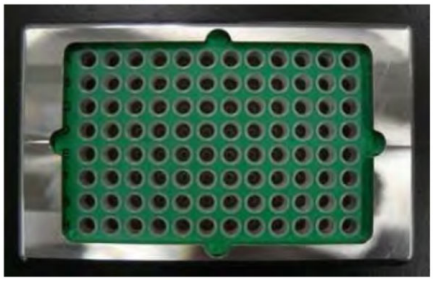
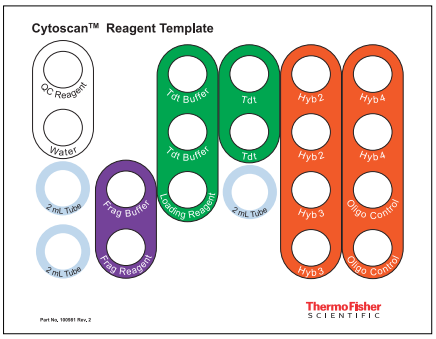
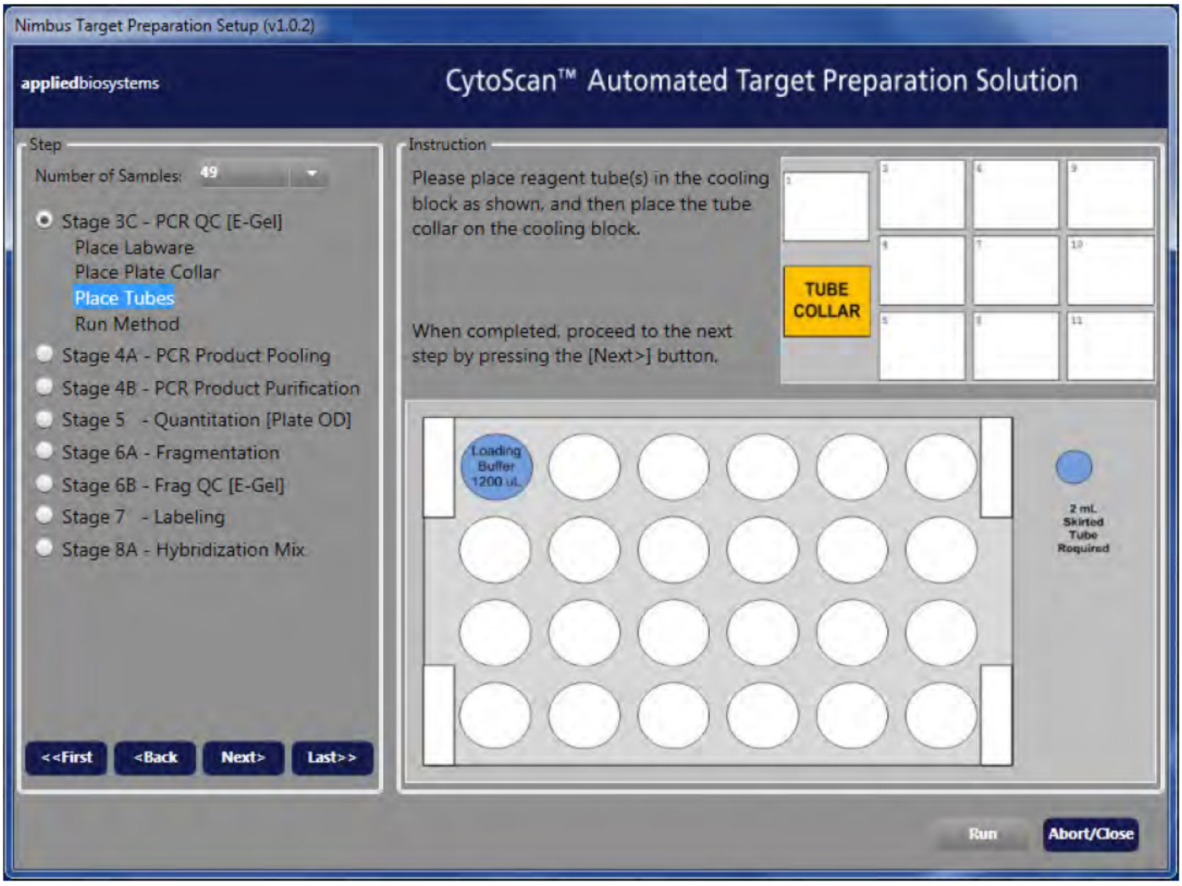


Plate collar on a sample plate

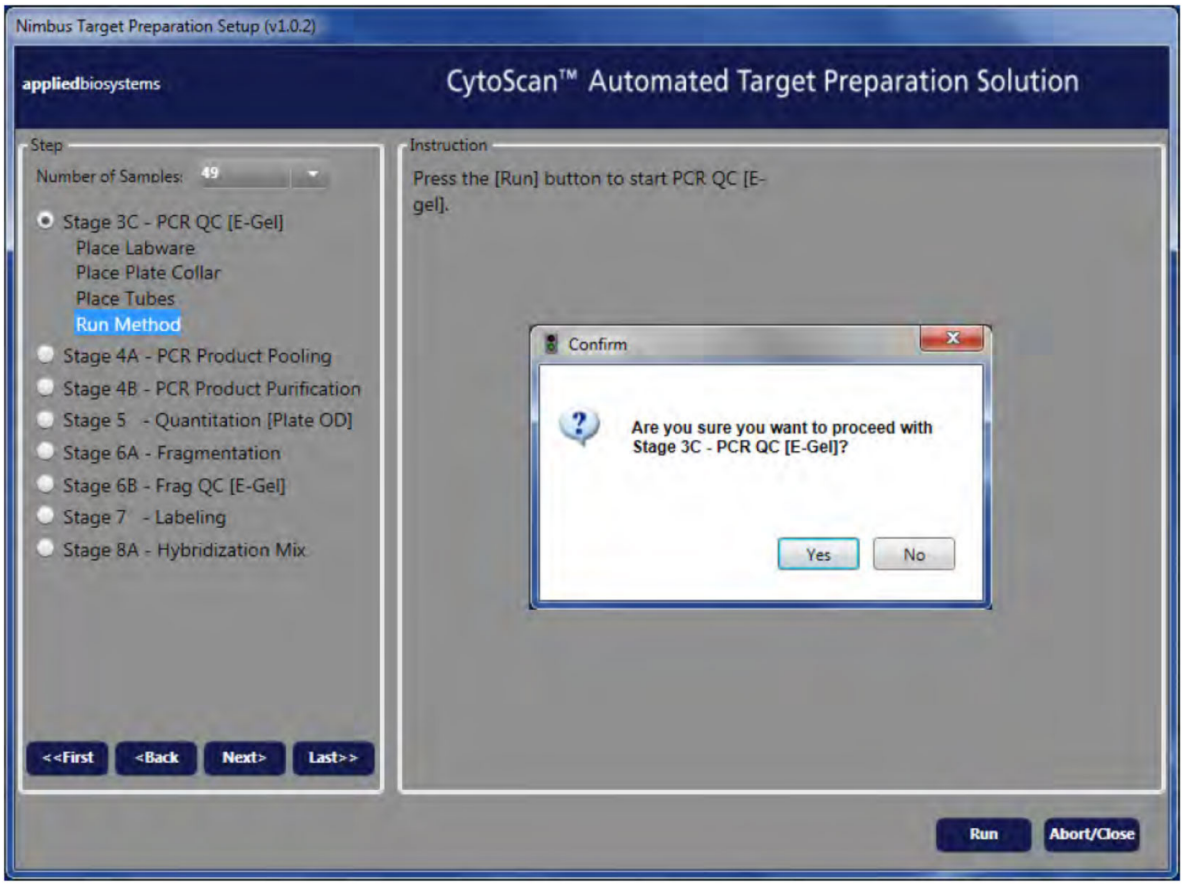
7. Place the tube(s) as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.

IMPORTANT! Use the CytoScan™ Reagent Template, Cat. No. 100981 Rev. 2 for this protocol. The template accommodates the fragmentation protocol and reagents.

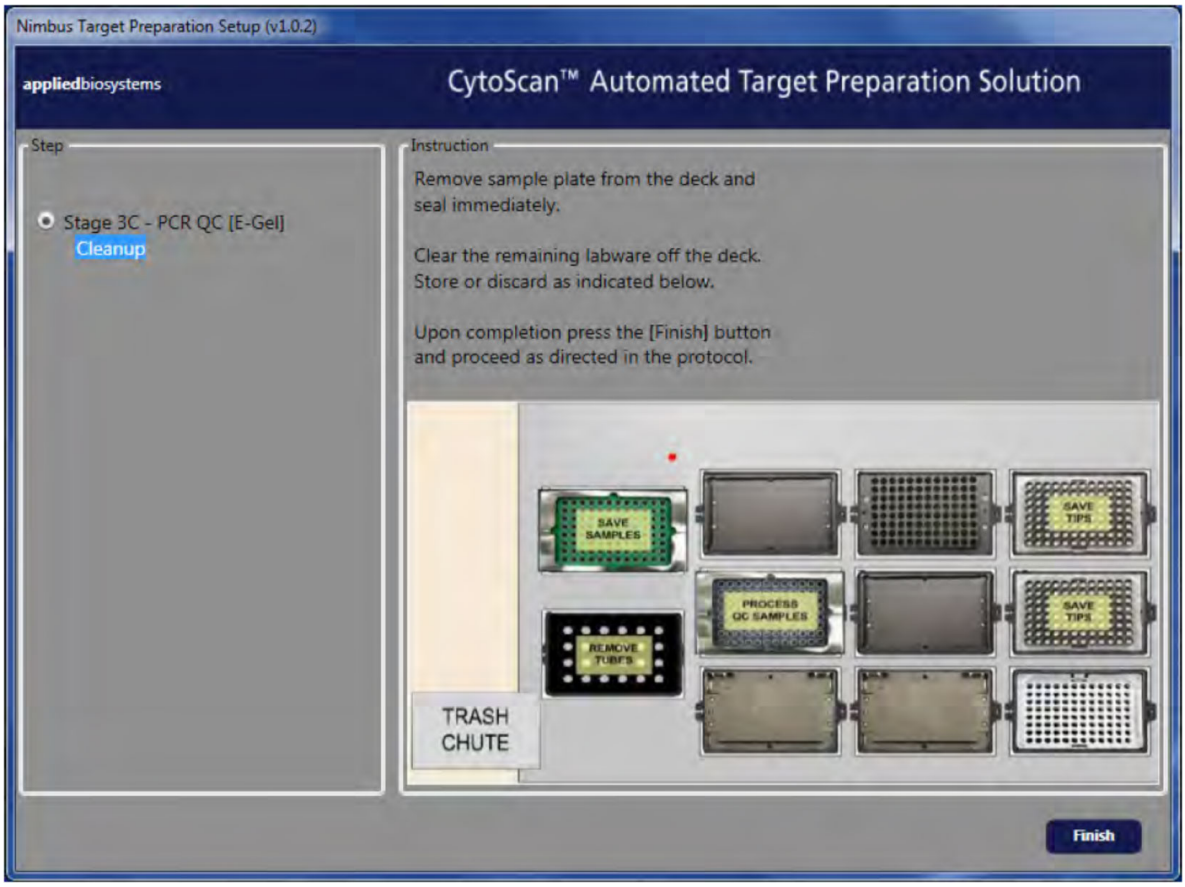




8. Click **Run** to start the method, the click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Remove the PCR plate from the NIMBUS™ Instrument deck and seal the plate tightly with a new seal. If not proceeding to the PCR Product Pooling step, store it at –20°C.

10. Remove the gel QC plate from the NIMBUS™ Instrument deck and seal the plate tightly with a new seal.

11. Remove the labware as shown on the **Cleanup** screen.

12. Click **Finish**, then click **Yes** to confirm.
    The method closes.

Run the E-Gel™ Agarose Gel

1. Power on the power for 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 and wipe away any buffer that comes out of the gel or is on the surface.

4. Insert the E-Gel™ *48* Agarose Gels, 2% into the slot (12-well E-Gel™ Agarose Gels can also be used if running a smaller number of samples).

5. Briefly vortex, then centrifuge the diluted samples before loading onto the E-Gel™ Agarose Gel.

6. Load 20 µL of the samples from the QC sample plate onto the E-Gel™ *48* Agarose Gels, 2%.

7. Dilute the 25 bp DNA Ladder (1:6 dilution, 4 µL in 20 µL of Nuclease-Free Water), then load all 15 µL into each of the marker wells (as needed). Fill empty wells with 20 µL water.

8. Set the run time to 21 minutes.

9. Push the Power/Prg button again (the light changes from red to green).

   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.

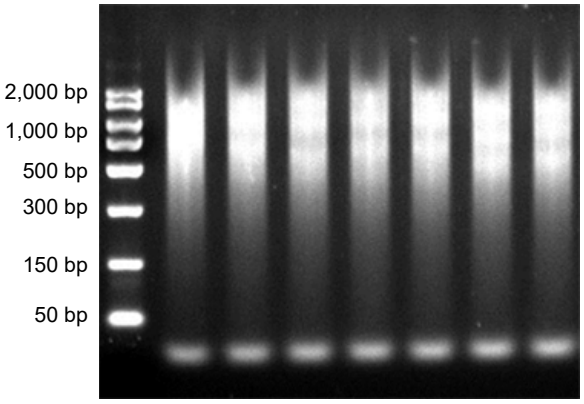


Figure 29 Gel image of the PCR product (from genomic DNA control) on E-Gel™ *48* Agarose Gels, 2%.

10. Do one of the following:

    - If the PCR has been verified, proceed to "Stage 4A—PCR product pooling" on page 230.
    - If not proceeding directly to the next stage, seal the plate with PCR product, then store the plate at –20°C.

Stage 4A—PCR product pooling

This stage uses the NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration. Use the PCR plate layout shown in the following image. Matching the format allows the NIMBUS™ Instrument to pool the PCR reactions for each sample.

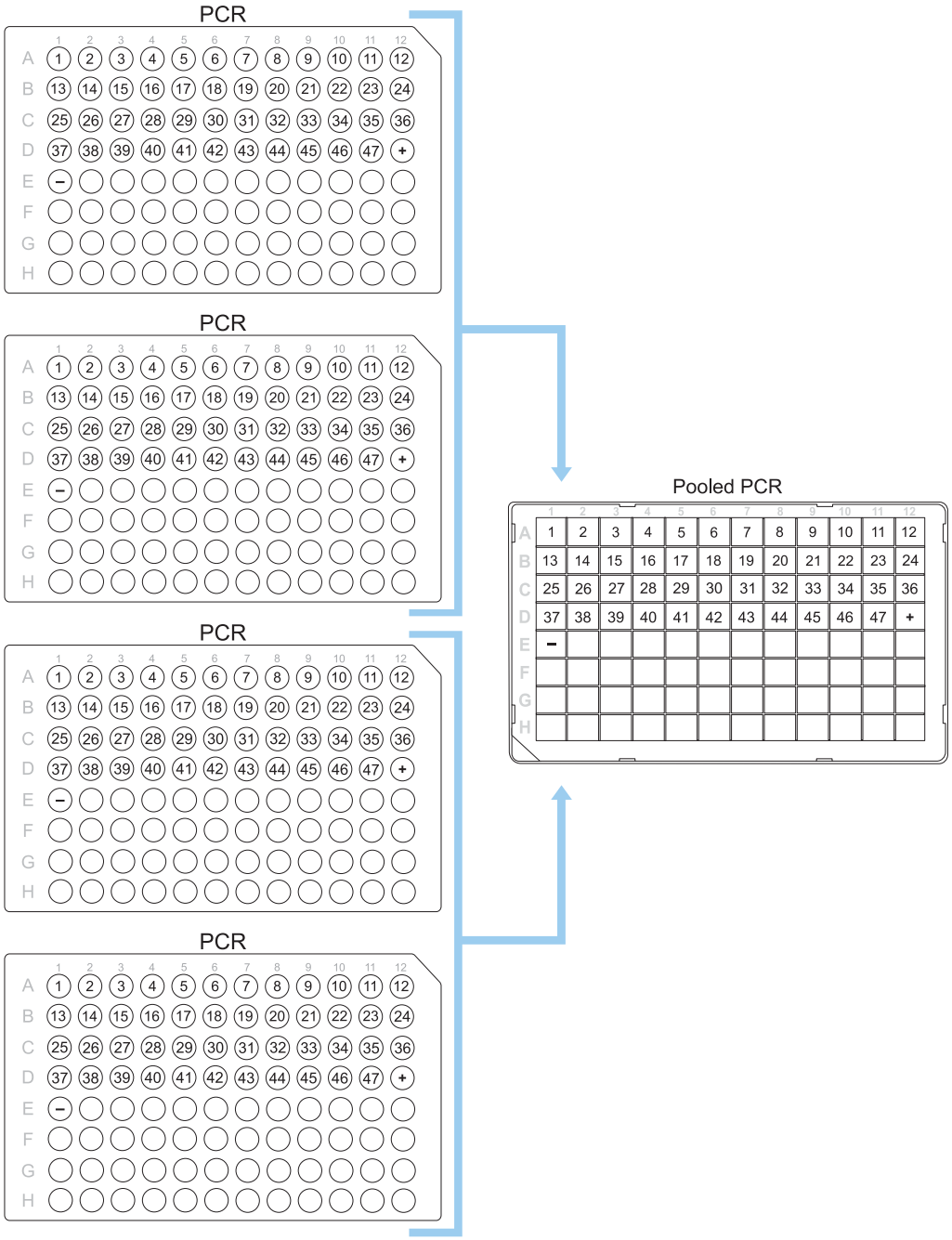


Figure 30 Pooling PCR products for 49 samples.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 4A—PCR product pooling

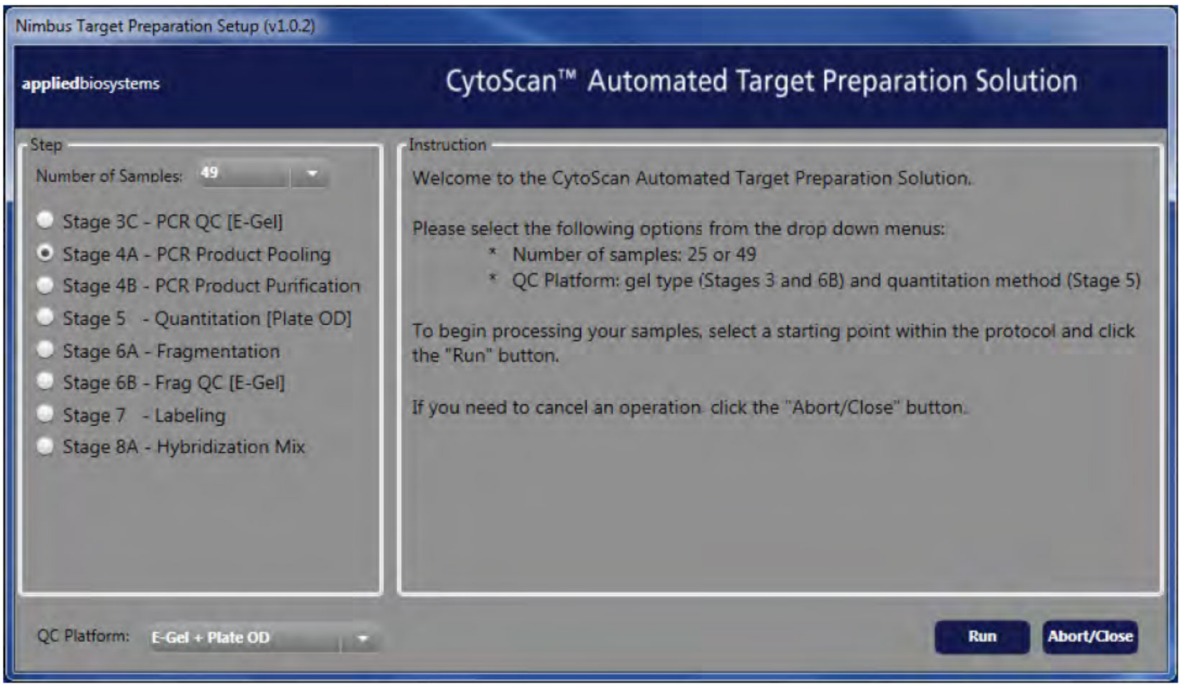
IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 44 Materials required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
1	2.2 mL Square Well Storage Plate
1 rack	Conductive 300-µL filter tips in frames
4	PCR tube racks

Pool PCR products

1. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 4A–PCR Product Pooling**
   - **QC Platform**—The gel type (TBE or E-Gel) and quantitation method (Plate OD or Nanodrop); for example, "E-Gel + Plate OD" .

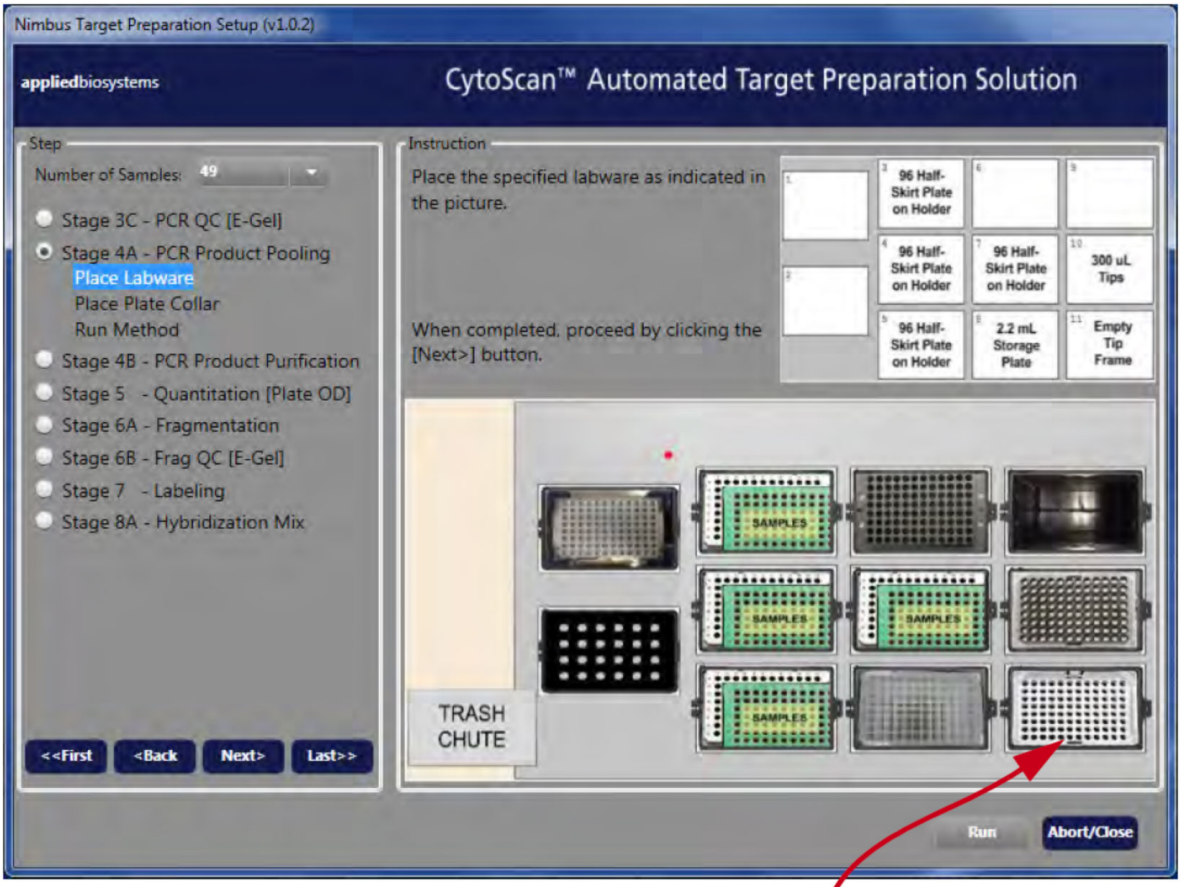


2. Click **Run** to start the method, then click **Yes** to confirm.

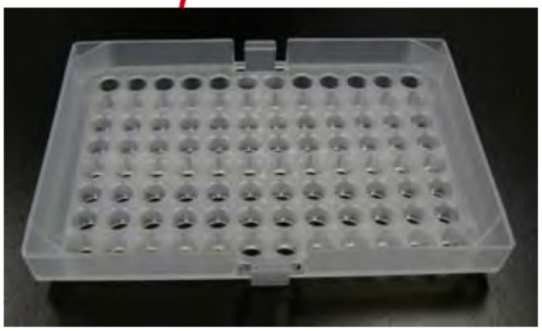
3. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions.

Note: Place an empty tip frame on deck position 11.

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.



Empty tip frame for position 11



4. Unseal the 4 PCR plates, then place them on the GeneMate PCR tube storage rack. Discard the seals. Place the plates on the NIMBUS™ Instrument deck as shown, then click **Next**.

   IMPORTANT! Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

5. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**.

   Labware at deck positions 3, 4, 5, and 7 require a plate collar.

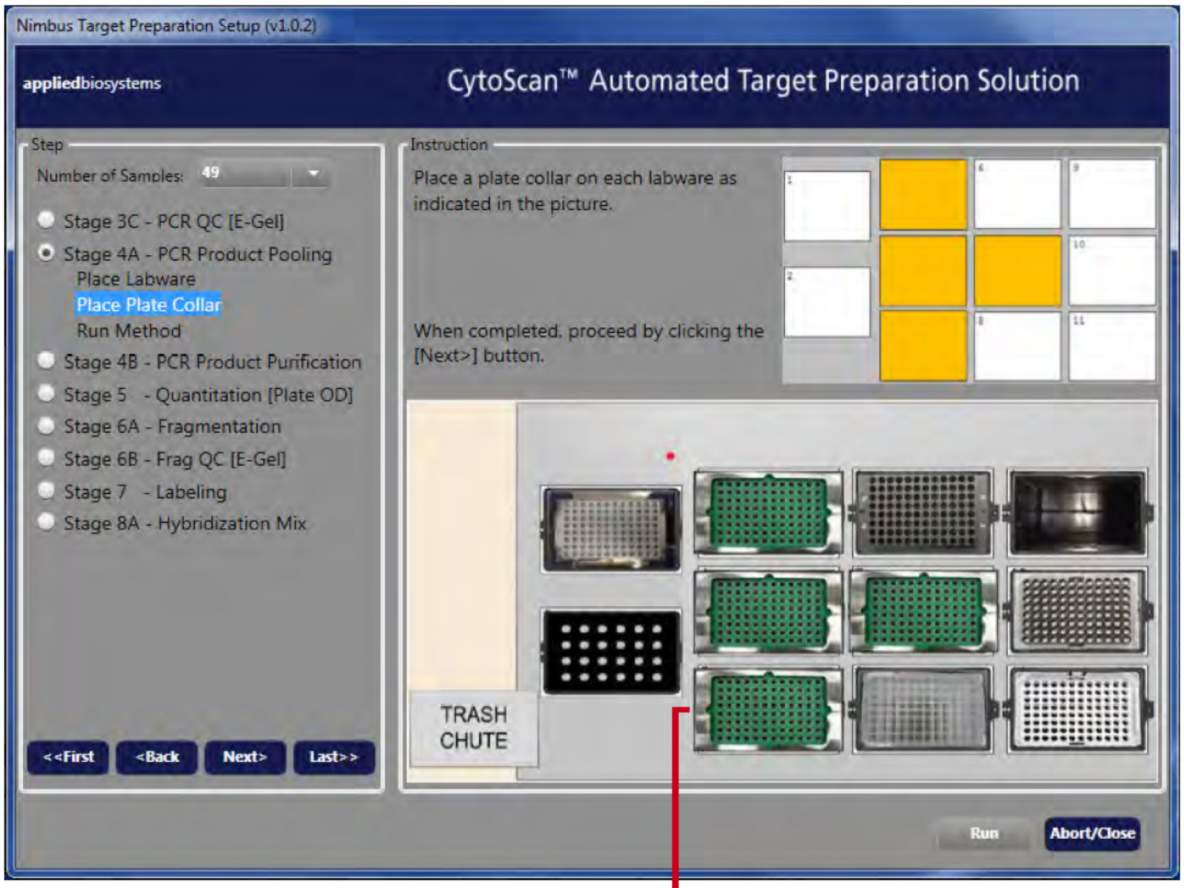
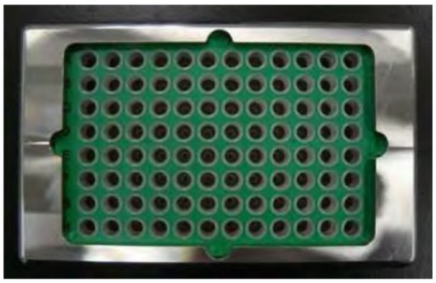
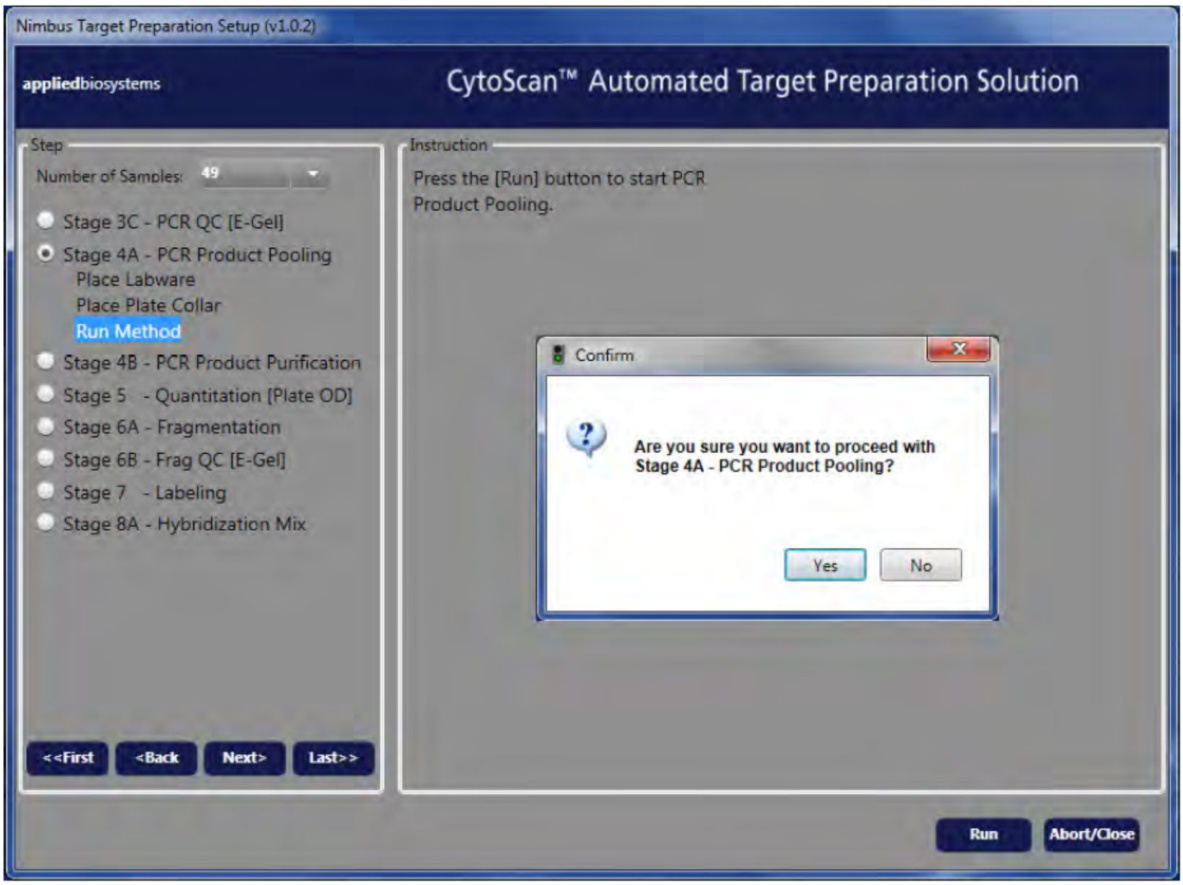


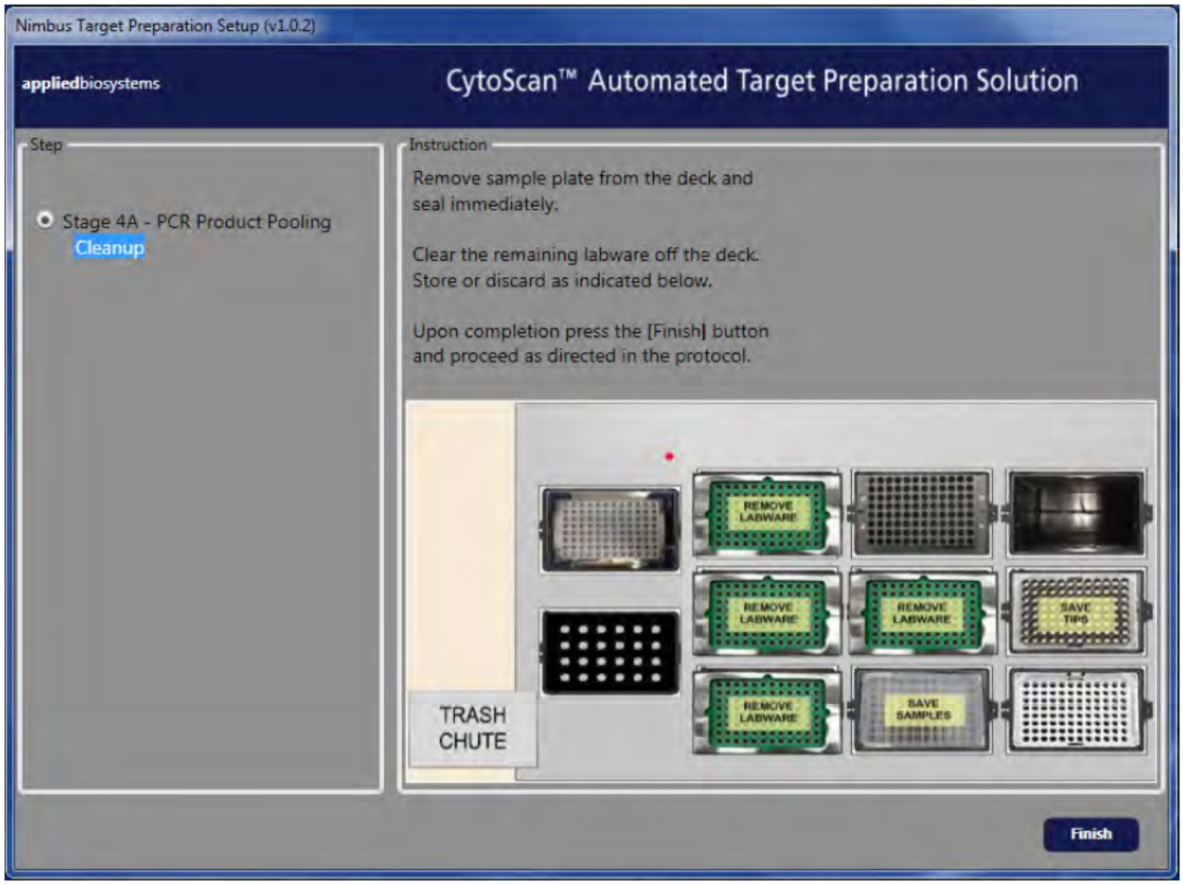
Plate collar on a sample plate



6. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



7. Remove labware from the NIMBUS™ Instrument deck as shown in the on-screen instructions.
8. Click **Finish**, then click **Yes** to confirm.
   The method closes.
9. Proceed to "Stage 4B—PCR product purification" on page 236.

Stage 4B—PCR product purification

This stage uses the NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 4B—PCR product purification

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 45 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Micro Plate 96 PCR Adapter and Plate Retainer (fixed on the Thermoshake device)
1	96S Super Magnet Plate
1	PCR Tube Rack
2 racks	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	Plates, 96-well, semi-skirted
1	Reservoir 4 ROW PYR PP 73 mL
2	2.2 mL Square Well Storage Plate
1	1.2 mL Square Well Round Bottom Storage Plate

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

Table 46 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
2	◯Purification Beads	Module 4	901807
2	◯Elution Buffer	Module 5	901738
2	◯Purification Wash Buffer		901372
As required	Absolute ethanol	—	—

Prepare the Purification Wash Buffer

Sample purification for 49 samples uses 2 bottles of Purification Wash Buffer.

1. Add 45 mL of absolute ethanol to each bottle of Purification Wash Buffer.

   IMPORTANT! Ensure that the correct amount of ethanol has been added to the Purification Wash Buffer bottle.

2. Cap the bottle tightly, then invert 10 times.

3. Enter the preparation date on the bottle label, then put a checkmark in the checkbox.

Prepare the Purification Beads

1. Thoroughly mix the Purification Beads stock by inverting the bottle 10 times.

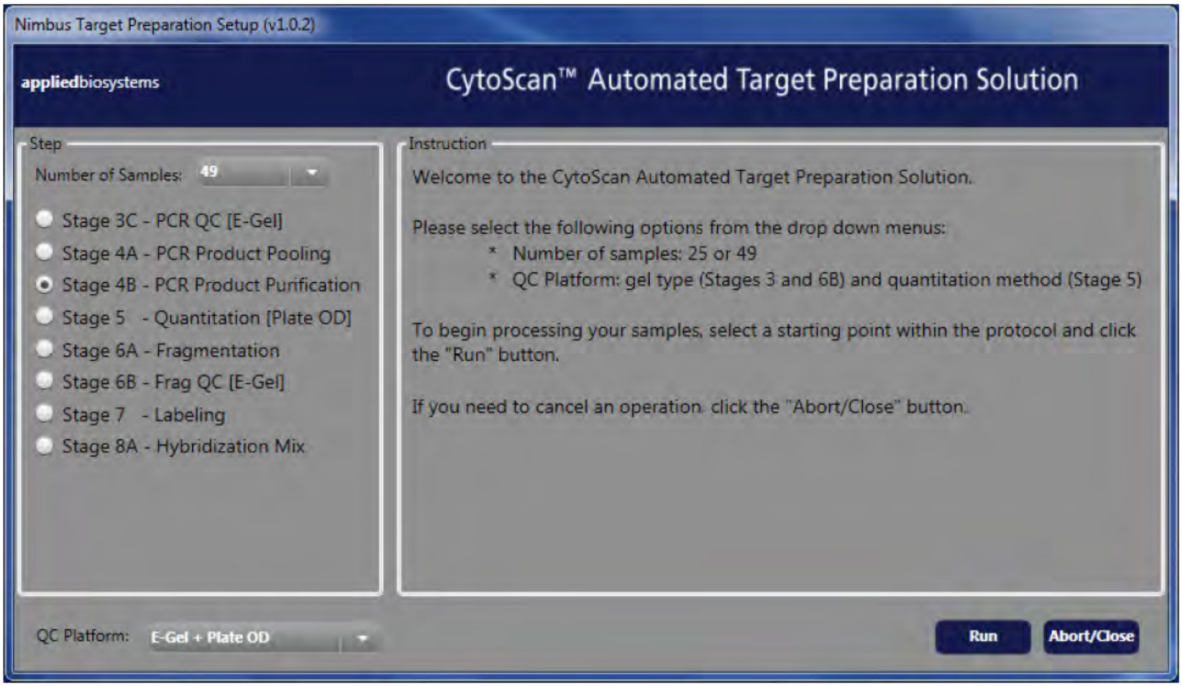
2. Examine the bottom of the bottle to ensure that the solution appears homogeneous.

Purify the pooled PCR products

Note: Controls can be placed in any well from A1 through E1 because the NIMBUS™ Instrument processes all samples.

PCR wells (4):	100 µL from each well	= 400 µL
Total volume in each well of the deepwell plate		**= 400 µL/well – 3 µL aliquoted for PCR gel in PCR QC step**

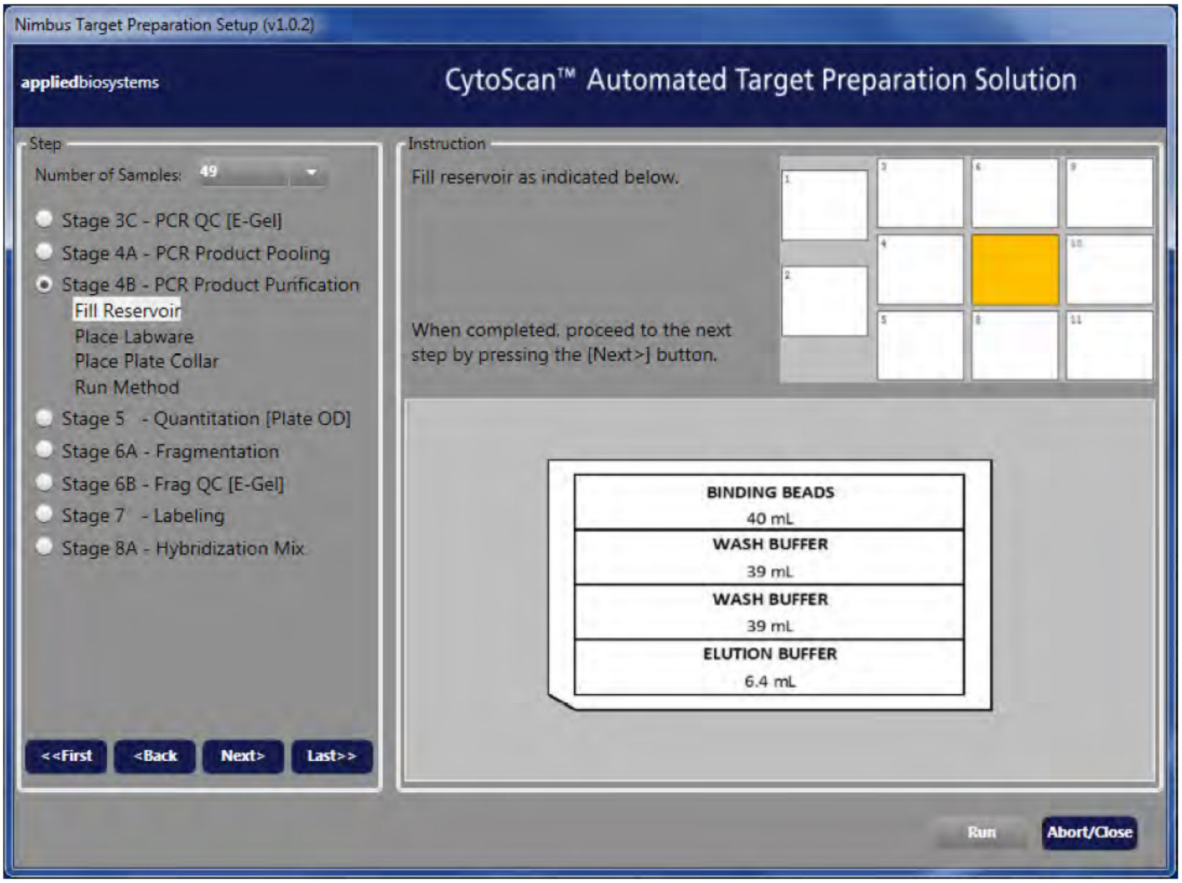
1. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 4B–PCR Product Purification**
   - **QC Platform**—For example, "E-Gel + Plate OD".



2. Click **Run** to start the method, then click **Yes** to confirm.

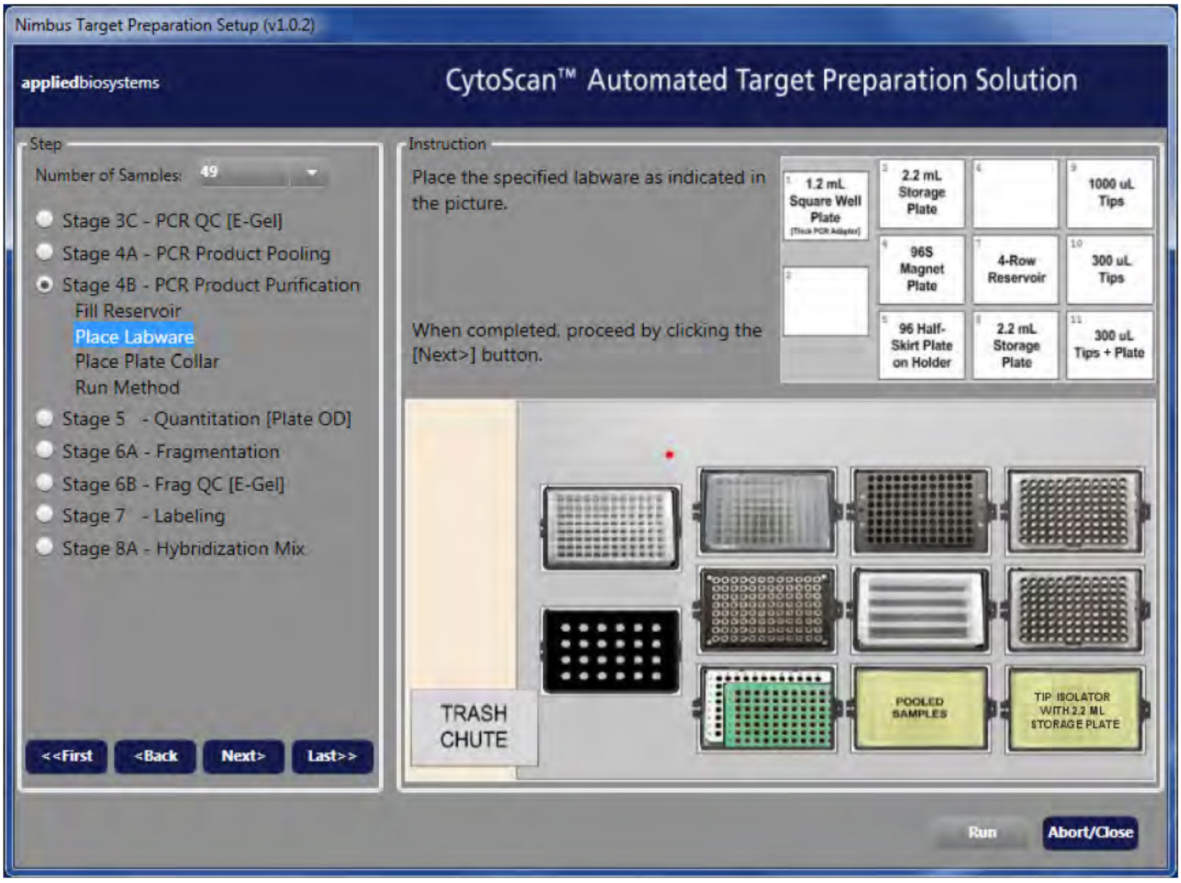
3. Transfer the entire content of each reagent bottle into its respective reservoir position. Ensure that the reservoir contains the minimum volumes shown in the on-screen instructions, then click **Next**.

Note: Each Elution Buffer bottle contains 3.3 mL of buffer. Transfer the entire content of 2 Elution Buffer bottles. Ensure that the buffer is evenly spread across the reservoir.



4. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions.

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.



5. Place a 2.2 mL Square Well Storage Plate under the tip isolator and place a rack of 300 µL tips on top as shown, then click **Next**.

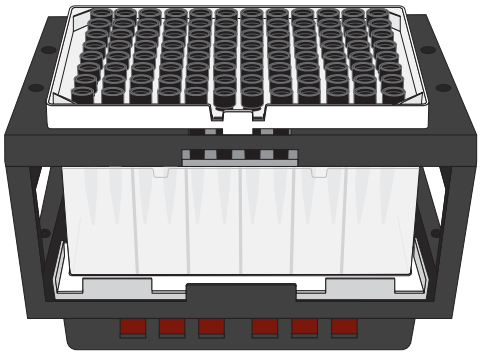


Figure 31 Tip Isolator with 2.2 mL Square Well Storage Plate underneath and 300 µL tips on top.

6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 5 and 7 require a plate collar.

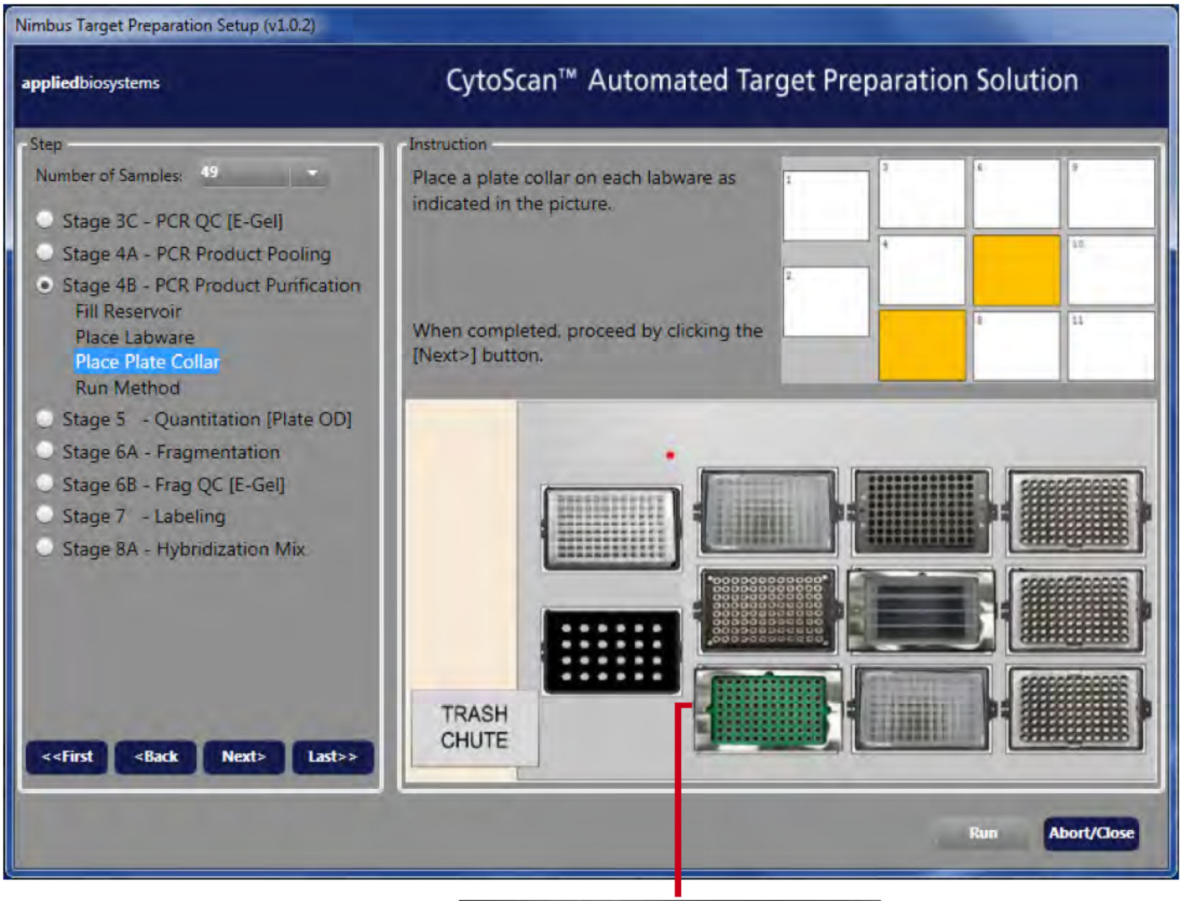
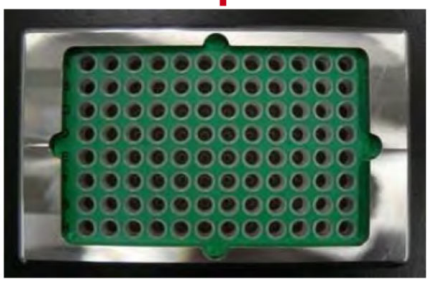
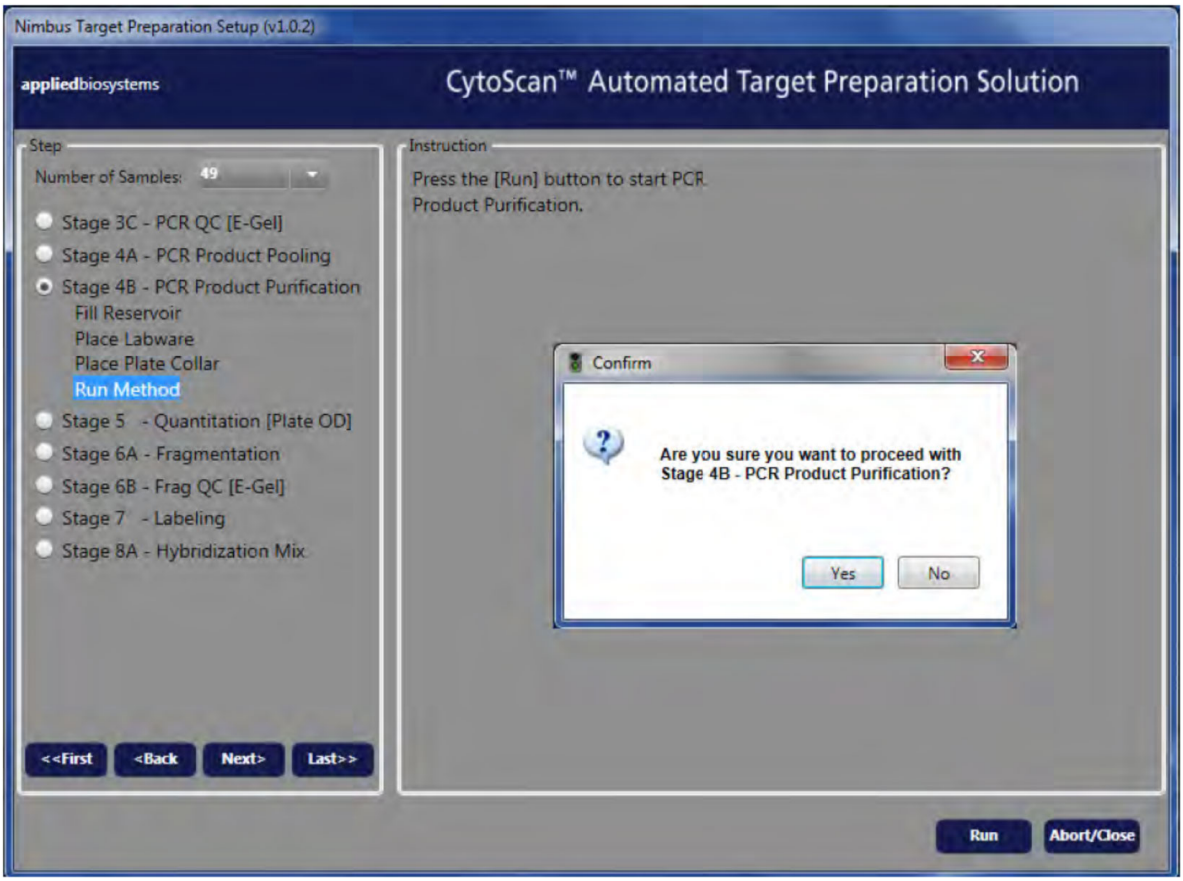


Plate collar on a sample plate



7. Click **Run** to start the method, then click **Yes** to confirm.



Timers show the duration and elapsed time for each step during PCR purification.

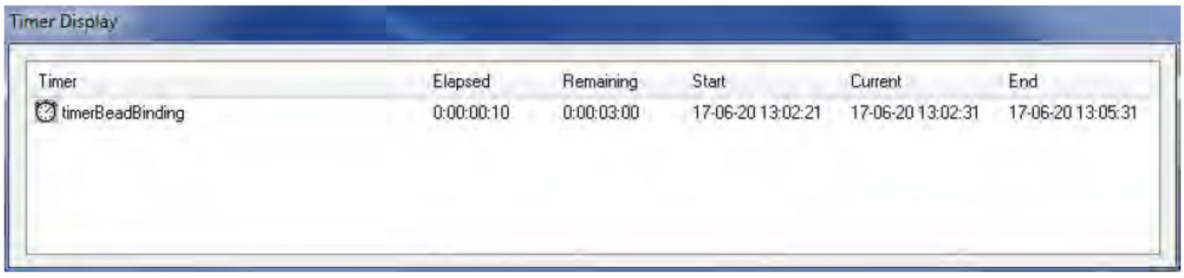


Figure 32 Example timer.

The NIMBUS™ Instrument transfers 47 µL of eluted sample to the appropriate well on a new 96-well plate.

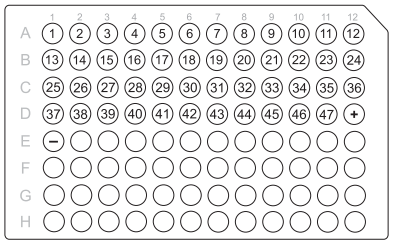


Figure 33 Purified PCR product sample format.

8. After the purification program is complete, visually examine the purified sample plate to determine whether the wells contain the same volume.

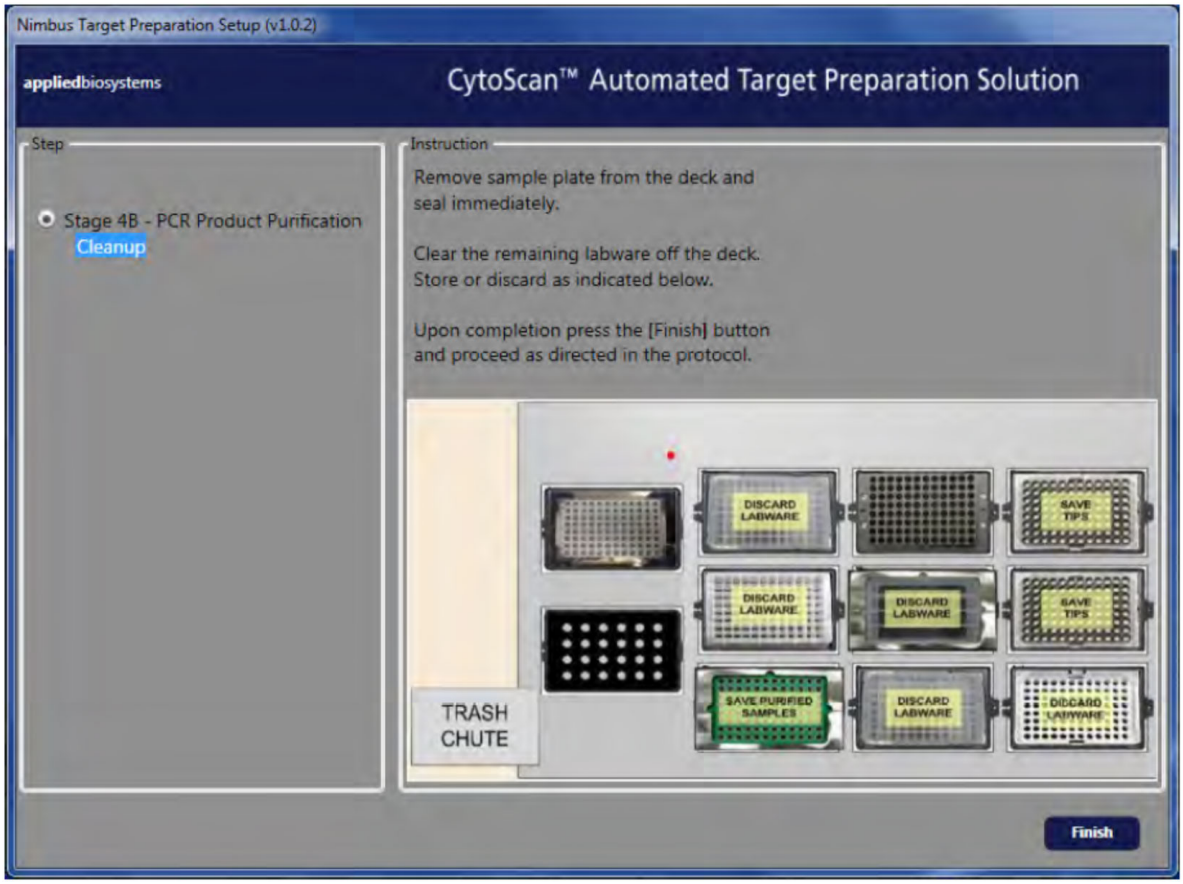
   If a well has lower volume:

   a. Measure the volume using a pipette.

   b. If the volume is less than 47 µL: With the Cleanup Plate still on the magnet, check if there is residual volume in the corresponding well. If so, carefully retrieve the eluent using a manual pipette.

9. Tightly seal the plate, then vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge at 2,000 rpm for 1 minute.

10. Remove labware from the NIMBUS™ Instrument deck as shown on **Cleanup** screen.



11. Click **Finish**, then click **Yes** to confirm.
    The method closes.

12. Proceed to “Stage 5—Quantitation” on page 245.

Stage 5—Quantitation

This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration. Perform quantitation using one of these methods:

- "Quantitation procedure using the SpectraMax® Plus 384 Microplate Spectrophotometer" on page 246.
- "Quantitation procedure using the NanoDrop™ Spectrophotometer" on page 252.

To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 5—Quantitation

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 47 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Marker, fine point, permanent
1	Mini centrifuge
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
2	96-well Full Skirt PCR Plate, clear (if using the NanoDrop™ Spectrophotometer)
1	Corning™ UV-Transparent Microplate (if using the SpectraMax® Plus 384 Microplate Spectrophotometer)
1	Spectrophotometer
1	Reservoir 4 ROW PYR PP 73 mL

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

Table 48 Reagent required.

Quantity	Reagent		
		Module	Part No.
As required	Nuclease-Free Water	Module 4	901781

Important information about this stage

IMPORTANT! To ensure the best results, carefully read the following information before you start this stage of the assay.

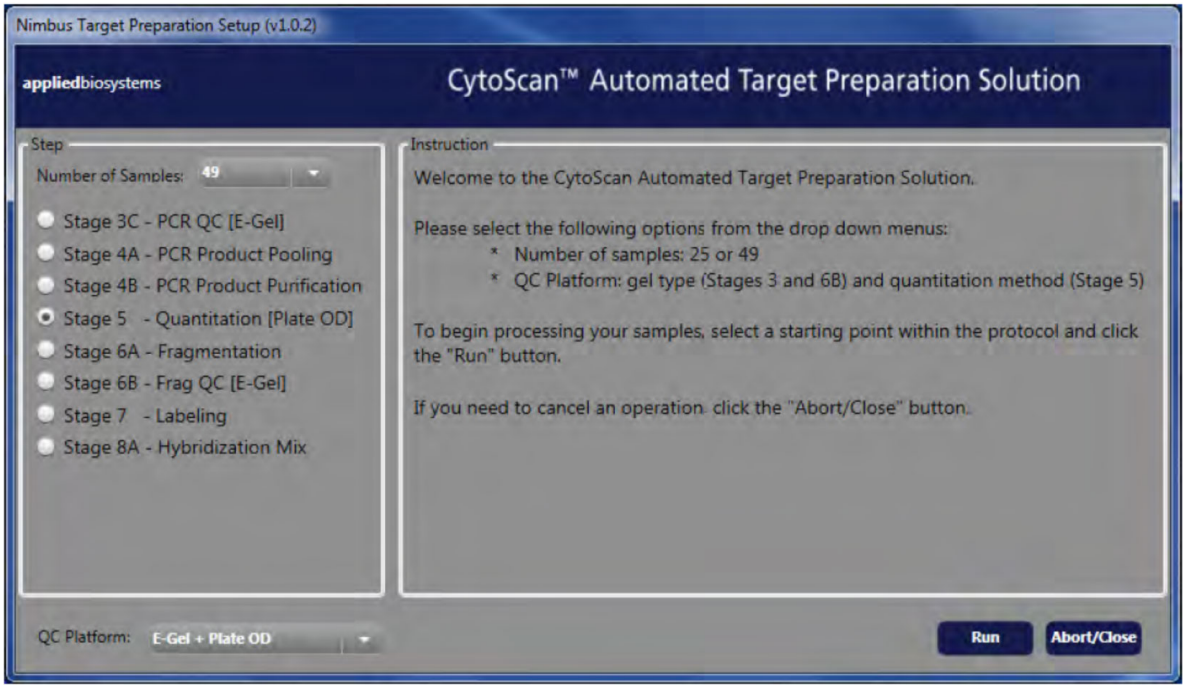
- The accuracy of the optical density (OD) measurement is critical. Carefully follow this procedure and be sure the OD measurement is within the linear range of the instrument.
- Calibrate the spectrophotometer regularly to ensure correct readings.
- This assay has been optimized using a UV spectrophotometer for quantitation.
- The controls can be placed in any microplate well from A1 through E1 because the NIMBUS™ Instrument processes all samples.

Quantitation procedure using the SpectraMax® Plus 384 Microplate Spectrophotometer

1. Power on the SpectraMax® Plus 384 Microplate Spectrophotometer, then allow it to warm for at least 10 minutes before use.

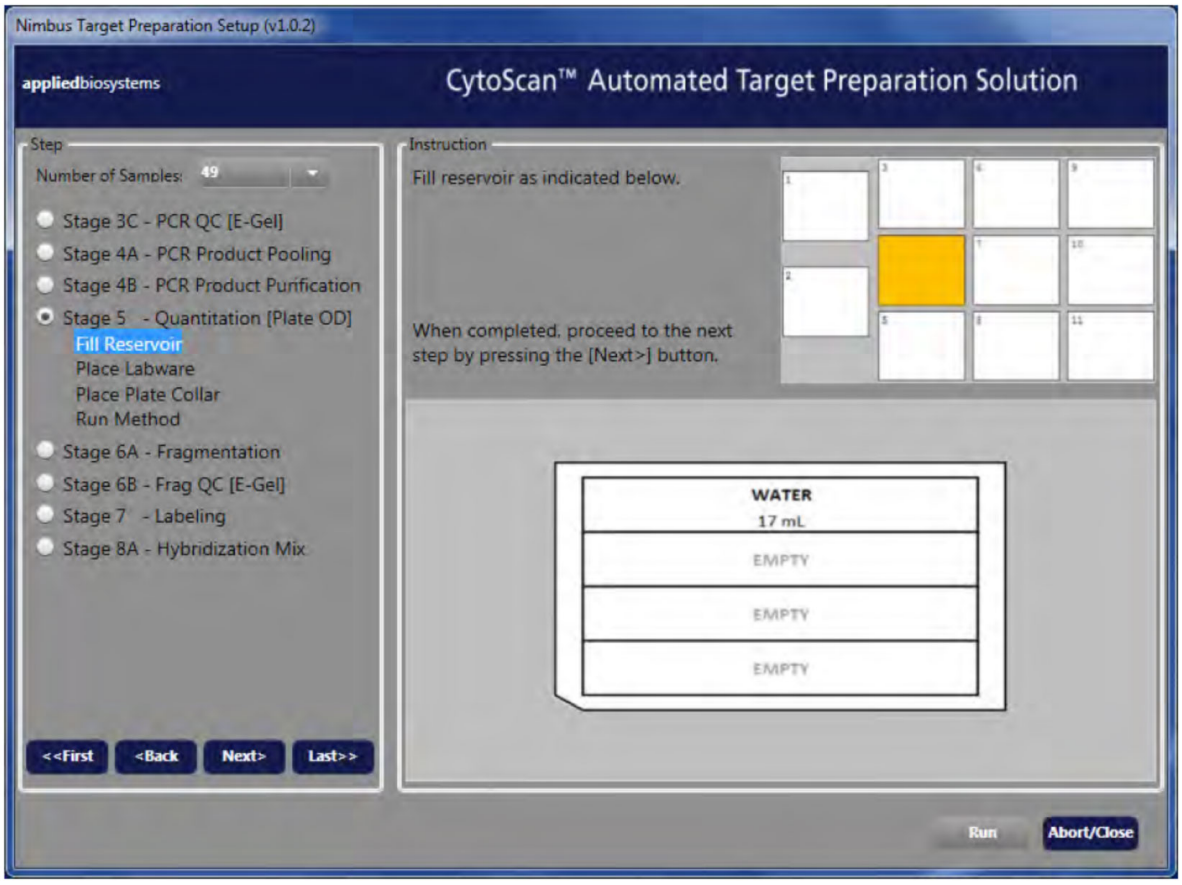
2. Verify that the plate is tightly sealed. Vortex, then centrifuge the purified samples at 2,000 rpm for 1 minute, then put in a plate holder.

3. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 5–Quantitation**
   - **QC Platform**—For example, “E-Gel + Plate OD”.



4. Click **Run** to start the method, then click **Yes** to confirm.

5. Fill the reservoir as shown in the on-screen instructions, then click **Next**.

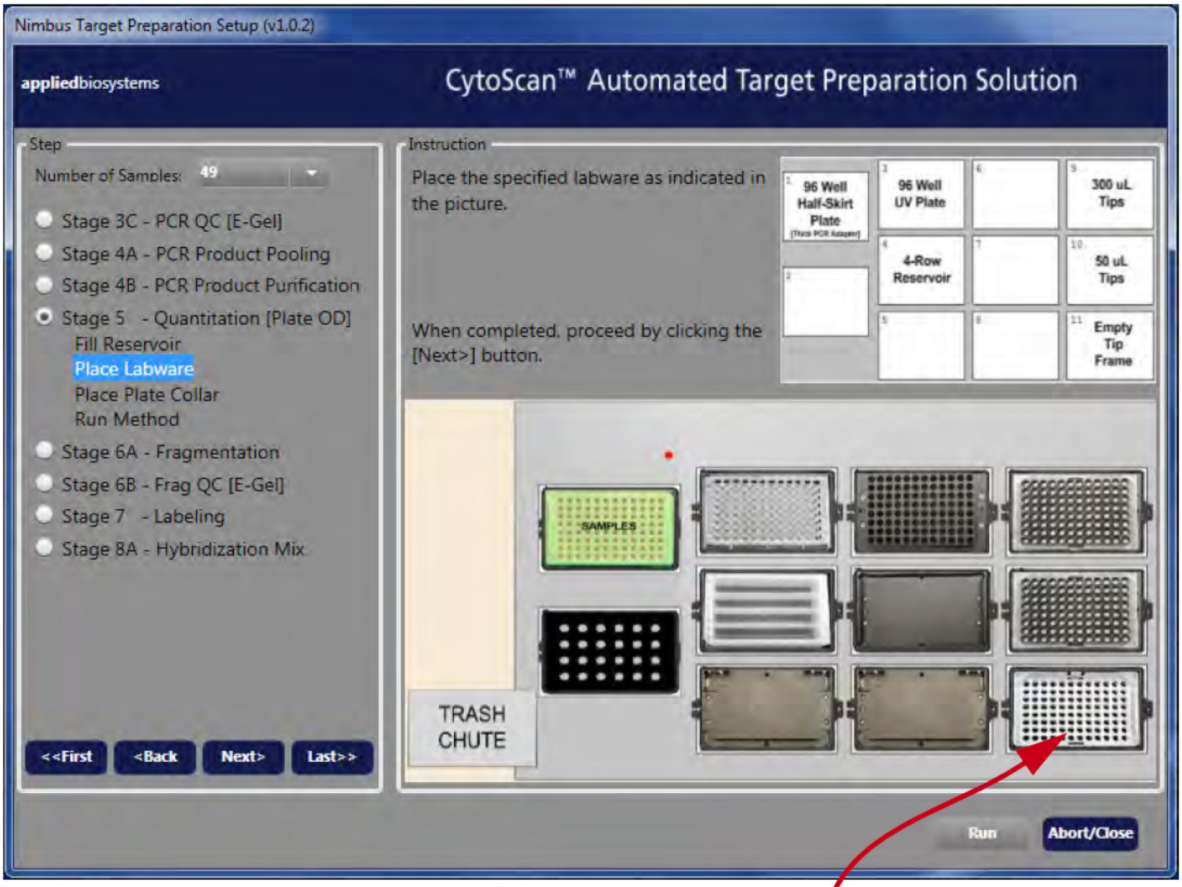


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

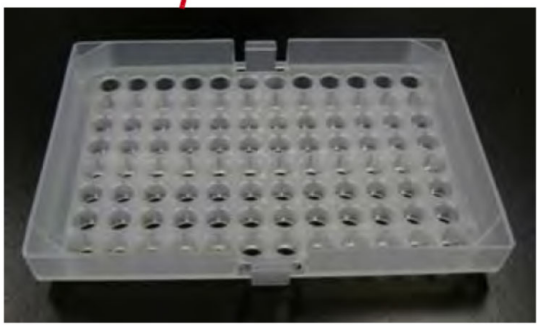
   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   Place an empty tip frame on deck position 11.

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.



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1 and 4 require a plate collar.

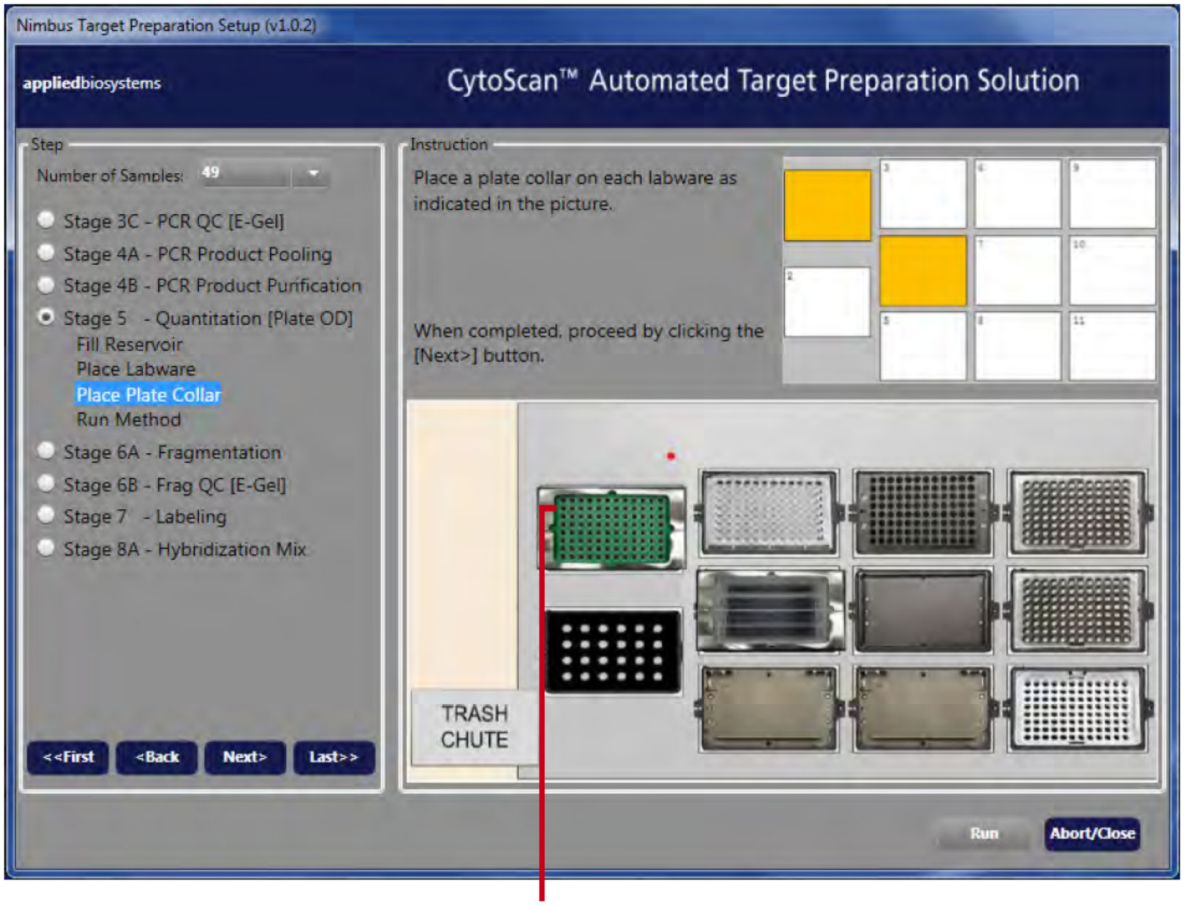
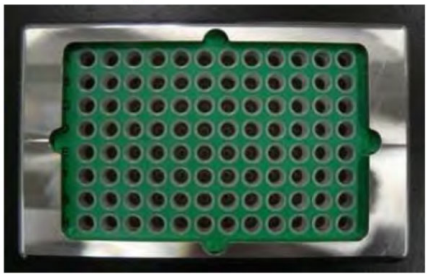
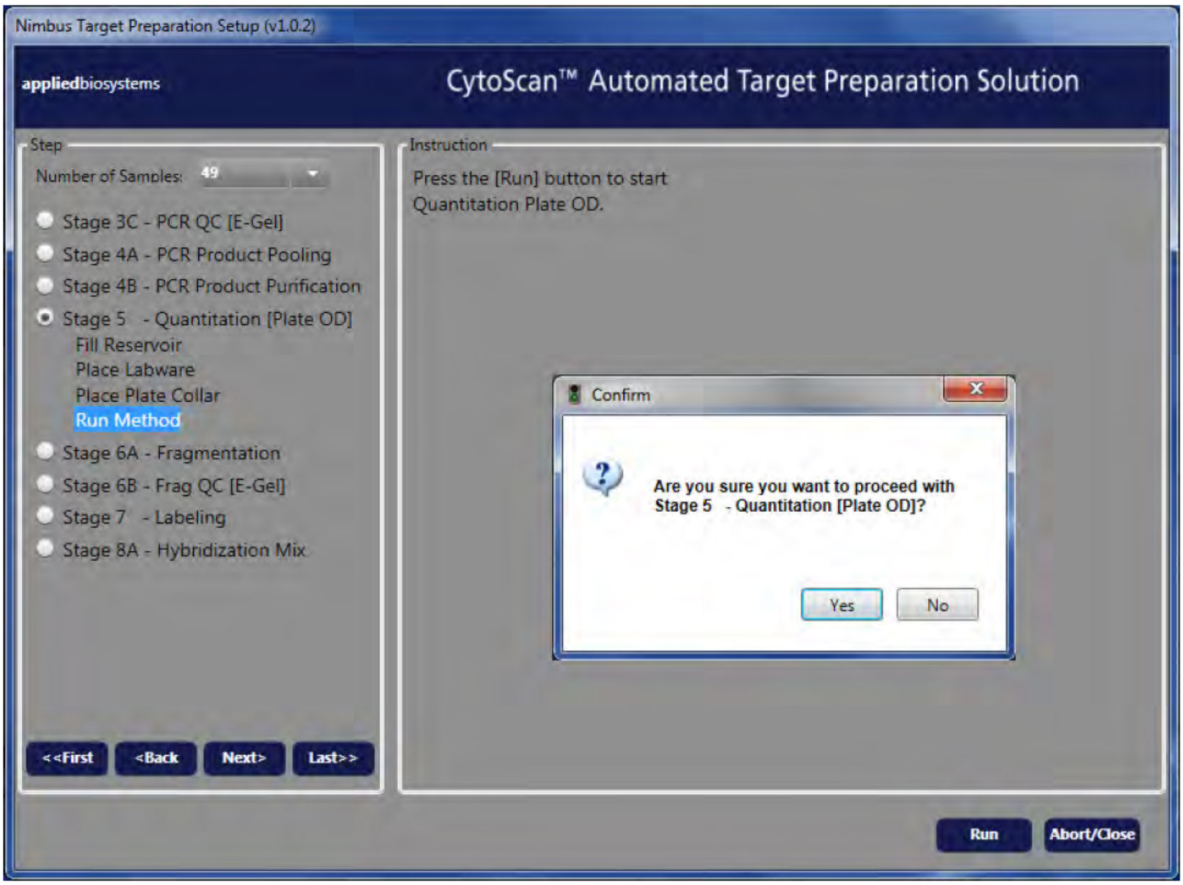


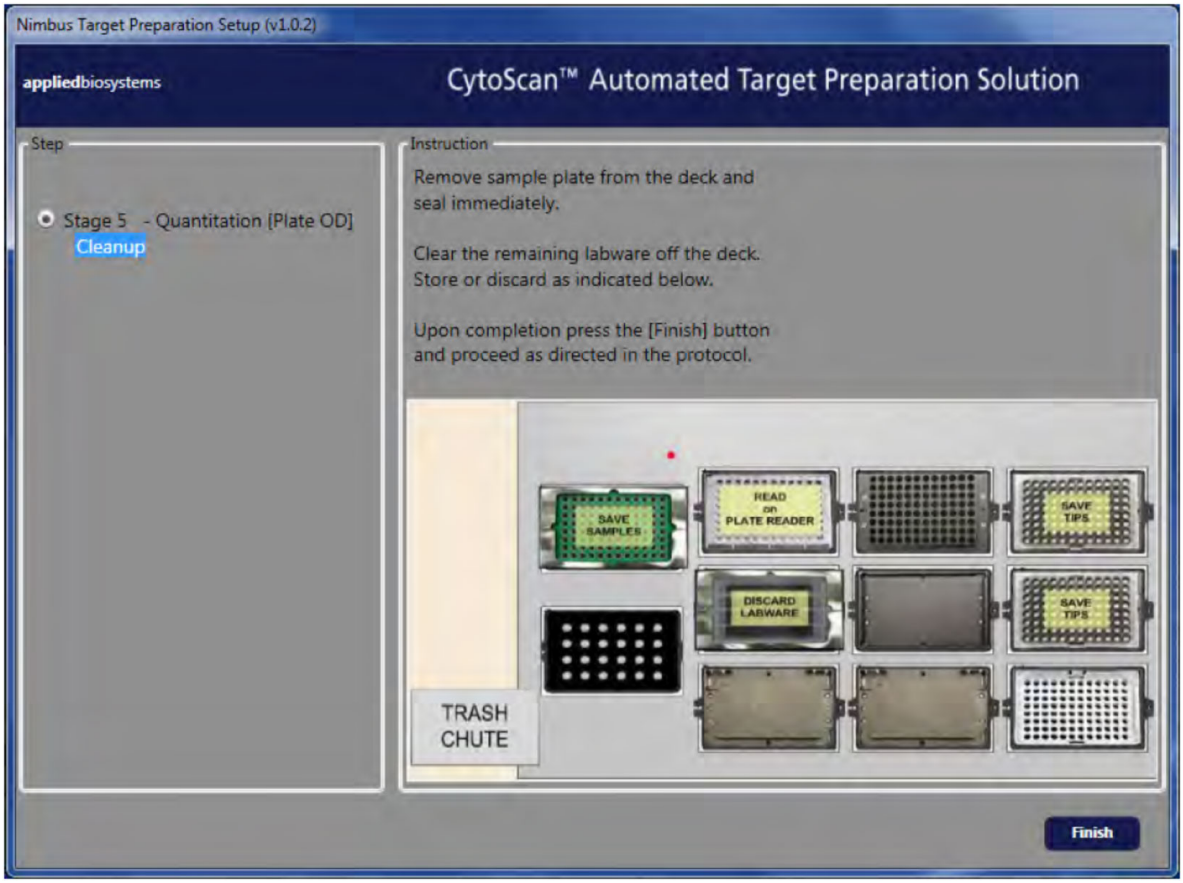
Plate collar on a sample plate



8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Tightly seal the plate with purified samples with a new seal, then store at –20°C. The plate can be stored at –20°C for up to 10 days.

10. Seal the UV plate, and using a laboratory tissue on the adaptor surface, vortex, then centrifuge at 2,000 rpm for 1 minute.

11. Remove labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

12. Click **Finish**, then click **Yes** to confirm.

    The method closes.

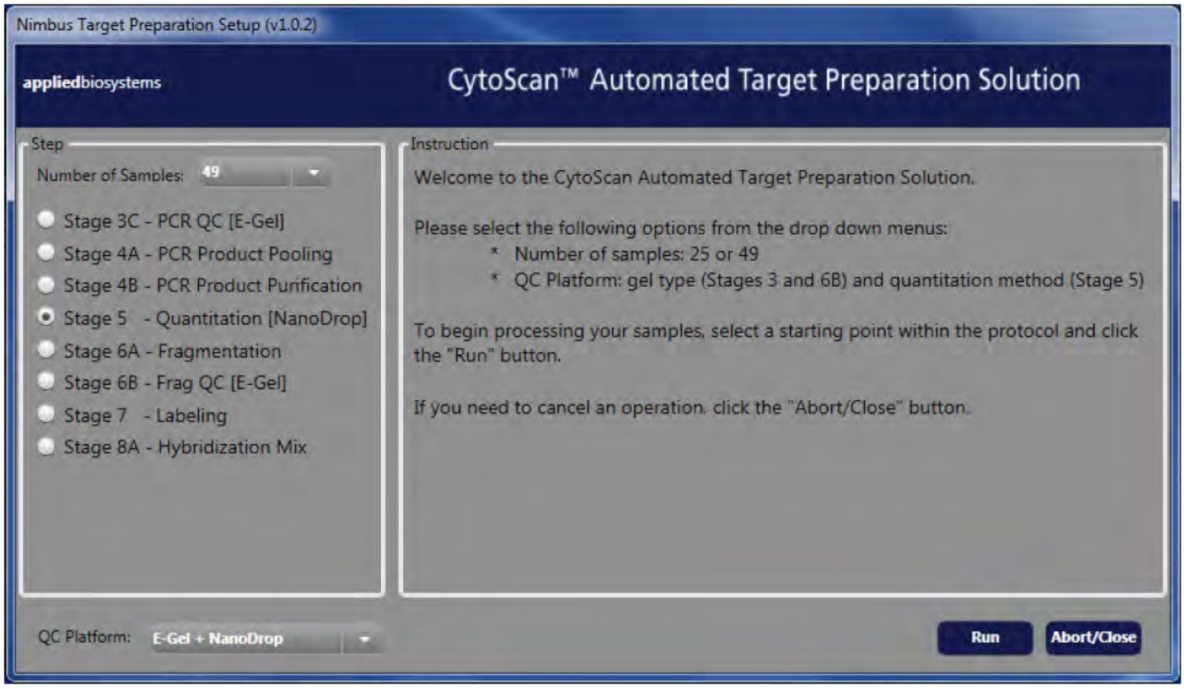
Quantitate the diluted PCR product

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.
   OD_{280} and OD_{320} are used as controls.
2. Determine the OD_{260} 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.

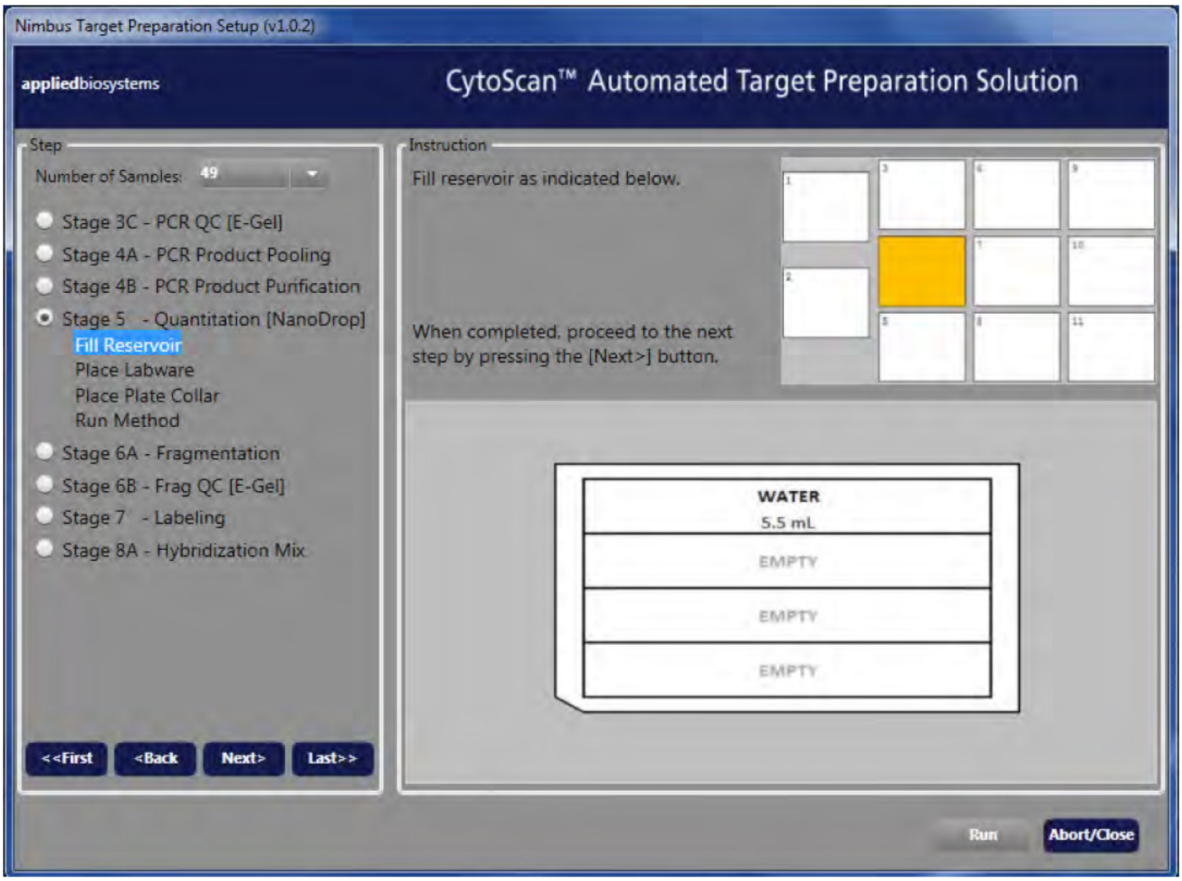
Quantitation procedure using the NanoDrop™ Spectrophotometer

1. Power on the NanoDrop™ Spectrophotometer, then allow it to warm for at least 10 minutes before use.
2. Verify that the plate is tightly sealed. Vortex, then centrifuge the purified samples at 2,000 rpm for 1 minute, then put in a plate holder.
3. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 5–Quantitation**
   - **QC Platform**—For example, “E-Gel + Nanodrop”.



4. Click **Run** to start the method, then click **Yes** to confirm.

5. Fill the reservoir as shown in the on-screen instructions, then click **Next**.

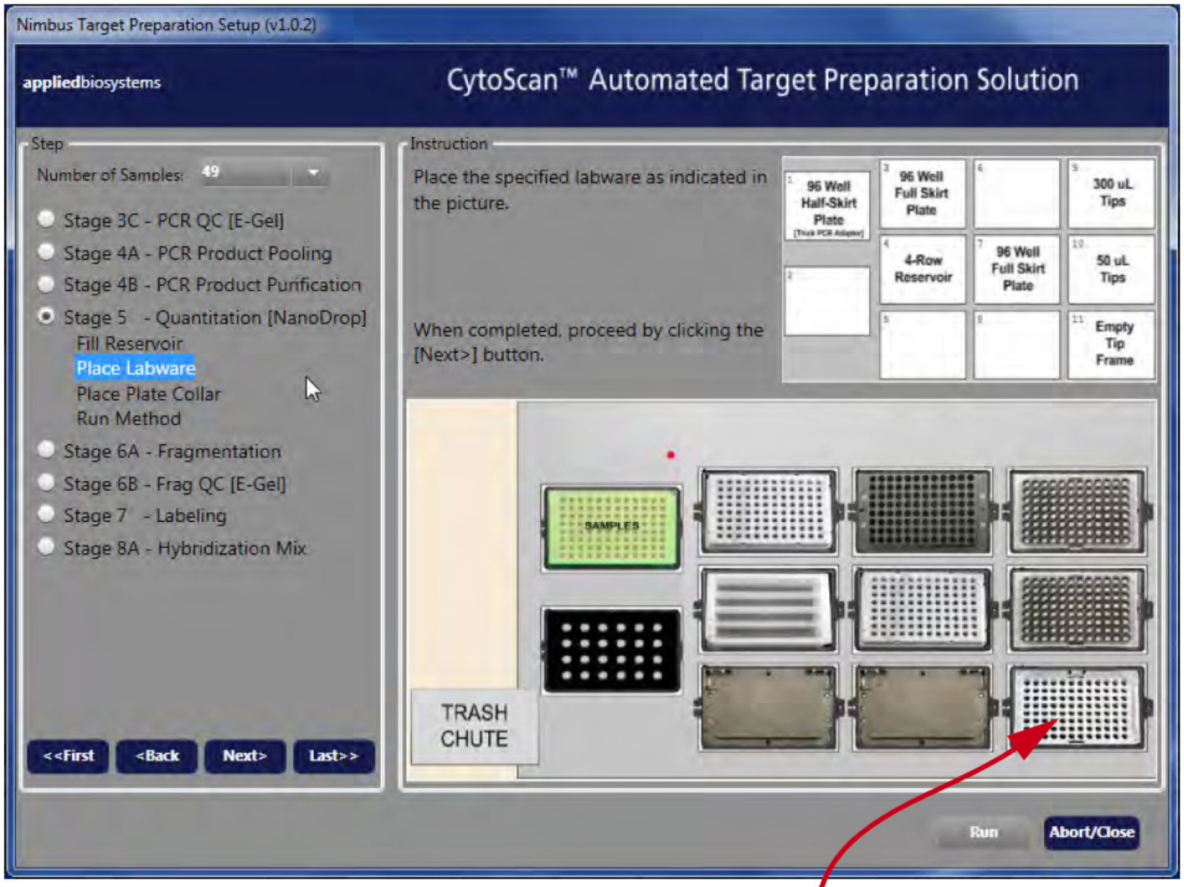


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

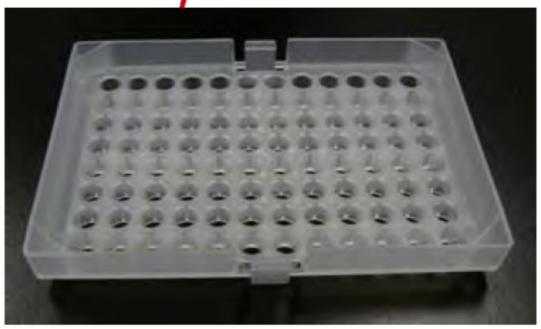
   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   Place an empty tip frame on deck position 11.

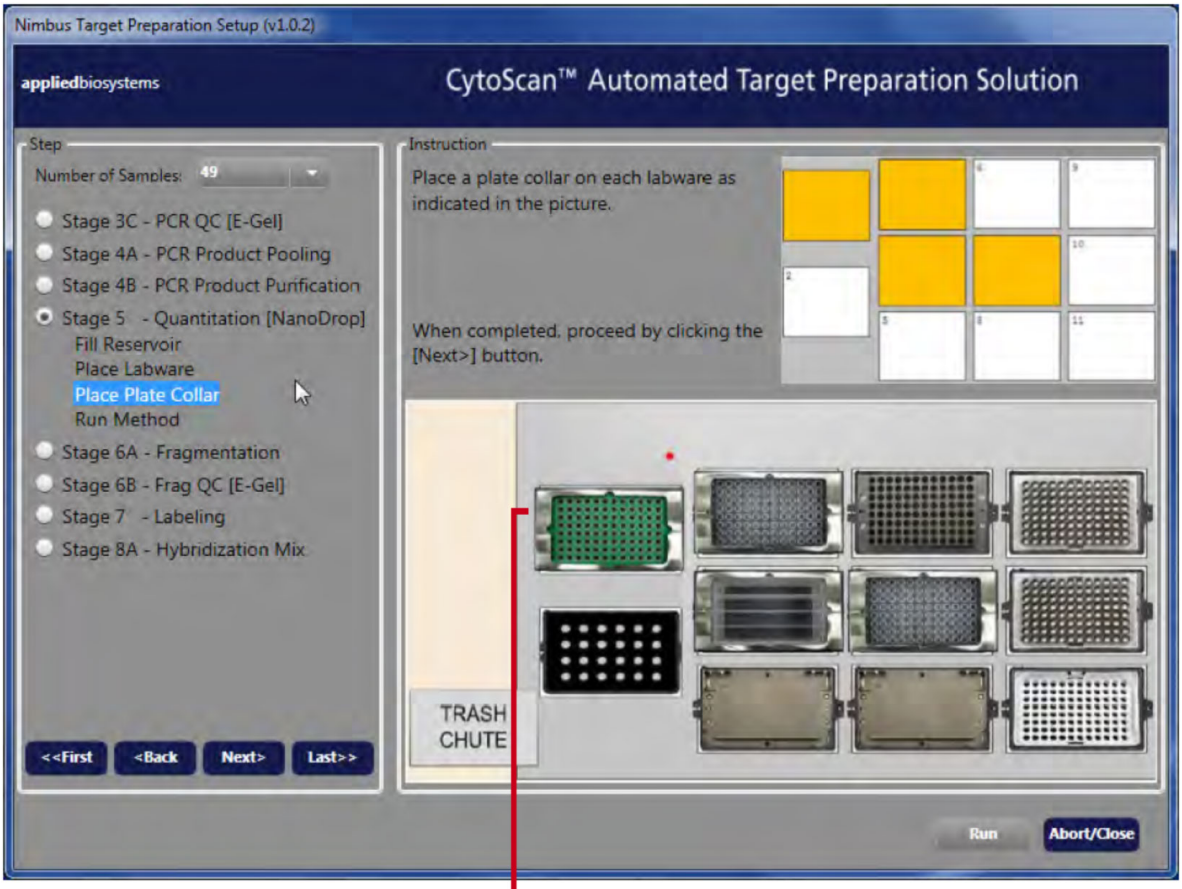
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 3, 4, and 7 require a plate collar.



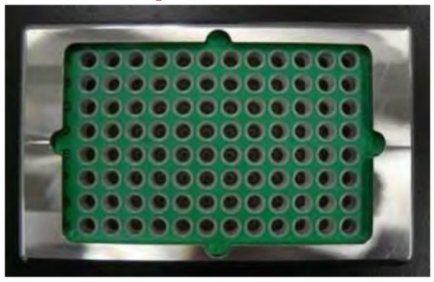
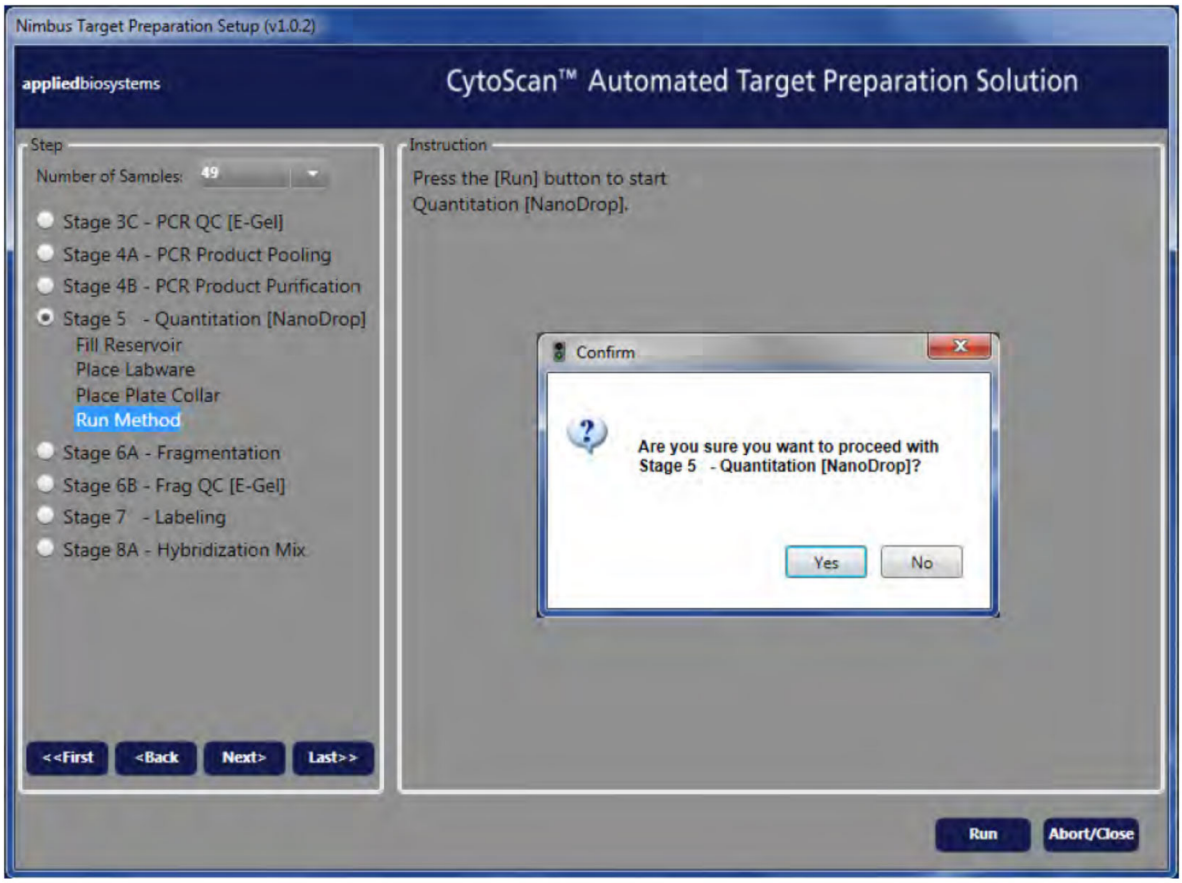
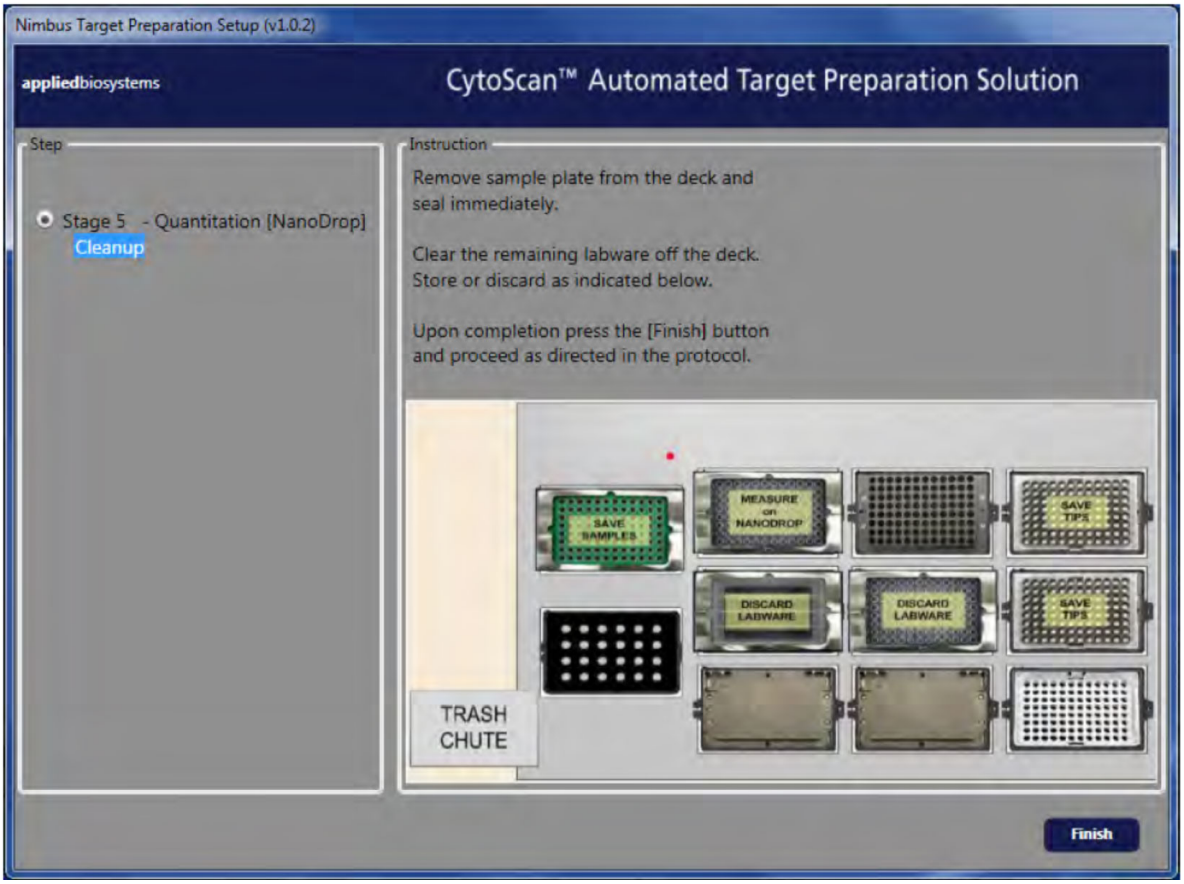


Plate collar on a sample plate

8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Tightly seal the plate with purified samples with a new seal, then store at –20°C. The plate can be stored at –20°C for up to 10 days.

10. Seal the UV plate. Using a laboratory tissue on the adaptor surface, vortex, then centrifuge at 2,000 rpm for 1 minute.

11. Remove labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

12. Click **Finish**, then click **Yes** to confirm.
    The method closes.

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.
      OD_{280} and OD_{320} 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).

Acceptable DNA yield

The average purification yield samples should be ≥3.0 µg/µL. If the average yield is <3.0 µg/µL, see the troubleshooting section. We do not recommend further processing of samples with yields <2.5 µg/µL.

The following OD ranges are based on the use of a conventional UV spectrophotometer plate reader and assume a path length of 1 cm.

- The OD_{260}/OD_{280} ratio should be >1.8.
- The OD_{320} measurement should be very close to zero (≤0.1).

If your OD readings are not within the acceptable range, see to Appendix B, “Troubleshooting”.

What to do next

Do one of the following:

- Proceed immediately to “Stage 6A—Fragmentation” on page 260.
- If not proceeding immediately to the next step, seal the plate of purified samples, and store at –20°C. The plate can be stored at –20°C for up to 10 days.

Stage 6A—Fragmentation

This stage uses the NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration. The instrument:

- Prepares the Fragmentation Master Mix.
- Aliquots the Fragmentation Master Mix into a distribution plate.
- Adds the Fragmentation Master Mix to the samples and performs mixing.

Note:

- Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.
- To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..
- The controls can be placed in any microplate well from A1 through E1 because the NIMBUS™ Instrument processes all samples.

Materials required for Stage 6A—Fragmentation

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 49 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Refrigerated plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini centrifuge
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	96-well Full Skirt PCR Plate, clear (Axygen™)

Table 49 Equipment and consumables required. *(continued)*

Quantity	Item
1	Thermal cycler
2	Tube, screw cap, no ribs, 2 mL
1	Vortexer
2	PCR Tube Racks

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

Table 50 Reagents required.

Quantity	Reagent	CytoScan™ Reagent Kit	
		Module	Part No.
1	Chilled Nuclease-Free Water	Module 2	902976
1	Fragmentation Reagent	Module 3	902428
1	Fragmentation Buffer		903001

Important information about this stage

The degree of fragmentation is critical. Perform this stage carefully to ensure uniform, reproducible fragmentation. To ensure the best results, carefully read the following information before you start this stage of the assay.

IMPORTANT! All additions, dilutions, and mixing must be performed at 4°C. Ensure that all reagents reach equilibrium before use.

About the Fragmentation Reagent

This Fragmentation Reagent is *extremely temperature sensitive* and rapidly loses activity at higher temperatures. To avoid loss of activity:

- Handle the tube only by the cap. Do not touch the sides of the tube because the heat from your fingers raises the reagent temperature.
- Keep at –20°C until ready to use. Transport and hold in a –20°C cooler. Return to the cooler immediately after use.
- Centrifuge so that the contents of the tube are uniform.
- Perform all steps rapidly and without interruption.

Prepare the reagents, equipment, and consumables

1. Power on the thermal cycler to preheat the lid. **Leave the block at room temperature**.
2. Power on the plate centrifuge, then turn down to 4°C at least 15—20 minutes before proceeding into the fragmentation step. Close the centrifuge lid to facilitate effective cooling.

   IMPORTANT! Always centrifuge the fragmentation plate in a centrifuge that has been cooled to 4°C.
3. Ensure that the plate centrifuge is at 4°C.
4. Leave 2 plate racks in the centrifuge buckets to cool.
   The racks are to be used for centrifuging the plate.

Thaw and prepare the reagents

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

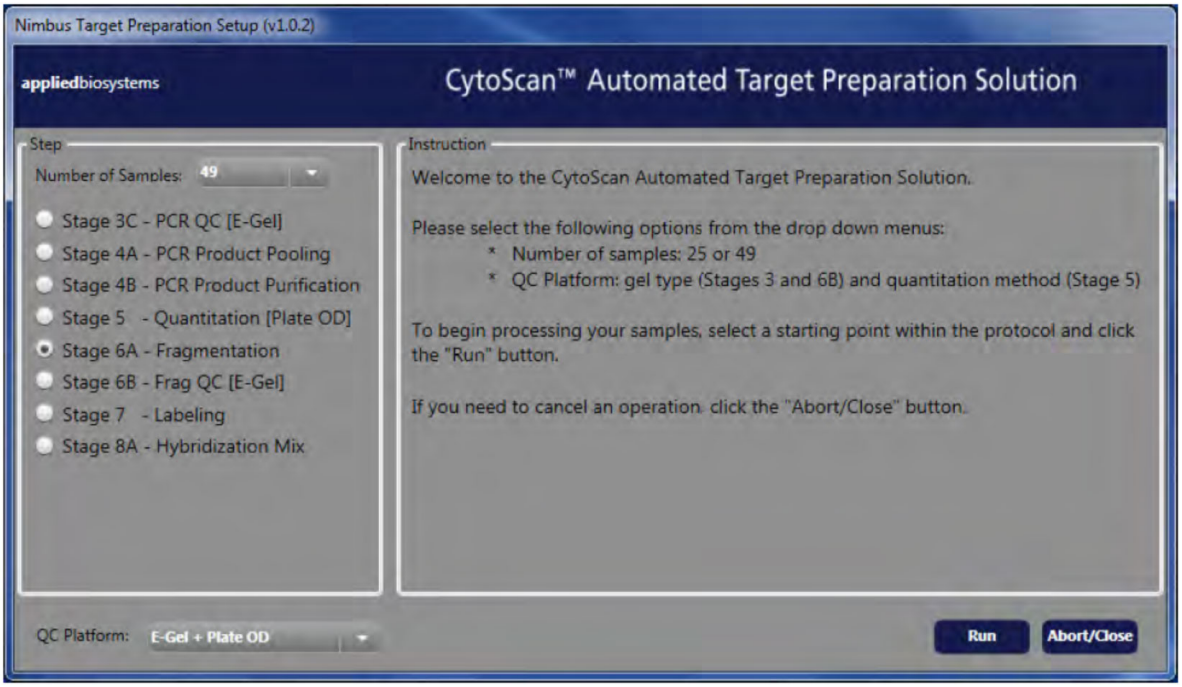
1. If the samples are frozen, remove the plate of purified, quantitated samples from the –20°C freezer, then thaw at room temperature. When thawed completely, ensure that the plate is sealed tightly, vortex, then centrifuge the plate.
2. Place the plate on the cooling block on ice, then chill for 10 minutes before use.
3. Thaw the Fragmentation Buffer (10X) at room temperature. When thawed, immediately place on ice.
4. Prepare the Fragmentation Buffer.
   a. Vortex 3 times, 1 second each time.
   b. Briefly centrifuge for 3 seconds.
   c. Place on ice.
5. Remove the Fragmentation Reagent from the freezer, then immediately place it in the cooler, chilled to –20°C.
   a. Vortex the Fragmentation Reagent at high speed 1 time for 1 second.
   b. Immediately briefly centrifuge for 3 seconds to bring down any reagent clinging to the top of the tube.
   c. Immediately place in the –20°C cooler.

Confirm these steps before the Fragmentation step run on the NIMBUS™ Instrument

- Cool the plate centrifuge to 4°C at least 15–20 minutes before proceeding with the fragmentation step. Place a plate holder rack in the centrifuge bucket so that the rack is already chilled when the fragmentation plate is ready to centrifuge.
- Pre-chill the reagents, empty the tube for master mix, and empty the strip tube before starting the fragmentation step.
- Leave the fragmentation reagent at –20°C until ready to load the NIMBUS™ Instrument deck.
- Always carry the sample plate to the centrifuge or the thermal cycler on the cooling block on ice.

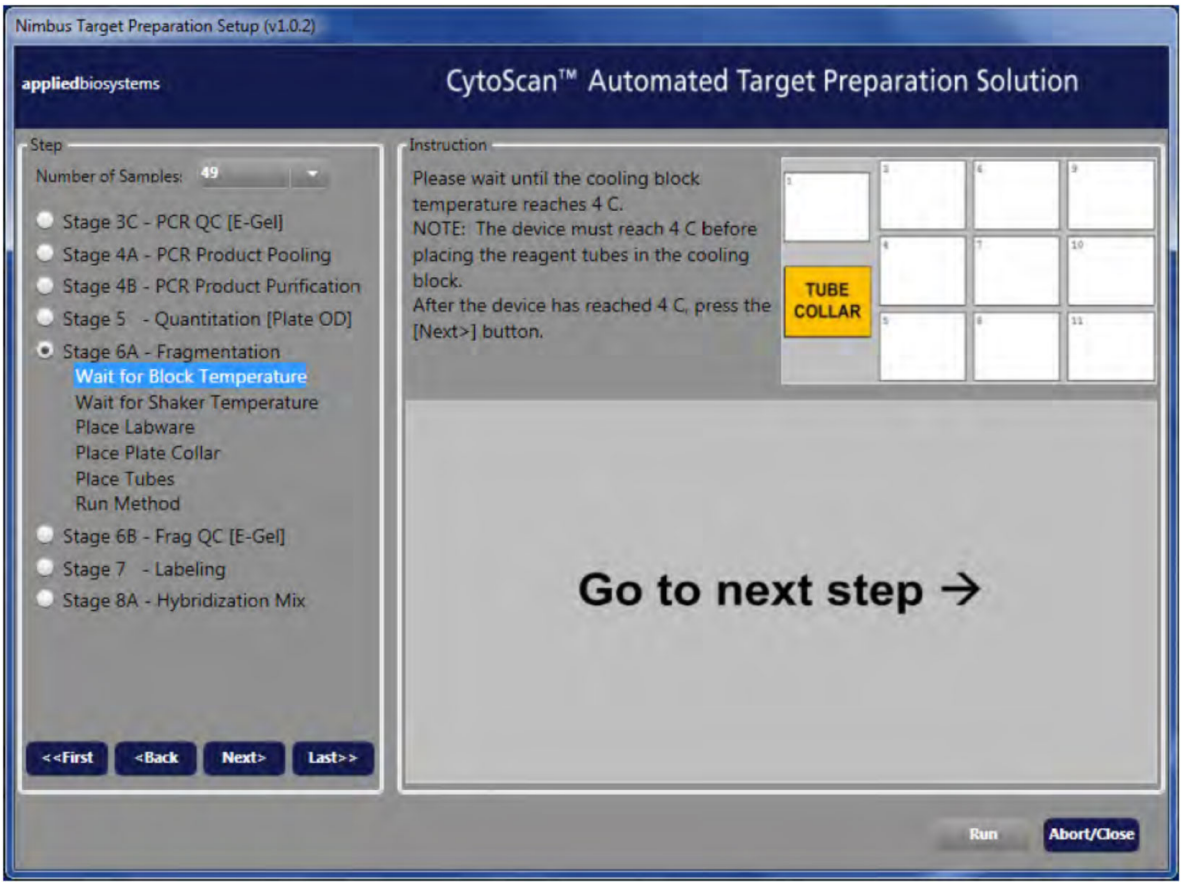
Add the Fragmentation Master Mix to the samples

1. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 6A–Fragmentation**

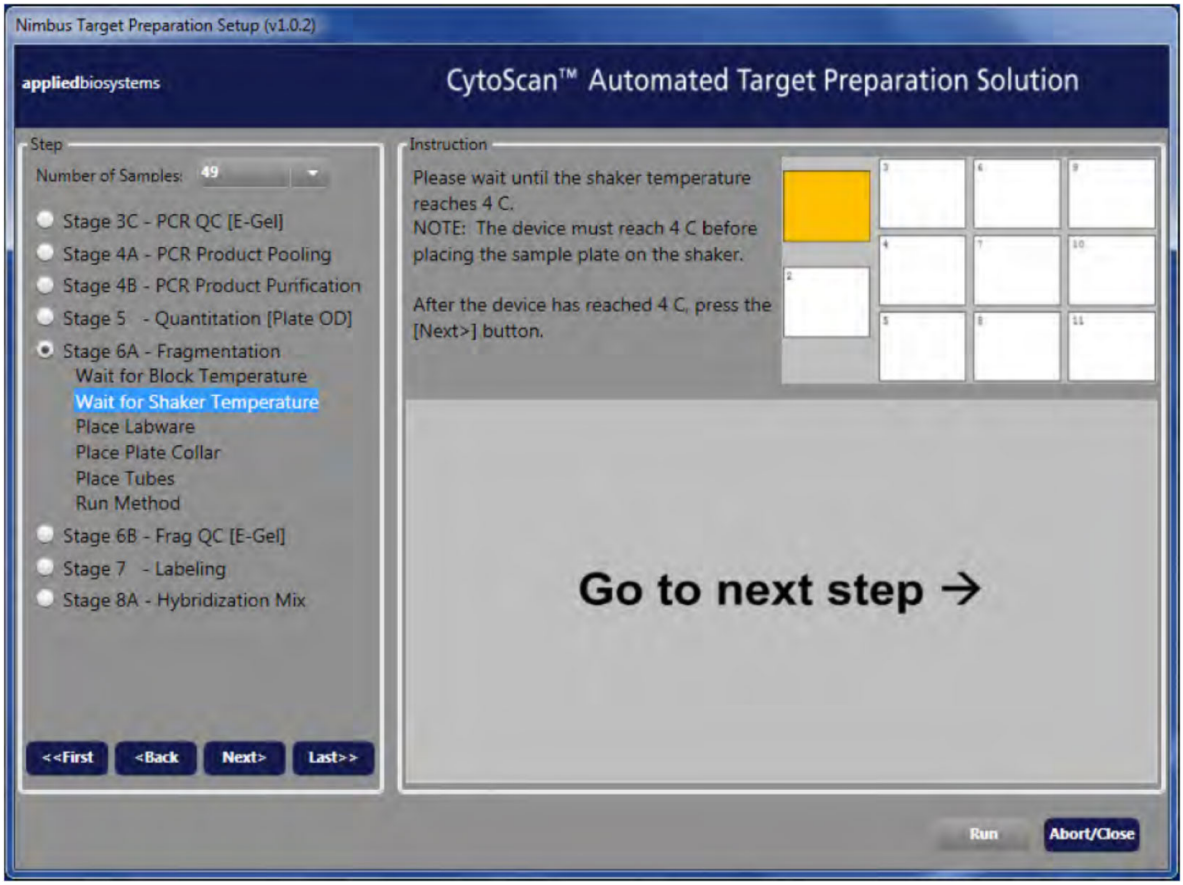


2. Click **Run** to start the method, then click **Yes** to confirm.

3. Wait until the cooling block temperature reaches 4°C, then click **Next**.



4. Wait until the shaker temperature reaches 4°C, then click **Next**.

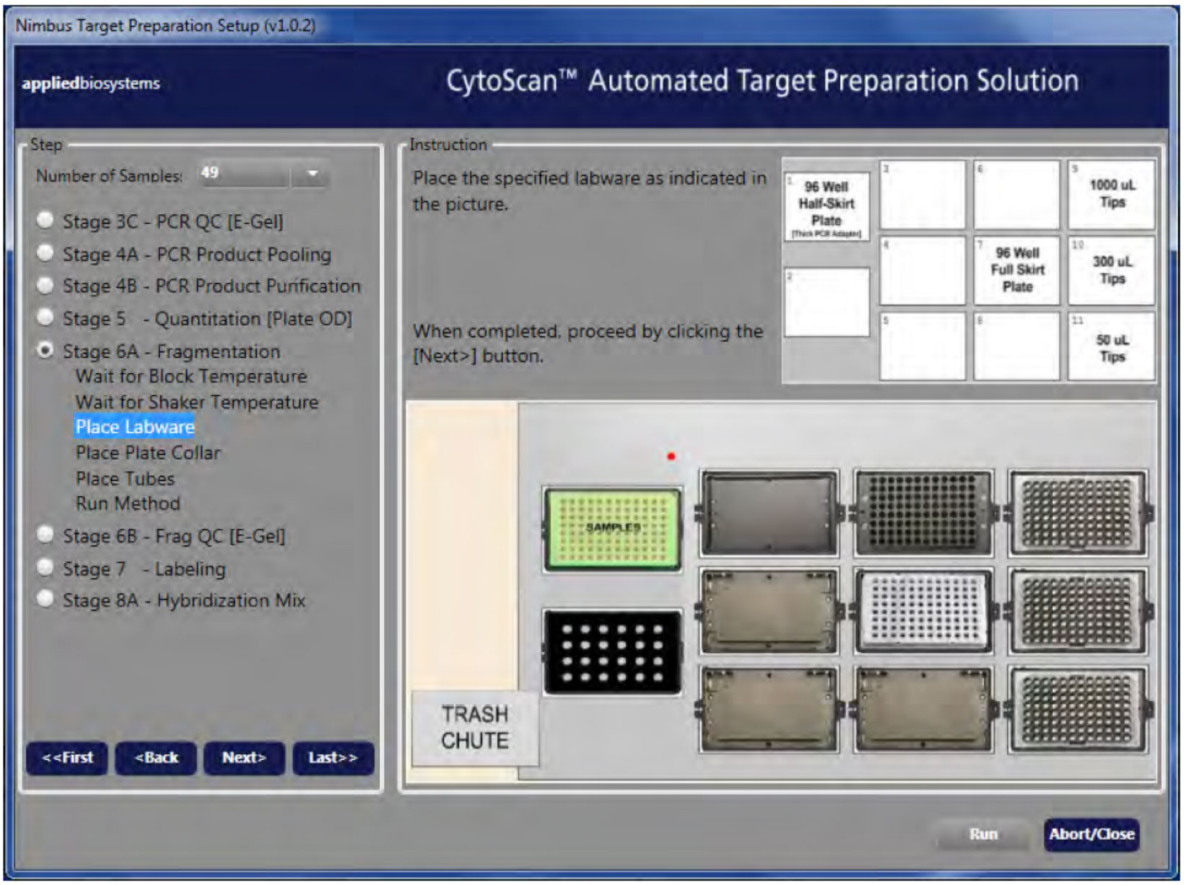


5. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   Place an empty tip frame on deck position 11.

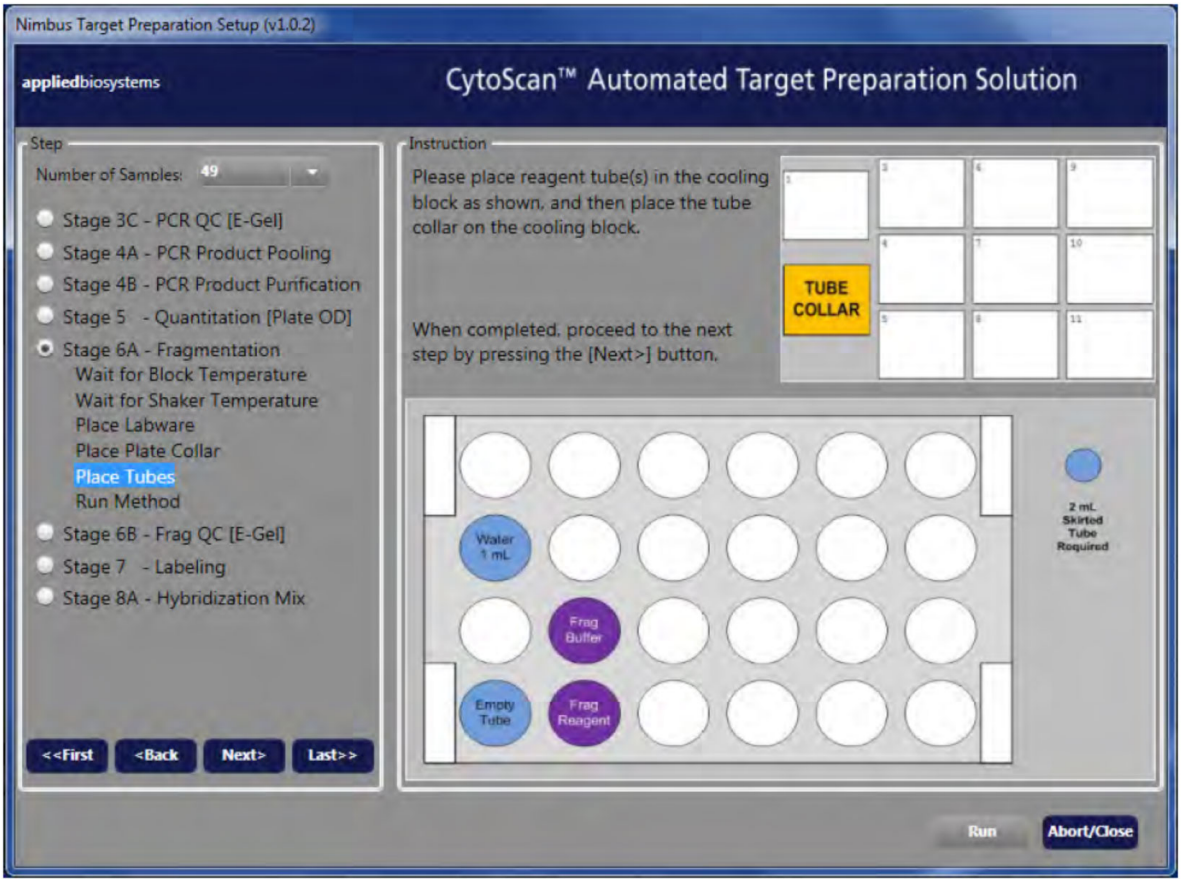
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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


Nimbus Target Preparation Setup (v1.0.2)
appliedbiosystems
CytoScan™ Automated Target Preparation Solution
Step
Number of Samples: 49
Stage 3C - PCR QC [E-Gel]
Stage 4A - PCR Product Pooling
Stage 4B - PCR Product Purification
Stage 5 - Quantitation [Plate OD]
Stage 6A - Fragmentation
Wait for Block Temperature
Wait for Shaker Temperature
Place Labware
Place Plate Collar
Place Tubes
Run Method
Stage 6B - Frag QC [E-Gel]
Stage 7 - Labeling
Stage 8A - Hybridization Mix
<<First
<Back
Next>
Last>>
Instruction
Place the specified labware as indicated in the picture.
When completed, proceed by clicking the [Next>] button.
96 Well Half-Skirt Plate
96 Well Full Skirt Plate
1000 uL Tips
300 uL Tips
50 uL Tips
SAMPLES
TRASH CHUTE
Run
Abort/Close

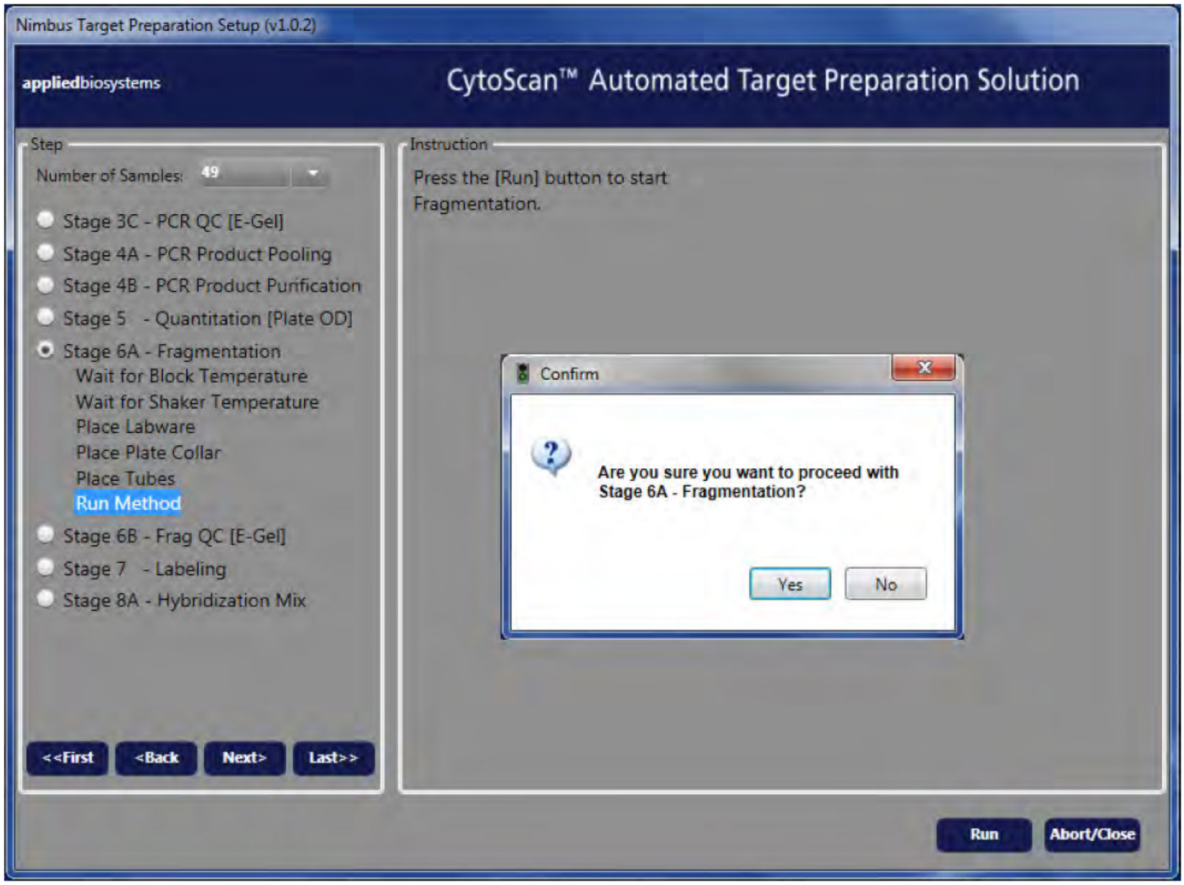
6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**.

Plate collar on a sample plate

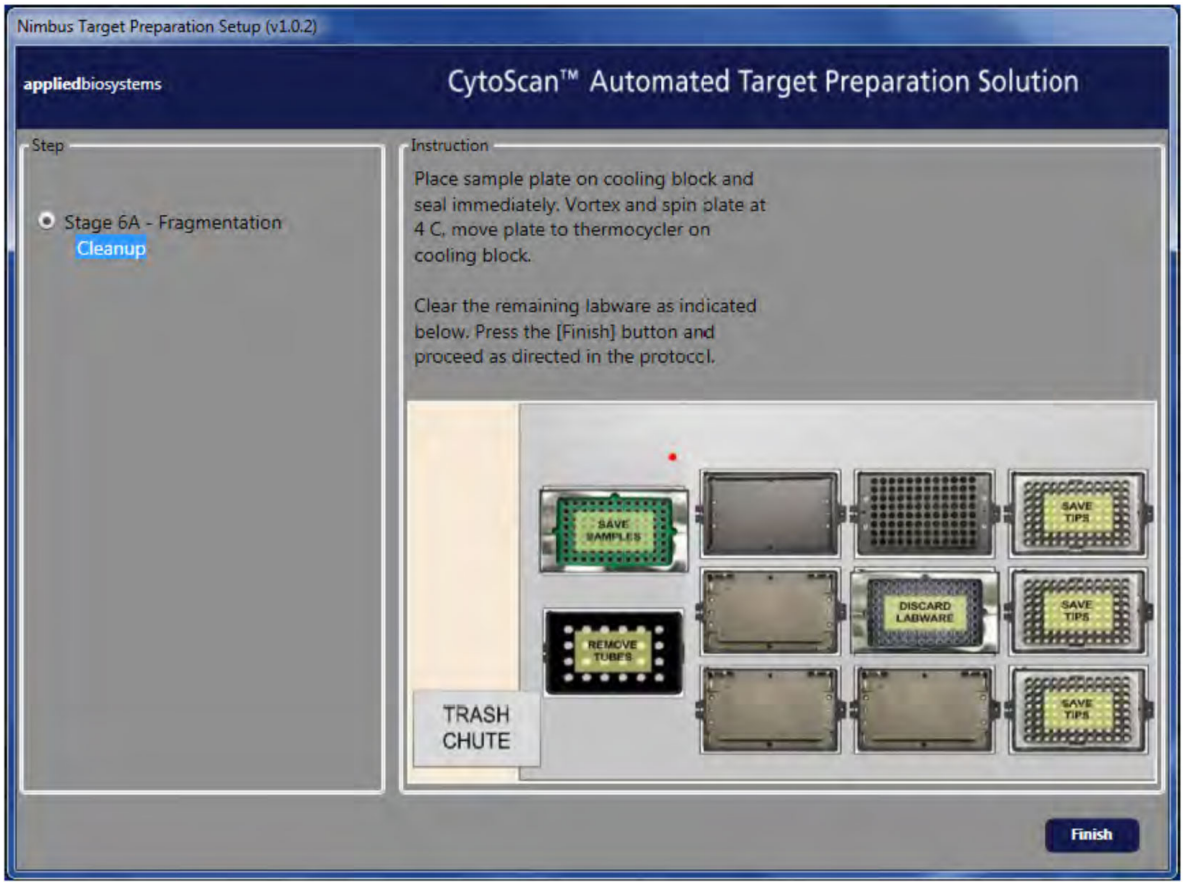
7. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. 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.

10. Vortex at high speed for 1 second in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40.

11. Bring the sample plate to the centrifuge on the cooling block in the ice box. Centrifuge the plate on chilled racks in the prechilled centrifuge at 2,000 rpm for 1 minute. Quickly remove the plate from the centrifuge, then place in the cooling block in the ice box.

12. Carry the sample plate on the cooling block in the ice box and immediately load the Fragmentation plate onto the thermal cycler with preheated lid.

13. Run the **CytoScan Fragment** thermal cycler protocol. Total volume for Fragmentation = 55 µL.

Temperature	Time
37°C	35 minutes
95°C	15 minutes
4°C	Hold

14. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

15. Remove and discard any remaining Fragmentation Master Mix.

IMPORTANT! Never reuse Fragmentation Master Mix.

Note: At this point the plate centrifuge can be turned back to room temperature.

16. Verify the fragmentation reaction by running gels as described in “Stage 6B—Fragmentation QC” on page 272.

Stage 6B—Fragmentation QC

This stage verifies the fragmentation by running a TBE gel or an E-Gel™ Agarose Gel using NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration.

Perform fragmentation QC using one of these methods:

- "Fragmentation QC procedure using TBE gels" on page 273.
- "Fragmentation QC procedure using E-Gel™ Agarose Gels" on page 282.

Note: Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.

Note: To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 6B—Fragmentation QC

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 51 Gels and related materials required for Fragmentation QC using TBE gels.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration (Cat. No. 00-0401)
As required	MicroAmp™ Clear Adhesive Film for 96-well plates (Cat. No. 4306311)
1 (Optional)	MicroAmp™ Adhesive Film Applicator (Cat. No. 4333183)
1	Mini centrifuge
1	Plate centrifuge
1	Electrophoresis gel box
1	Electrophoresis power supply
1	Gel, 4% TBE (precast or house-made)
1 liter	1X TBE Buffer
Few drops	Ethidium bromide solution
1	5X RapidRun™ Loading Dye
1 rack	Conductive 50 µL filter tips in frames
1 rack	Conductive 300 µL filter tips in frames
1	Reservoir 4 ROW PYR PP 73 mL
1	Tube, screw cap, no ribs, 2 mL

Table 51 Gels and related materials required for Fragmentation QC using TBE gels. *(continued)*

Quantity	Item
2	96-well Full Skirt PCR Plate, clear
1	25 bp DNA Ladder (25–300 bp)

Table 52 Gels and related materials required for Fragmentation QC using E-Gel™ Agarose Gels.

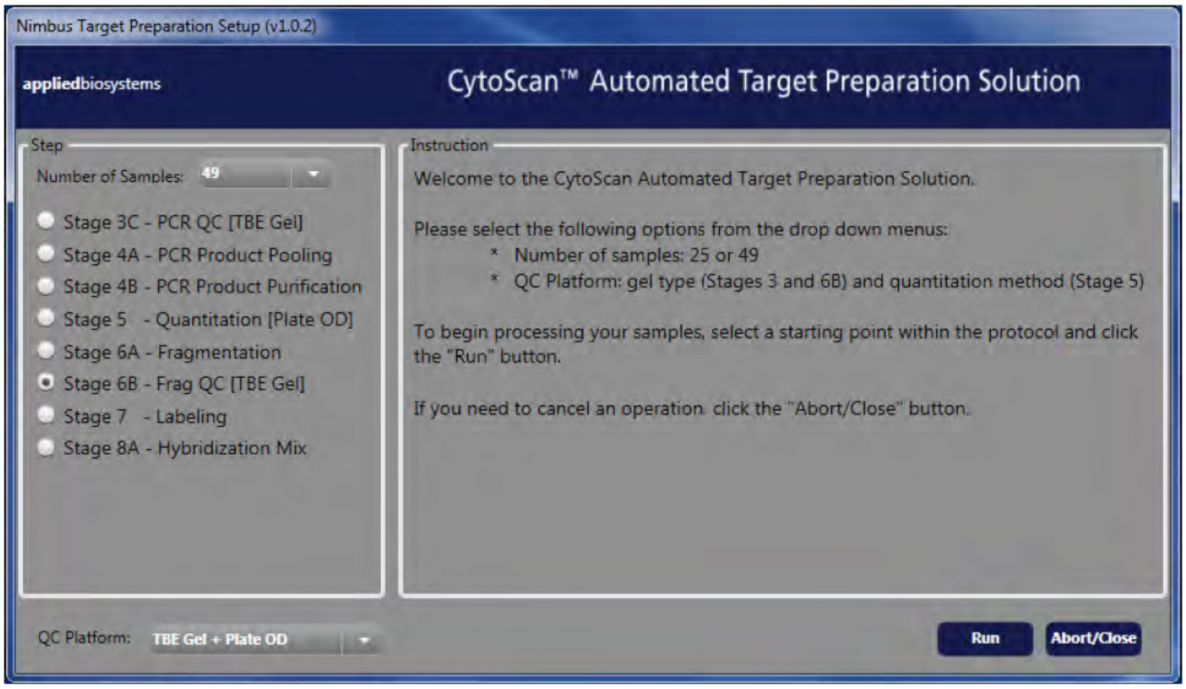
Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Mini centrifuge
1	Plate centrifuge
1	Mother E-Base™ Device
1	Daughter E-Base™ Device (optional for running multiple gels simultaneously)
2	96-well Full Skirt PCR Plate, clear
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1	Reservoir 4 ROW PYR PP 73 mL
1	Tube, screw cap, no ribs, 2 mL
2	E-Gel™ *48* Agarose Gels, 4%, Cat. No. G800804
1	TrackIt™ Cyan/Orange Loading Buffer
1	25 bp DNA Ladder (25–300 bp)

Fragmentation QC procedure using TBE gels

The controls can be placed in any well from A1 through E1 because the NIMBUS™ Instrument processes all of the samples.

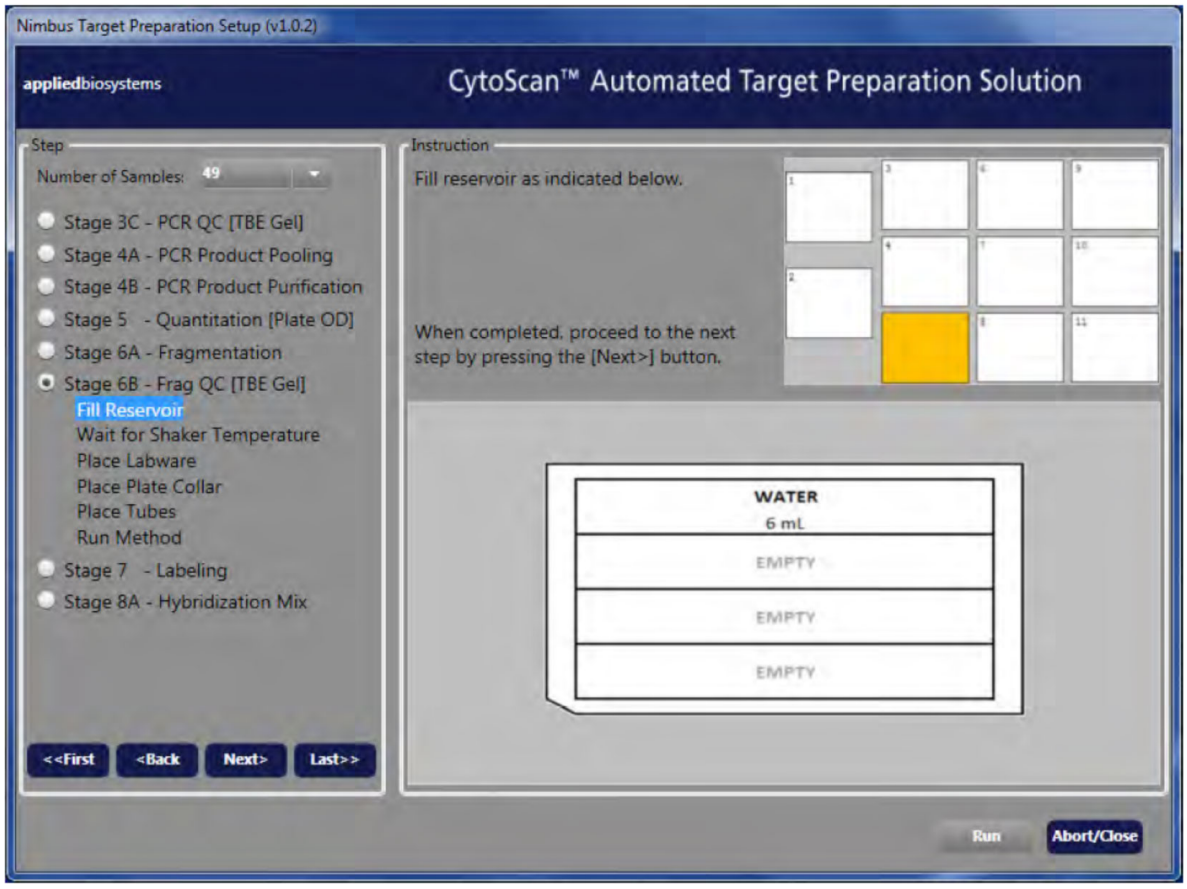
1. When the **CytoScan Fragment** thermal cycler protocol is finished:
   a. Remove the sample plate from the thermal cycler.
   b. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute. Place on a cooling block on ice.

2. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 6A–Fragmentation**
   - **QC Platform**—For example, “TBE Gel + Plate OD”.

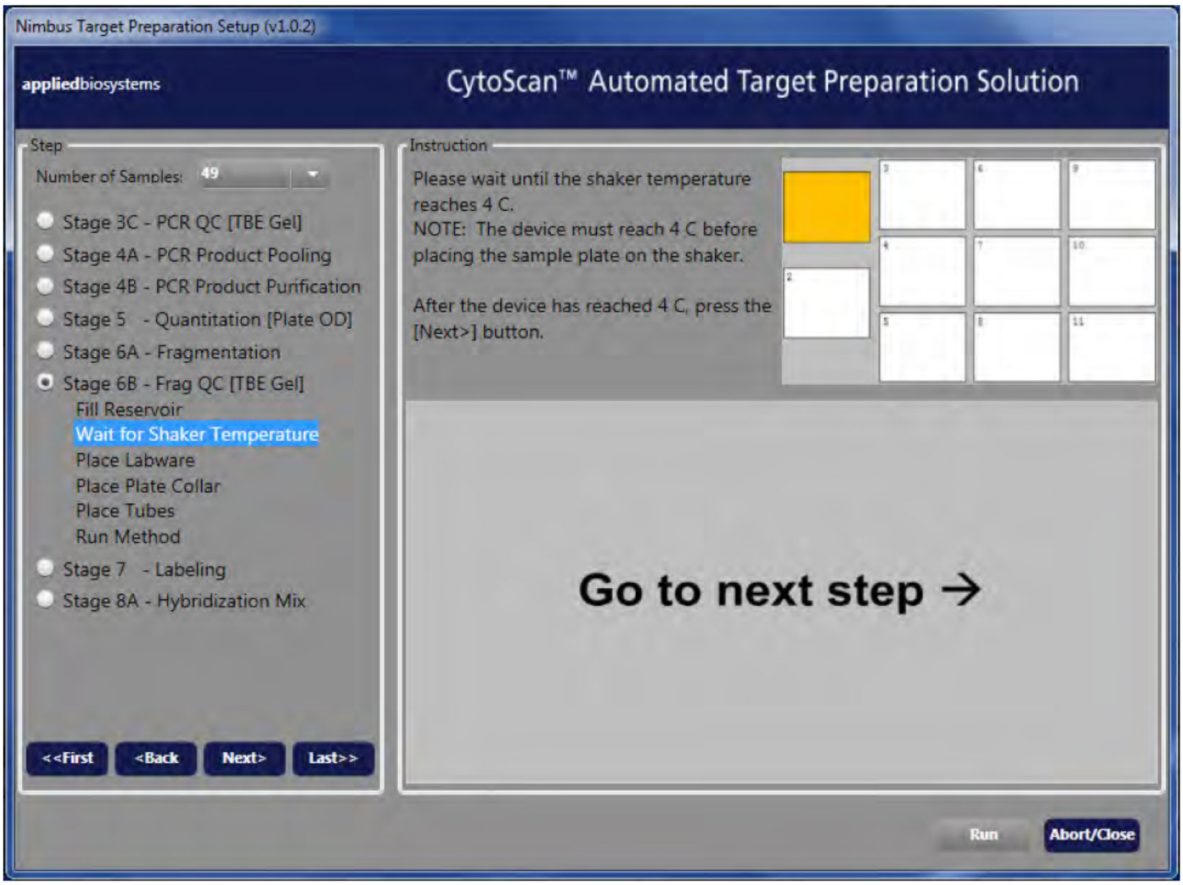


3. Click **Run** to start the method, then click **Yes** to confirm.

4. Fill the reservoir as shown in the on-screen instructions, then click **Next**.



5. Wait until the shaker temperature reaches 4°C, then click **Next**.

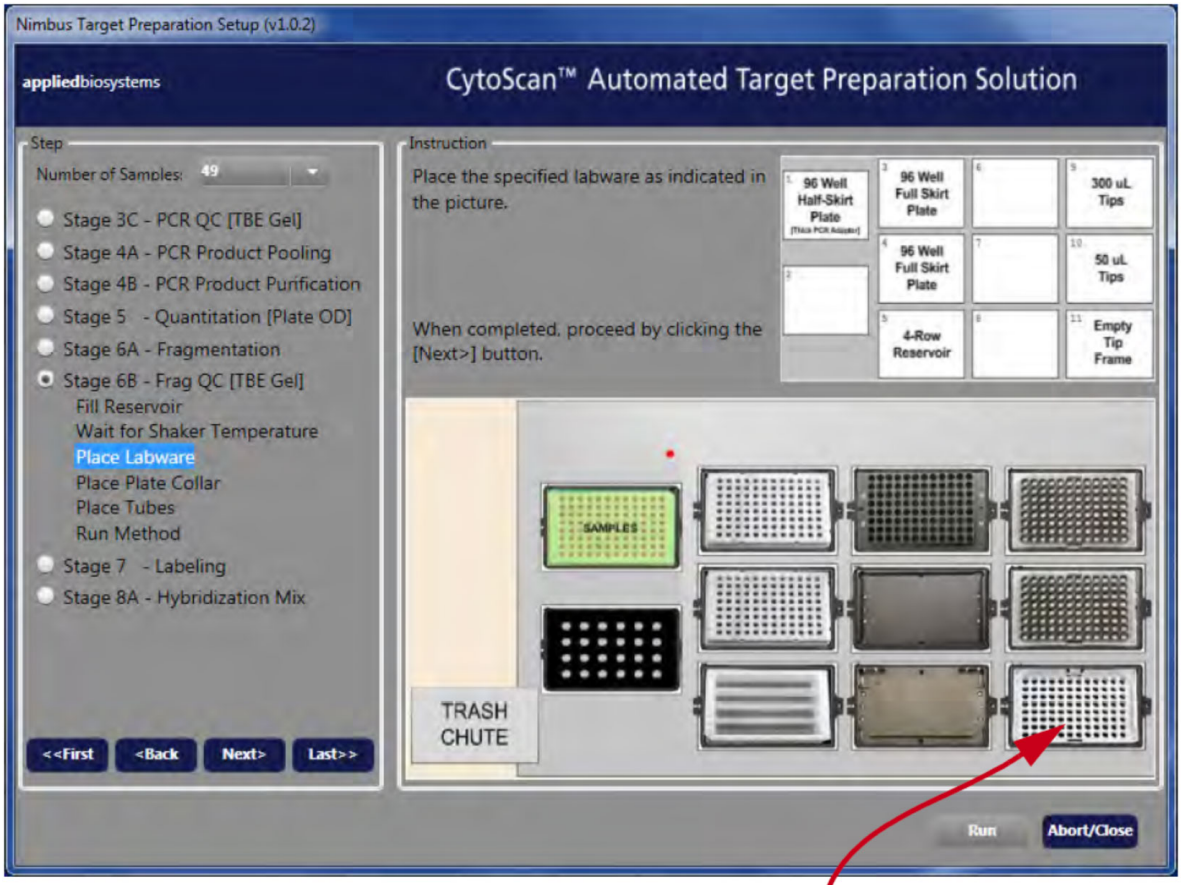


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

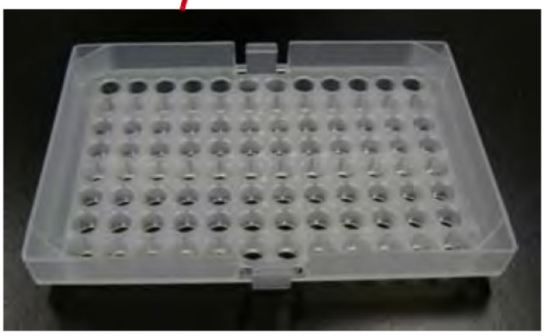
Note: Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

Place an empty tip frame on deck position 11.

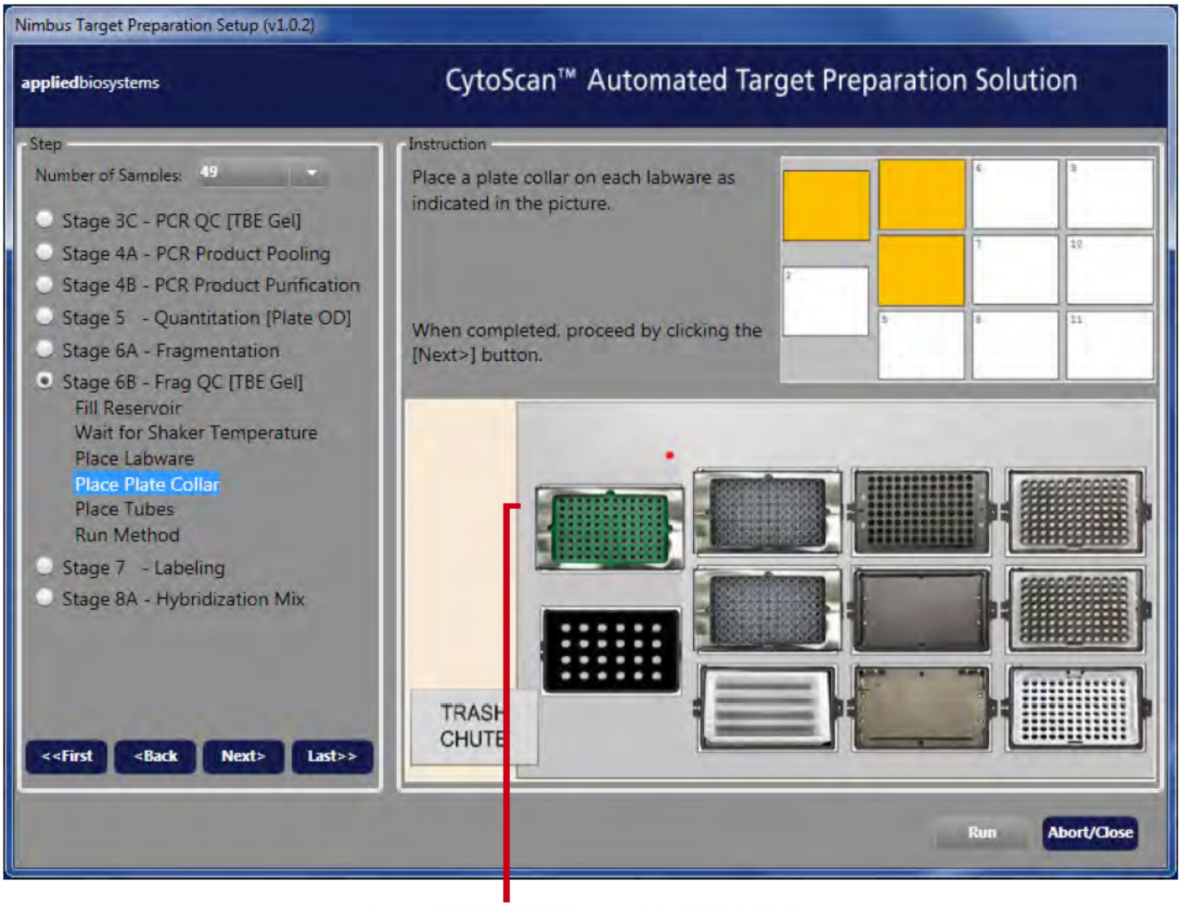
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 3, 4, and 5 require a plate collar.



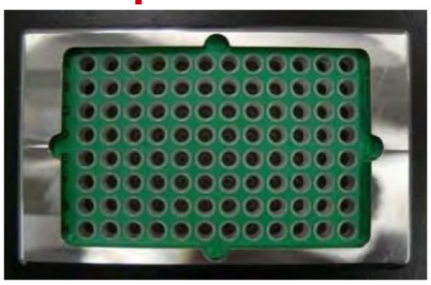
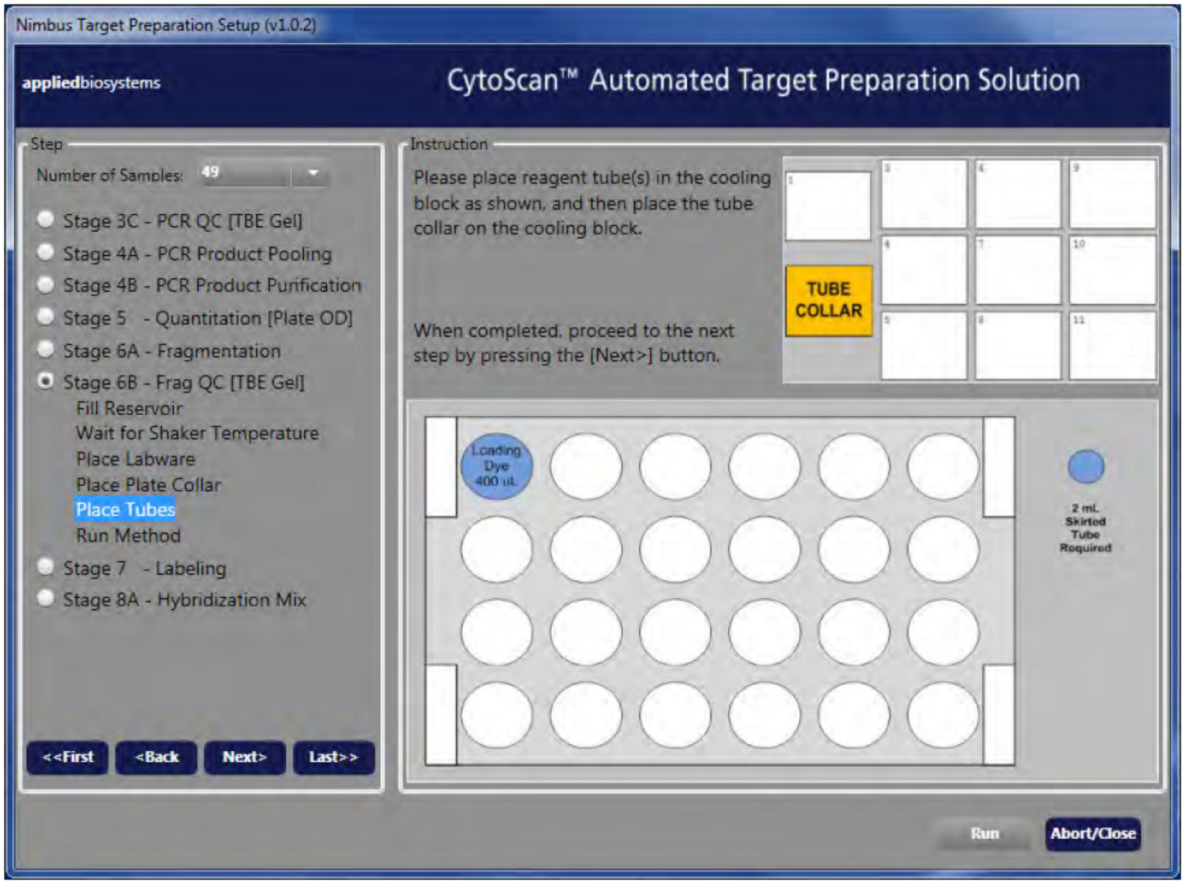
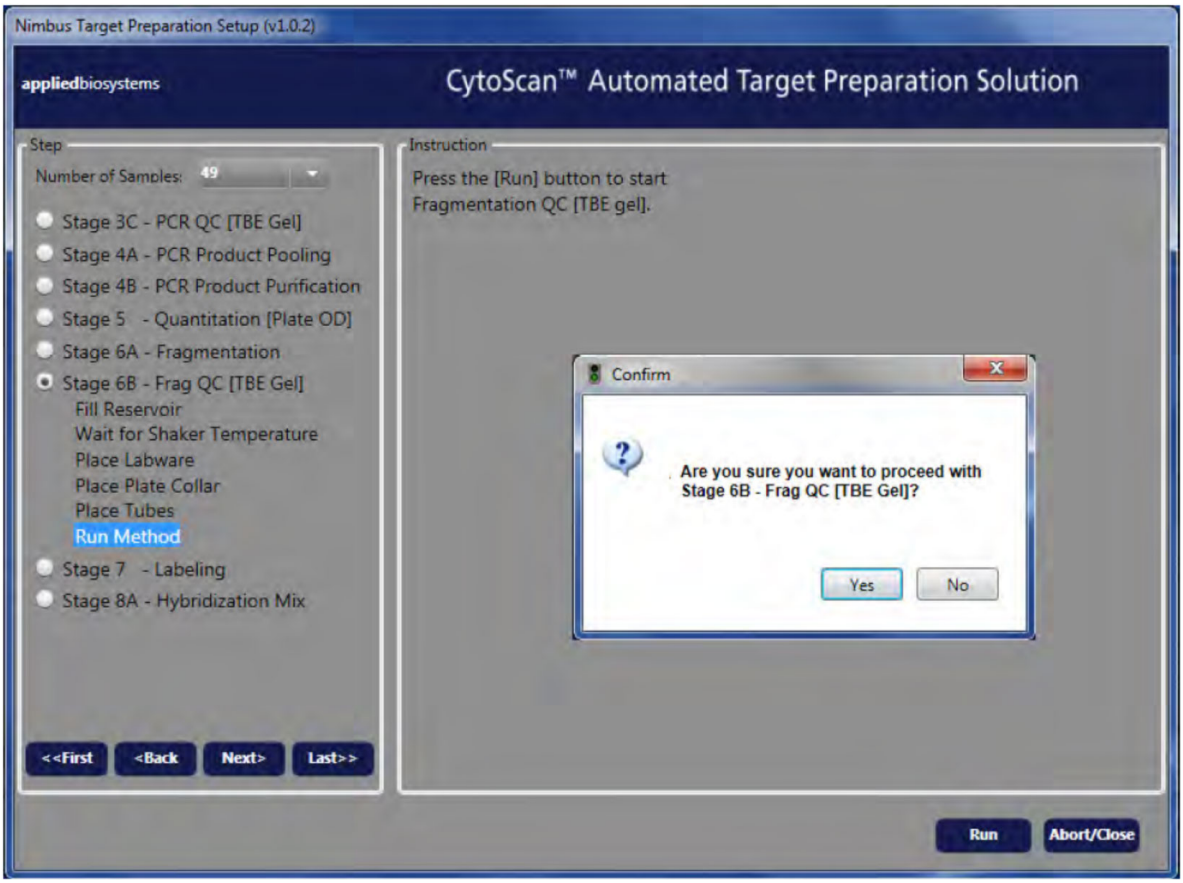


Plate collar on a sample plate

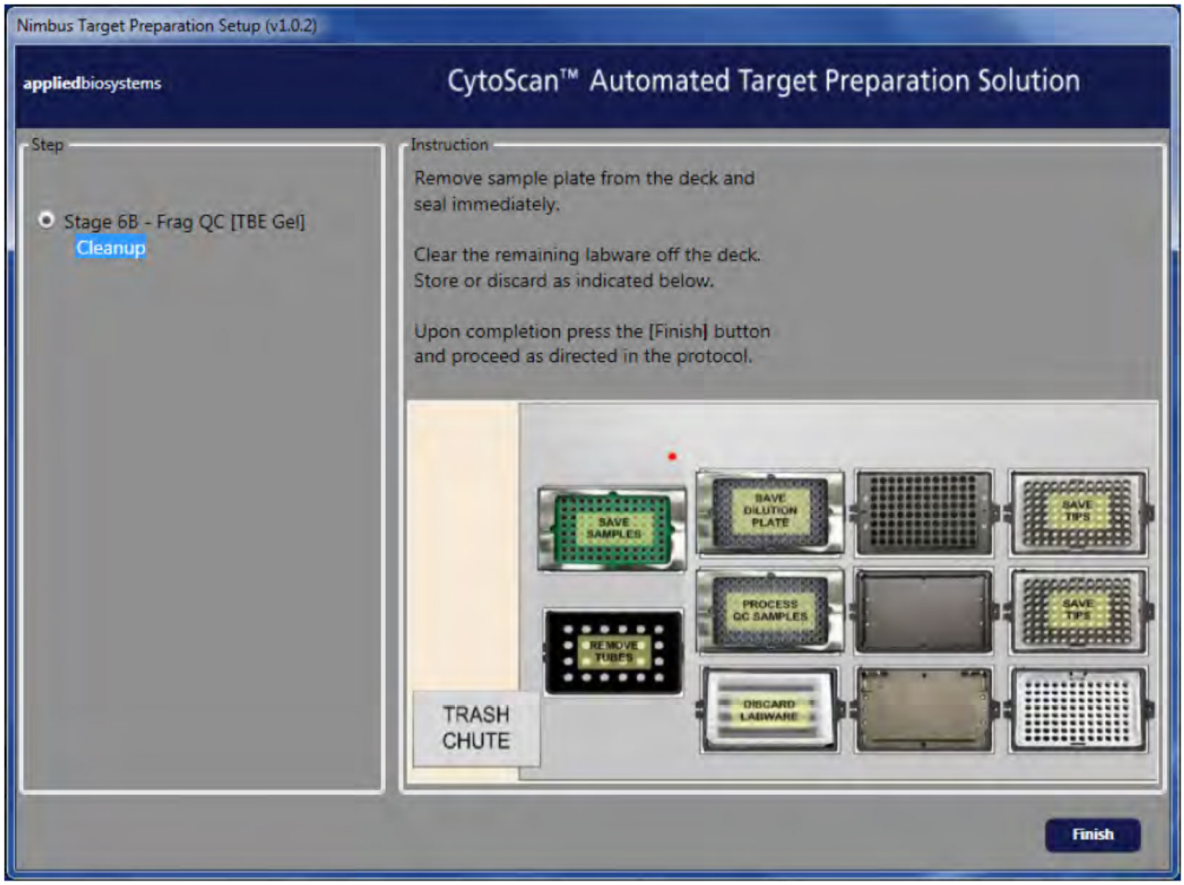
8. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



9. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



10. Remove the sample plate from the deck, then tightly seal the plate.
    The plate of fragmented DNA can be stored at –20°C for up to 10 days.

11. Seal and store the Fragmentation QC Sample Dilution plate at –20°C for further analysis using the Agilent™ 2100 Bioanalyzer™ Instrument.
    See Appendix C, “Analyzing sample fragmentation using the Agilent™ 2100 Bioanalyzer™ Instrument”.

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

13. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

14. Click **Finish**, then click **Yes** to confirm.
    The method closes.

Run the TBE gel

1. Load 8 µL of the samples from the QC sample plate onto the gel.

2. Load 2 µL of 25 bp DNA Ladder to the first and last lanes.

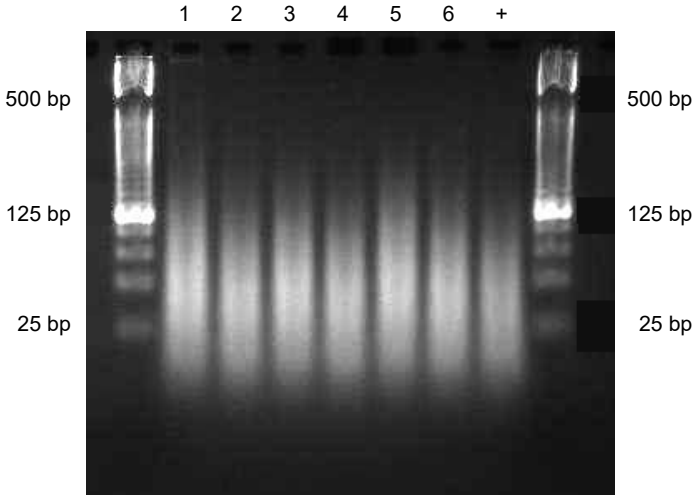
3. Run the samples on a 4% TBE gel at 5 V/cm for 45 minutes or until the dye front reaches at least 75% of distance down the gel.

   Run gels at 5 V/cm (5 volts X distance in cm between electrodes). For example, run a 33-cm electrophoresis box at 165 V; run a 16-cm electrophoresis box at 80 V.

4. Inspect the gel, then compare it against the example shown in the following example.

   The majority of fragment distribution must be between 25 and 125 bp.

Figure 34 Example of fragmented PCR products run on 4% TBE Gel at 5 V/cm for 45 minutes. Fragmentation is verified by the majority of distribution between 25 and 125 bp.



Fragmentation QC procedure using E-Gel™ Agarose Gels

The controls can be placed in any microplate well from A1 through E1 because the NIMBUS™ Instrument processes all of the samples.

Dilute the TrackIt™ Cyan/Orange Loading Buffer

The following instructions prepare a 1,000-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer.

1. Add 50 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.95 mL Nuclease-Free Water (total volume is 50 mL).

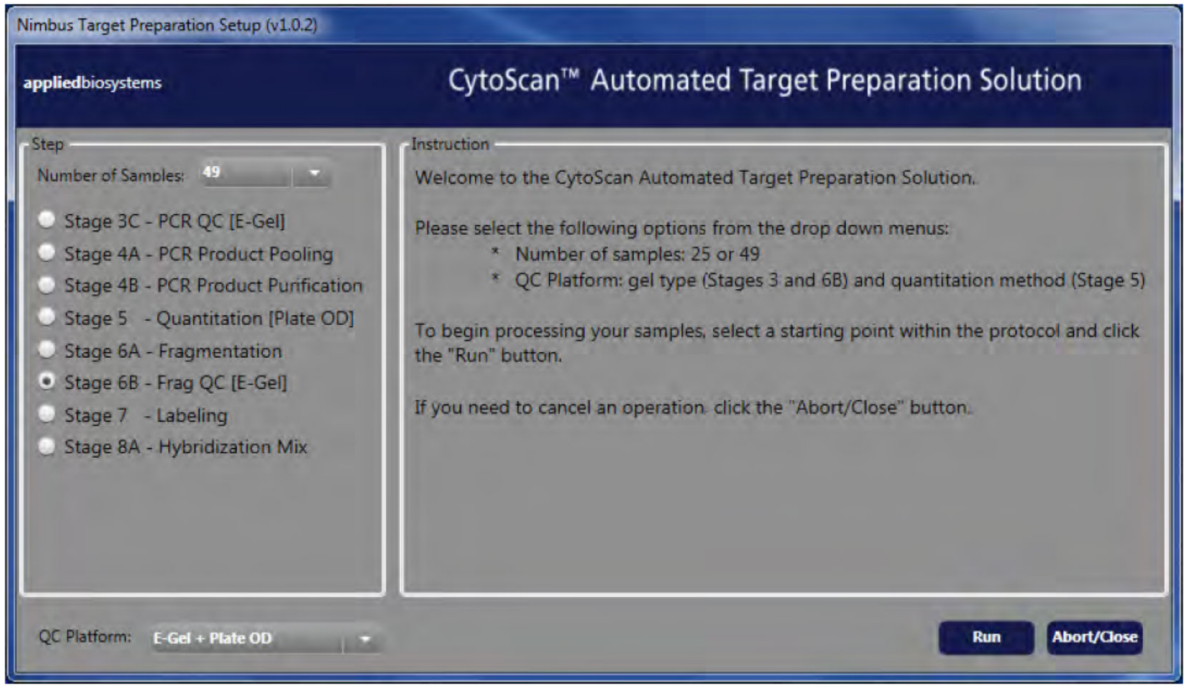
2. Mix well, then store at room temperature.

Prepare the QC gel plate

1. When the **CytoScan Fragment** thermal cycler protocol is finished:
   a. Remove the sample plate from the thermal cycler.

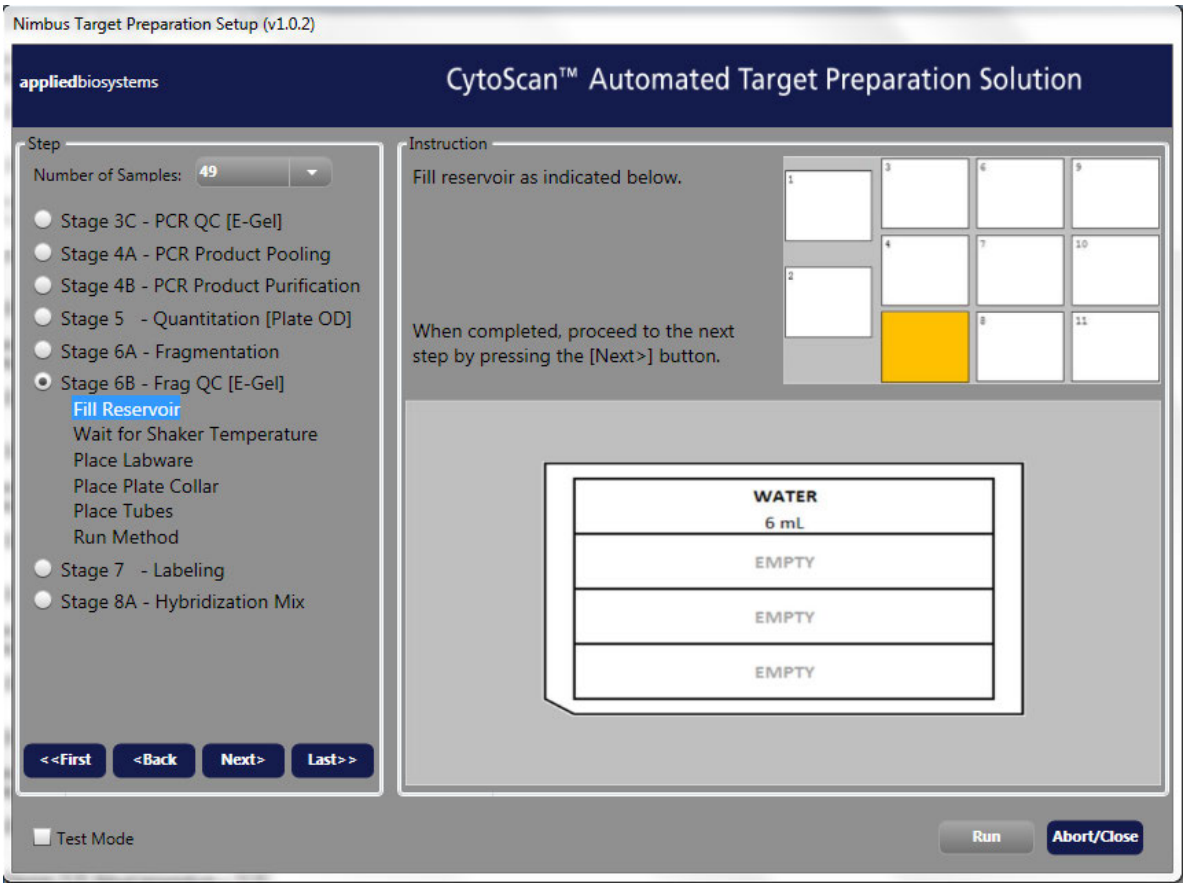
   b. Ensure that the plate is sealed tightly, then centrifuge at 2,000 rpm for 1 minute. Place on a cooling block on ice.

2. On the **Welcome** screen, select the following options:
   - **Number of samples**—49
   - **Stage 6B–Frag QC**
   - **QC Platform**—For example, "E-Gel + Plate OD"

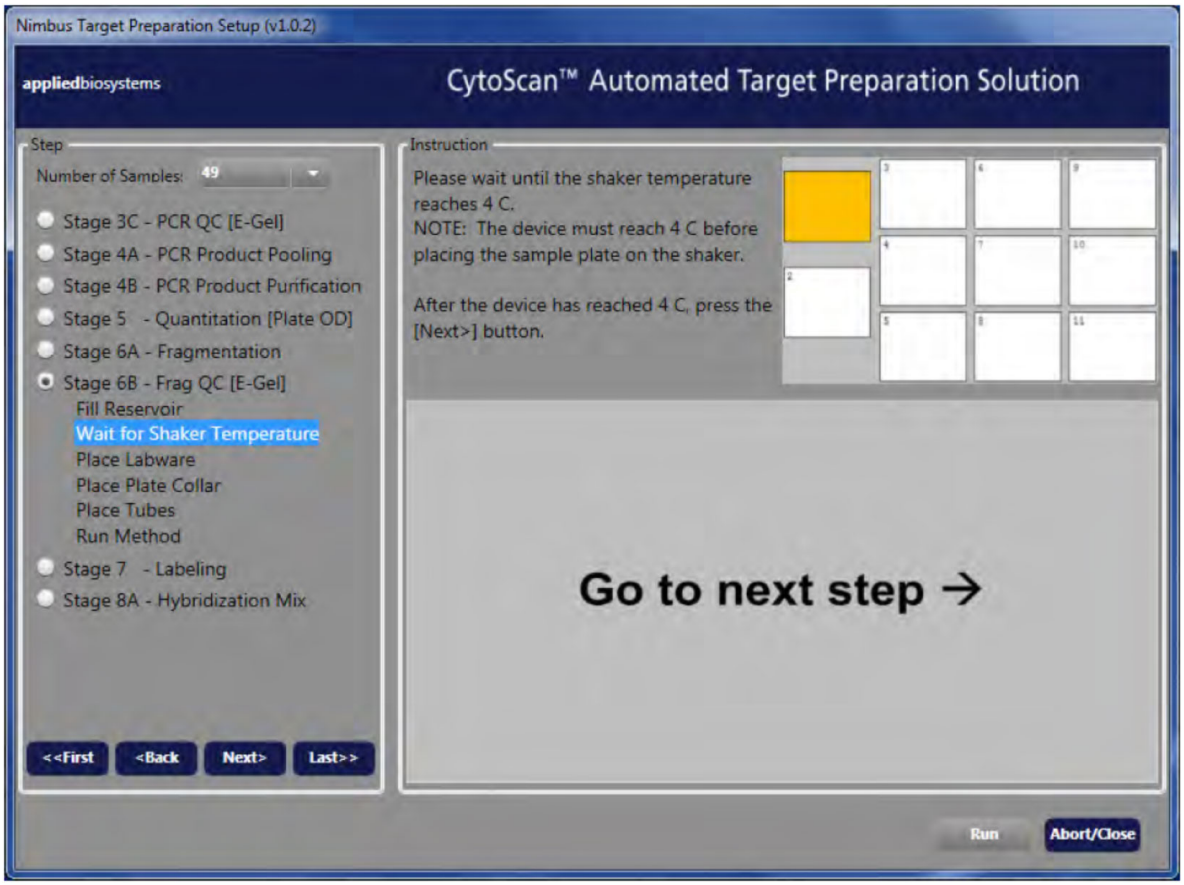


3. Click **Run** to start the method, then click **Yes** to confirm.

4. Fill the reservoir as shown in the on-screen instructions, then click **Next**.



5. Wait until the shaker temperature reaches 4°C, then click **Next**.

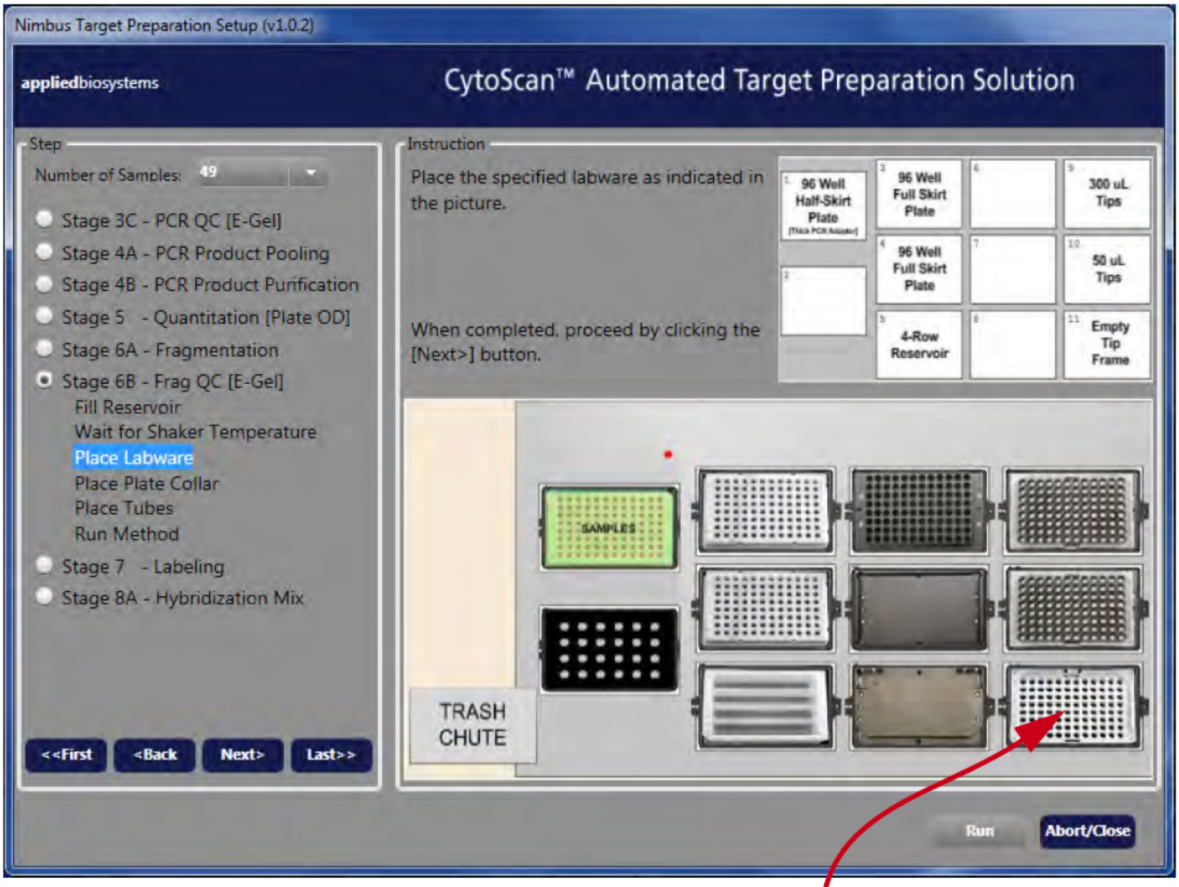


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

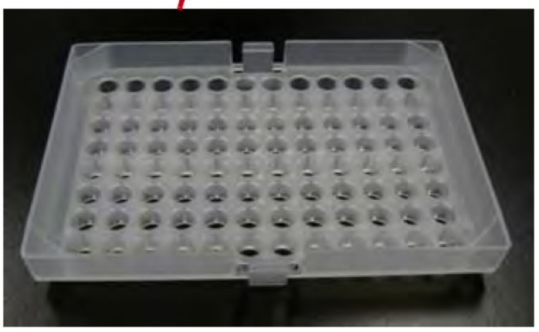
   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   Place an empty tip frame on deck position 11.

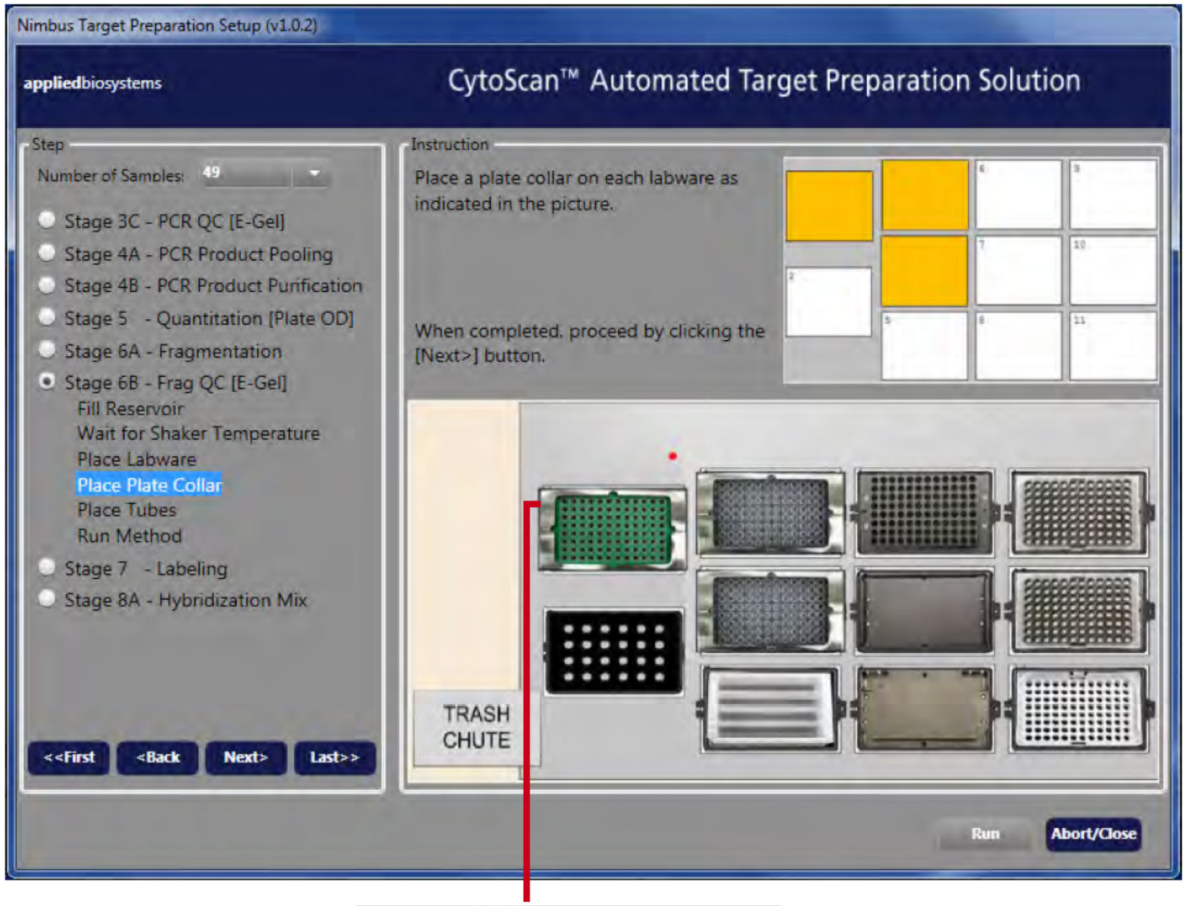
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 3, and 4 require a plate collar.



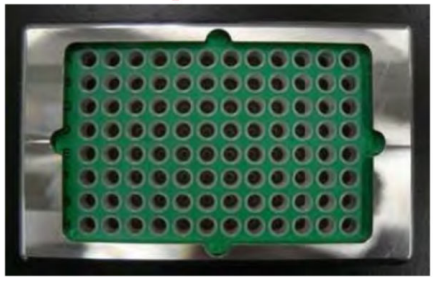
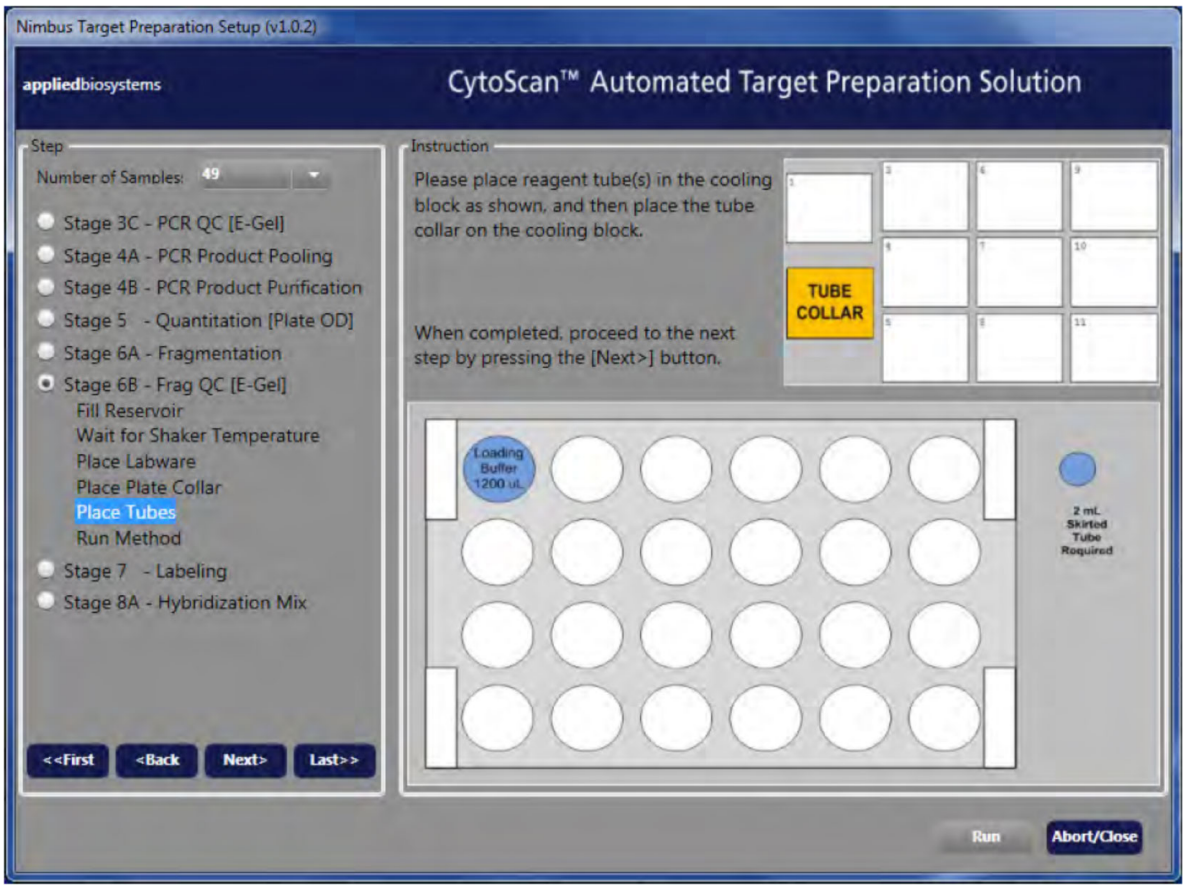
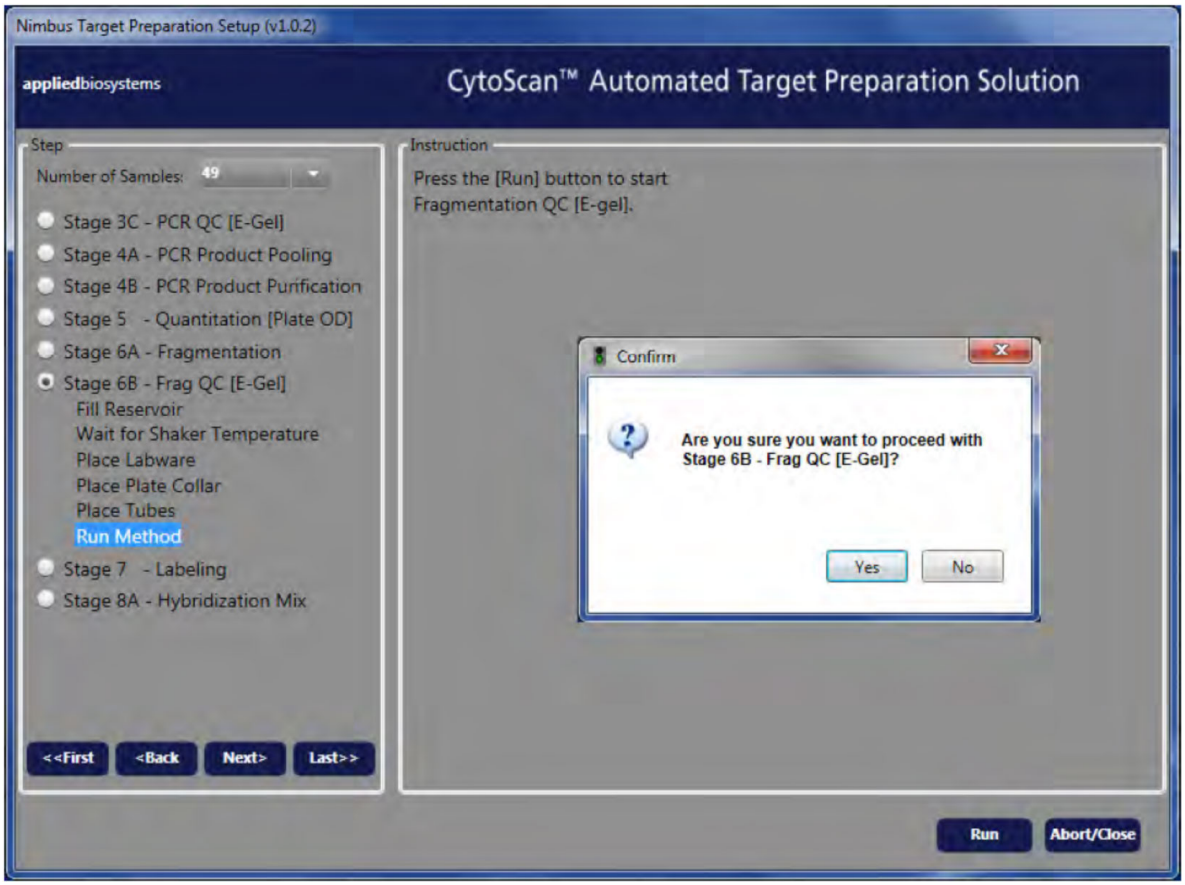


Plate collar on a sample plate

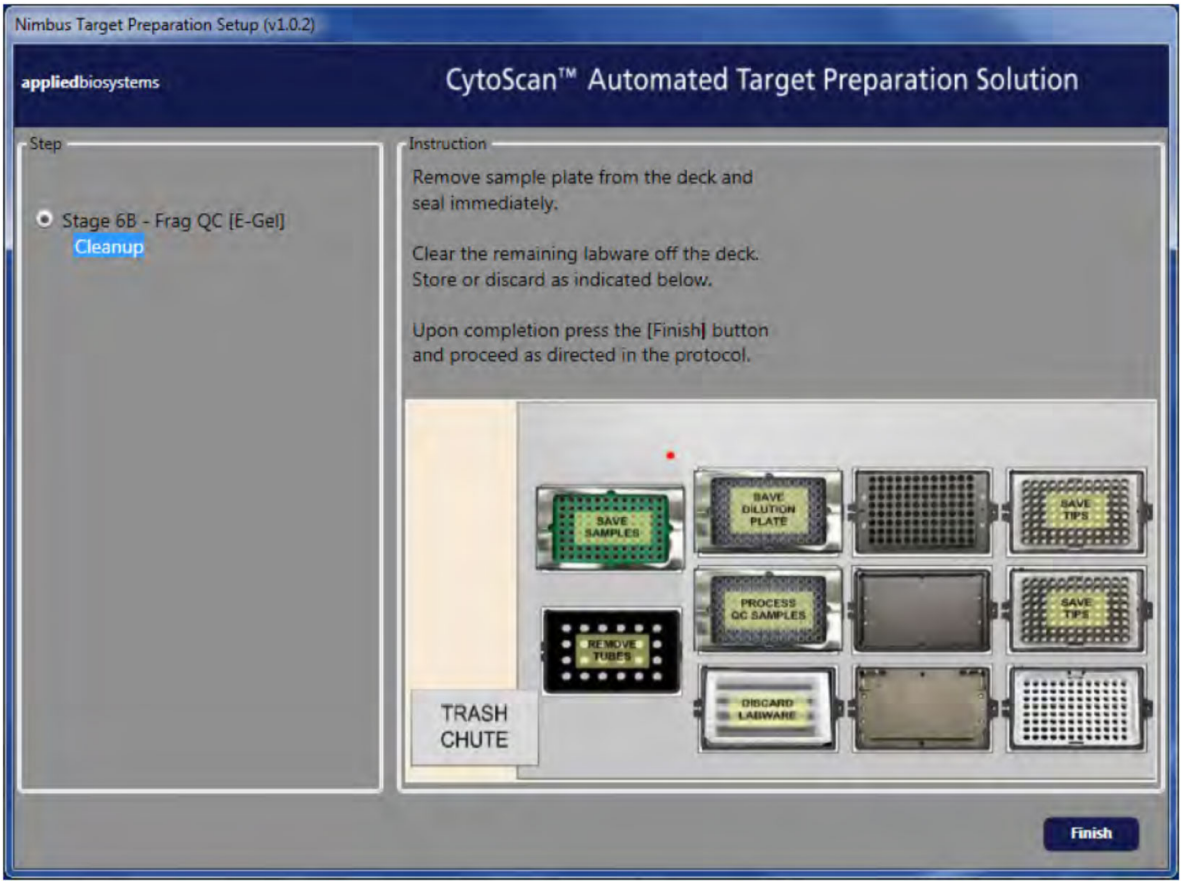
8. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



9. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



10. Remove the sample plate from the deck, then tightly seal the plate.

11. Seal and store the Fragmentation QC Sample Dilution plate at –20°C for further analysis using the Agilent™ 2100 Bioanalyzer™ Instrument.

    See Appendix C, “Analyzing sample fragmentation using the Agilent™ 2100 Bioanalyzer™ Instrument”.

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

13. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.

14. Click **Finish**, then click **Yes** to confirm.

    The method closes.

Run the E-Gel™ Agarose Gel

1. Power on the E-Base™ device 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.
   A 12-well E-Gel™ Agarose Gel can also be used if running a smaller number of samples.
5. Load 20 µL of the samples 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 of the marker wells (as needed). Fill empty wells with 20 µL water.
7. Set the run time to 19 minutes.
8. Push the Power/Prg button again (the light changes from red to green).
   When the run time is reached, the system automatically powers off (when the dye is near the end of the lane). The gel is then ready for imaging.

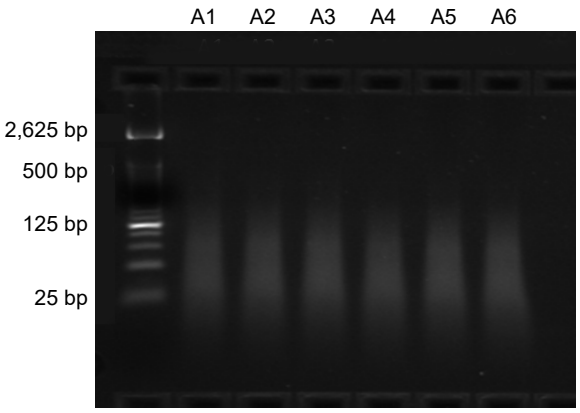


Figure 35 Gel image of fragmented product (from REF DNA 103 (50 ng/µL)) on E-Gel™ *48* Agarose Gels, 4%.

9. Proceed to "Stage 7—Labeling" on page 292.

Stage 7—Labeling

This stage uses the NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration. The instrument:

- Prepares the Labeling Master Mix.
- Aliquots the Labeling Master Mix into a distribution plate.
- Adds the Labeling Master Mix to samples and performs mixing.

Note:

- Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.
- To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..
- The controls can be placed in any microplate well from A1 through E1 because the NIMBUS™ Instrument processes all samples.

Materials required for Stage 7—Labeling

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 53 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Cooler, chilled to –20°C
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini centrifuge
1 rack	Conductive 50-µL filter tips in frames
1 rack	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	Thermal cycler

Table 53 Equipment and consumables required. *(continued)*

Quantity	Item
1	Tube, screw cap, no ribs, 2 mL
1	96-well Full Skirt PCR Plate, clear
1	Vortexer
2	PCR Tube Racks

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

Table 54 Reagents required.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
2	TdT Enzyme	Module 3	902675
2	TdT Buffer		902676
1	DNA Labeling Reagent		902677

Prepare the reagents, equipment, and consumables

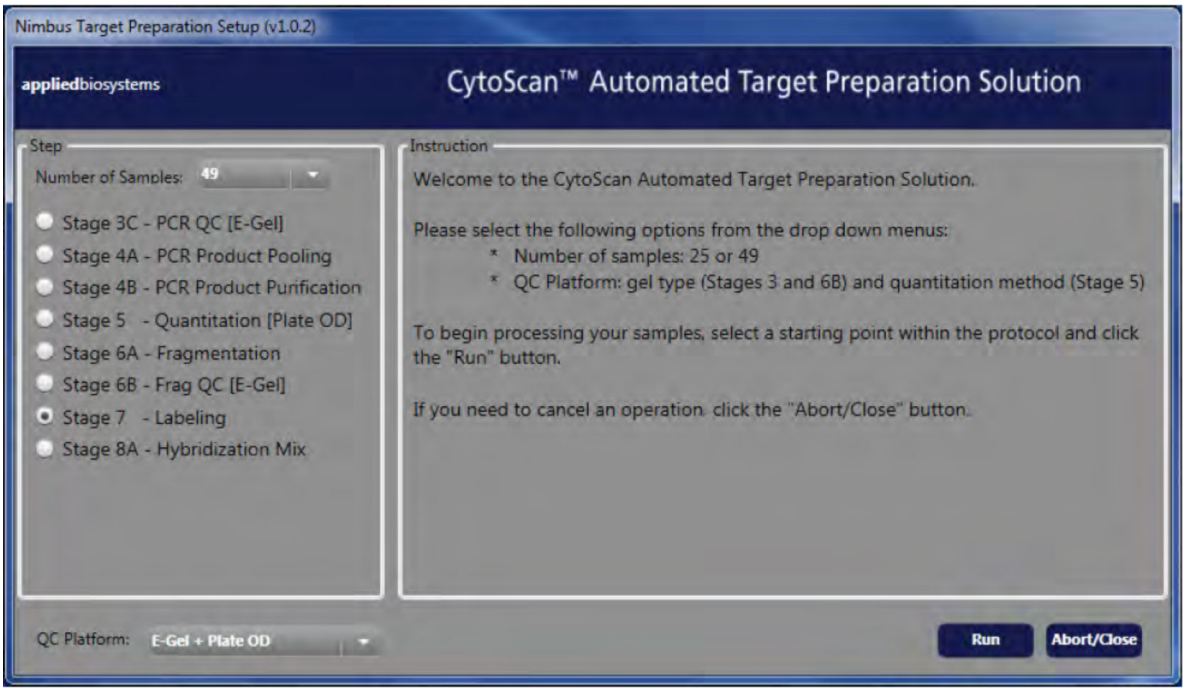
1. Power on the thermal cycler to preheat the lid. **Leave the block at room temperature.**
2. Place a double cooling block on ice.
3. Thaw the following reagents at room temperature. Immediately place on cooling block on ice when thawed.
   - TdT Buffer
   - DNA Labeling Reagent

   IMPORTANT! Leave the TdT Enzyme at –20°C until ready to use.
4. Prepare the TdT Buffer and DNA Labeling Reagent.
   a. Vortex each reagent at high speed 3 times, 1 second each time.
   b. Briefly centrifuge for 3 seconds, then place in the cooling block.
5. If the fragmented samples are frozen, allow them to thaw at room temperature.
6. When thawed, immediately centrifuge the plate at 2,000 rpm for 1 minute, then place on the cooling block on ice.
7. Remove the TdT Enzyme from the freezer, then immediately place in the cooler, chilled to –20°C.

8. Vortex the enzyme at high speed 1 time for 1 second.
9. Briefly centrifuge the enzyme for 3 seconds, then immediately place back in the –20°C cooler.

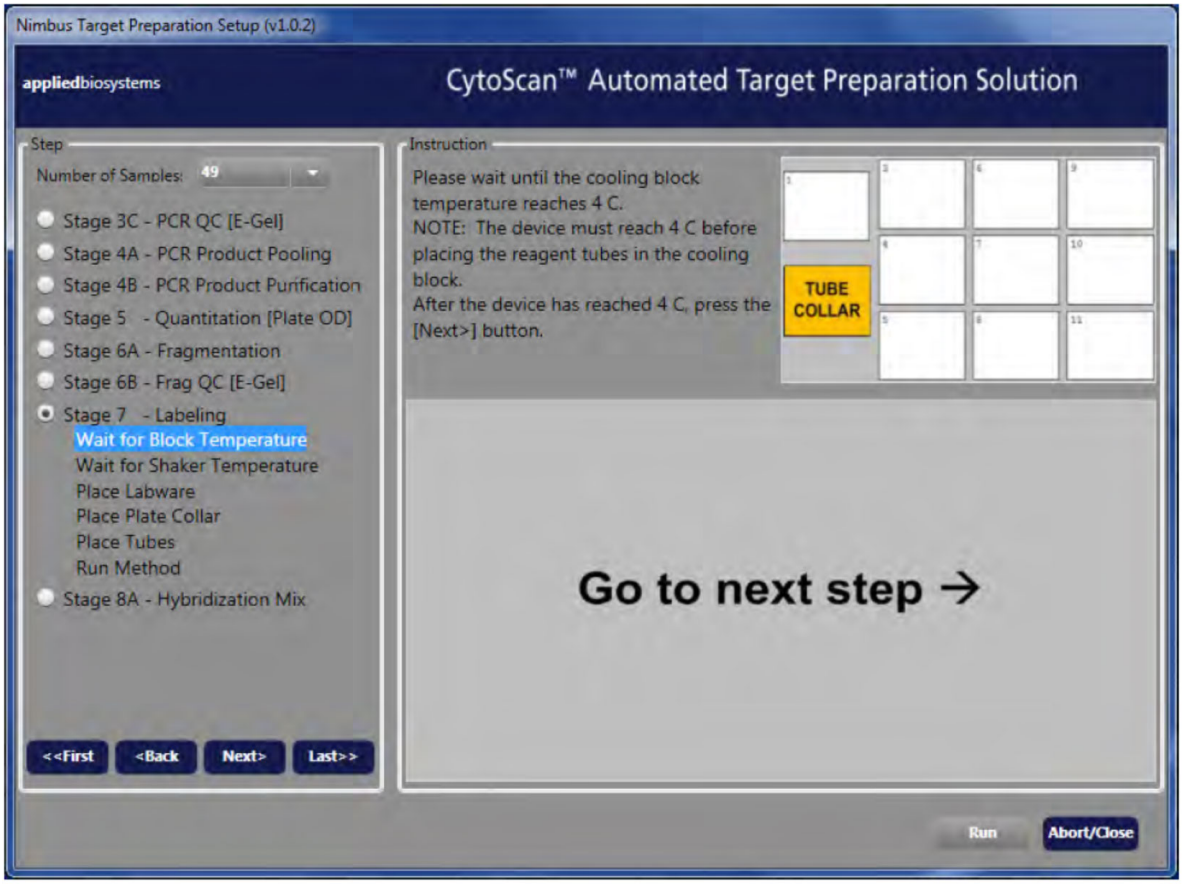
Run the Labeling method

1. On the **Welcome** screen, select the following options.
   - **Number of samples**—49
   - **Stage 7–Labeling**

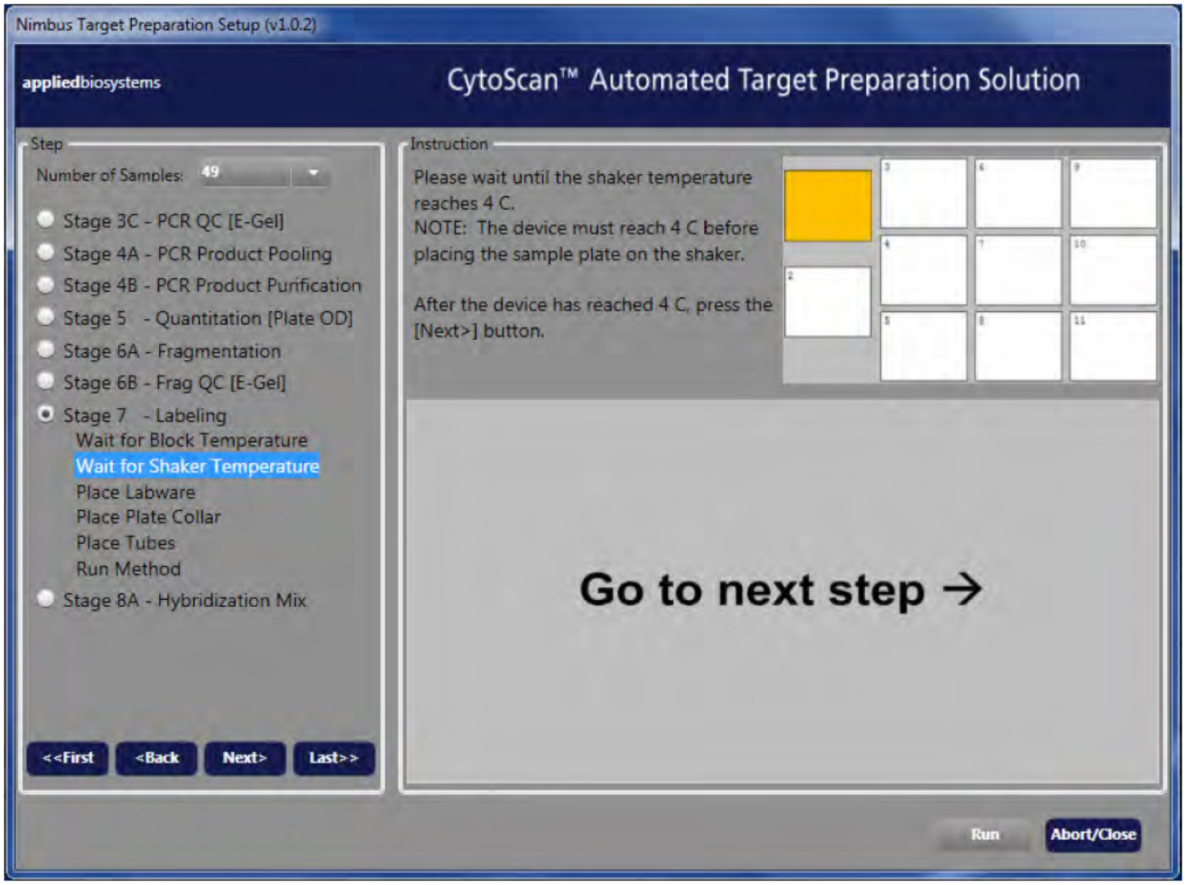


2. Click **Run** to start the method, then click **Yes** to confirm.

3. Wait until the cold block reaches 4°C, then click **Next**.



4. Wait until the shaker temperature reaches 4°C, then click **Next**.

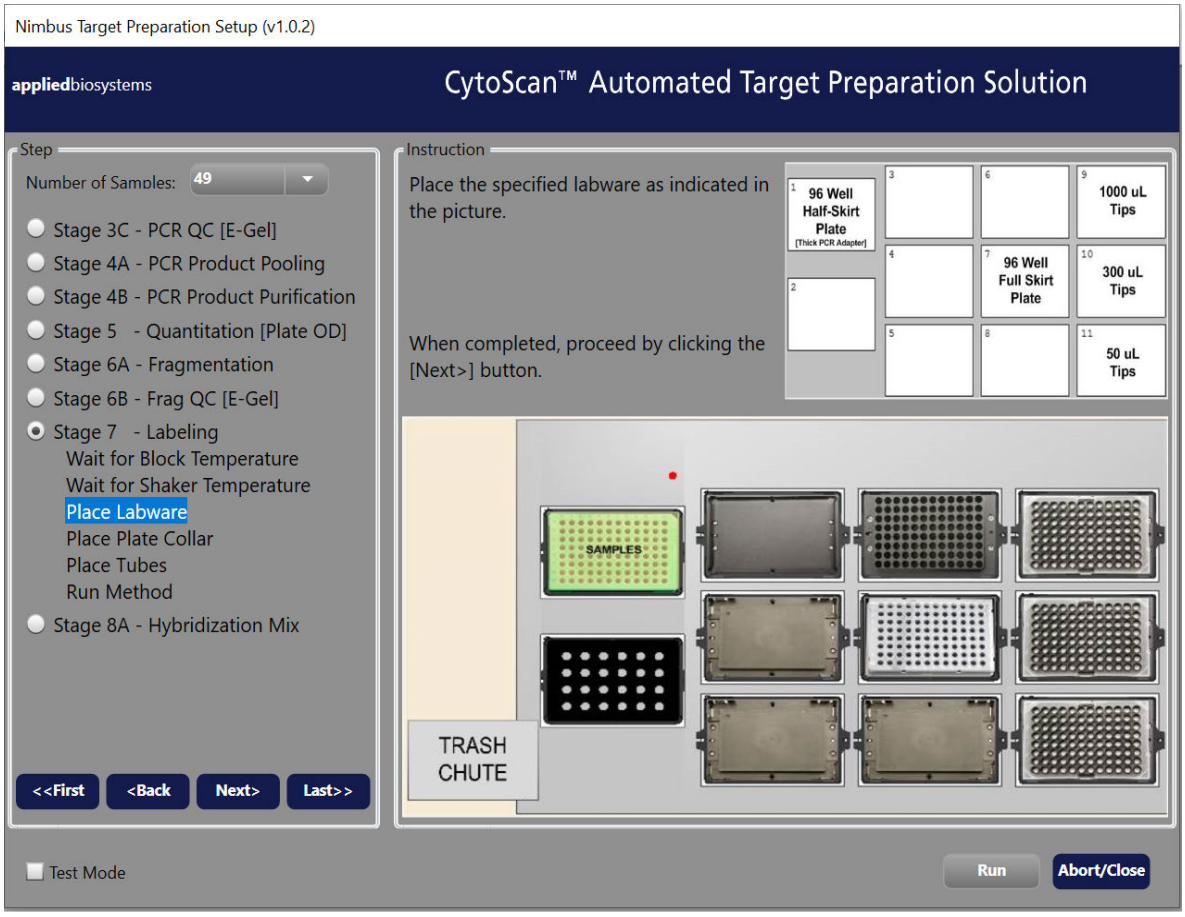


5. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

   Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

   Place an empty tip frame on deck position 11.

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.



6. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1 and 7 require a plate collar.

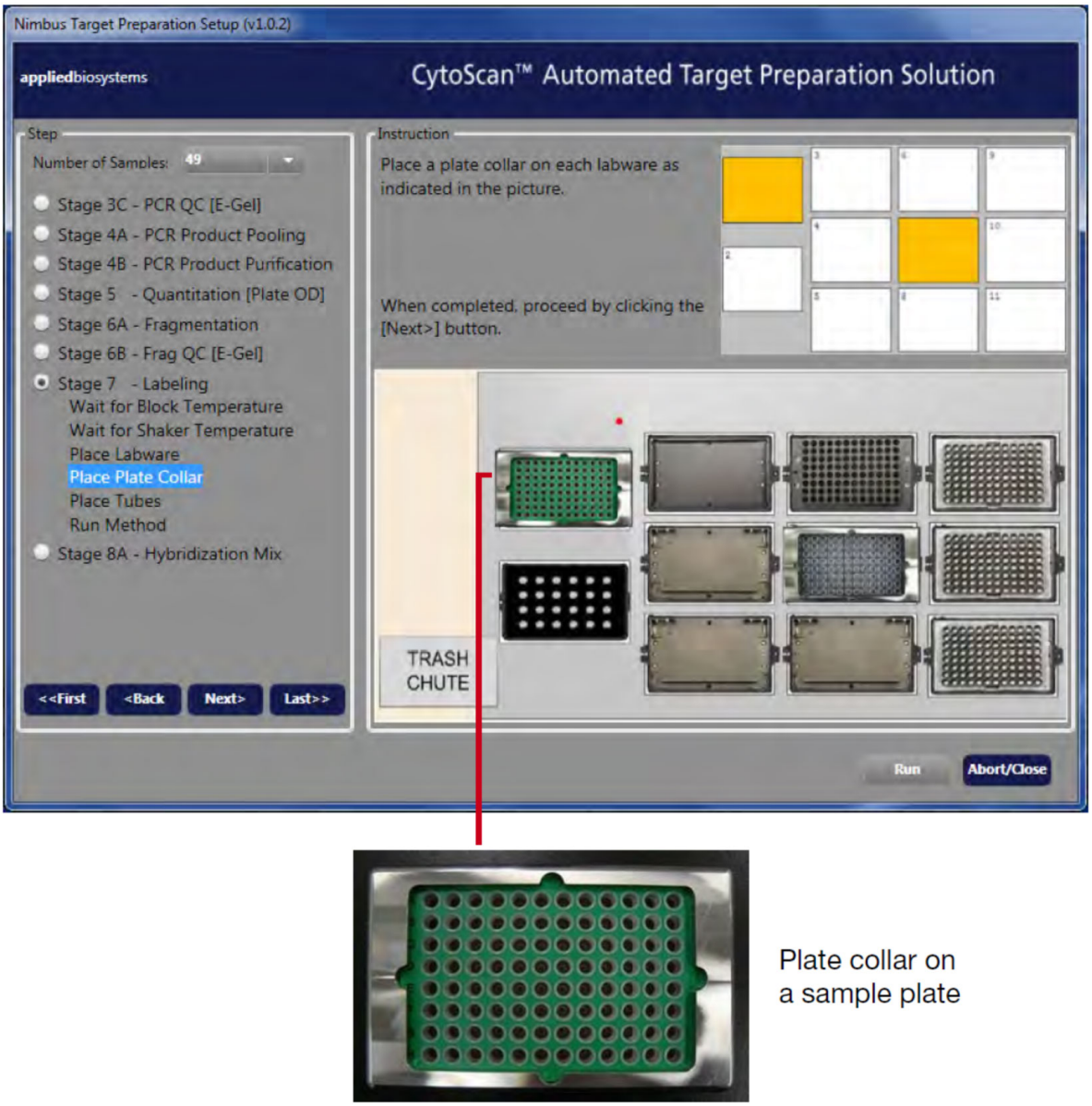
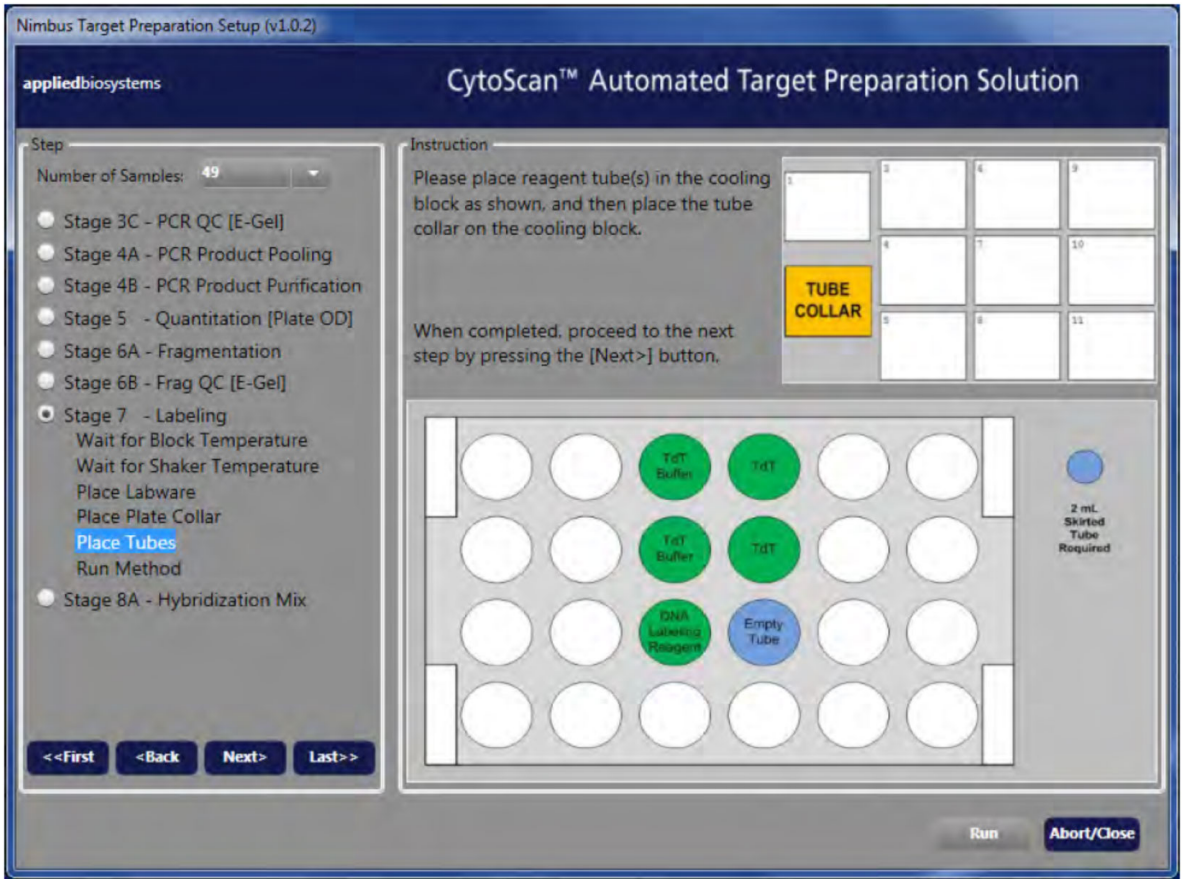
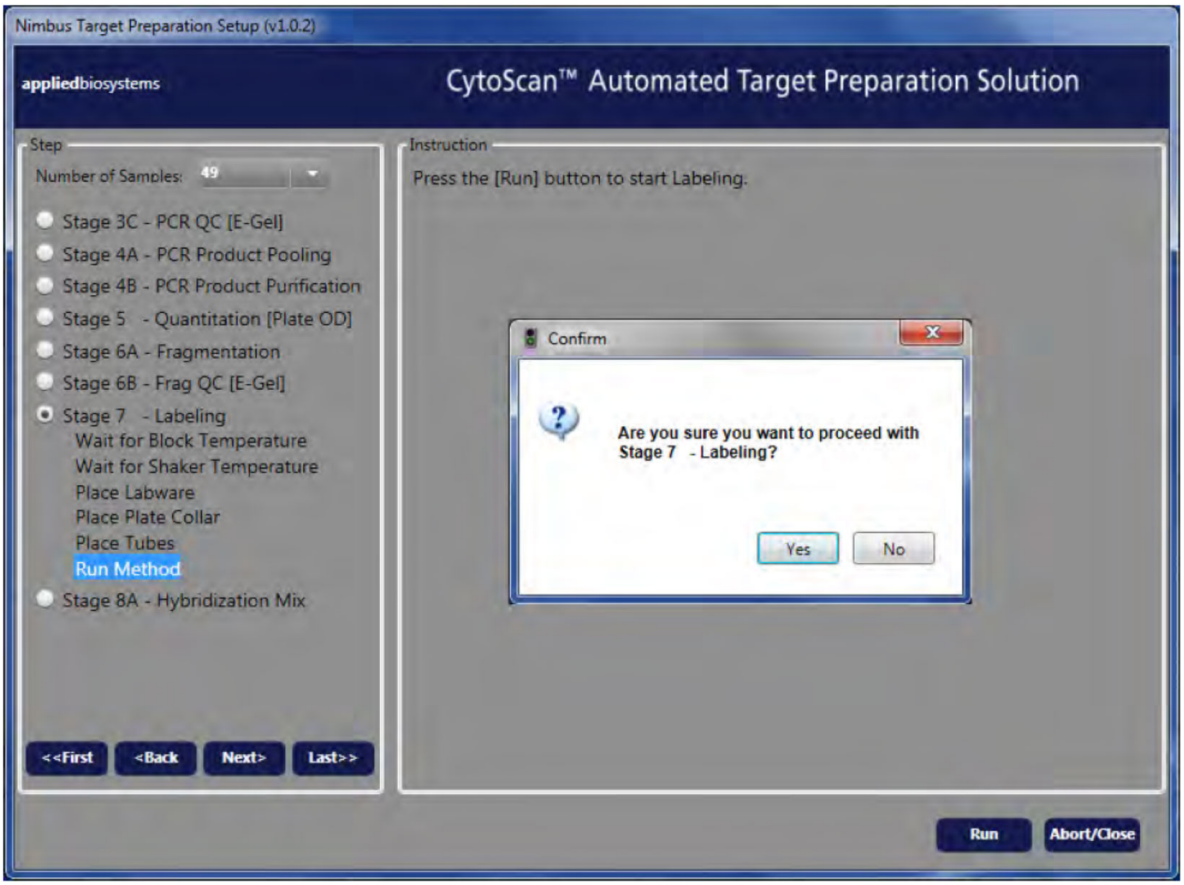


Plate collar on a sample plate

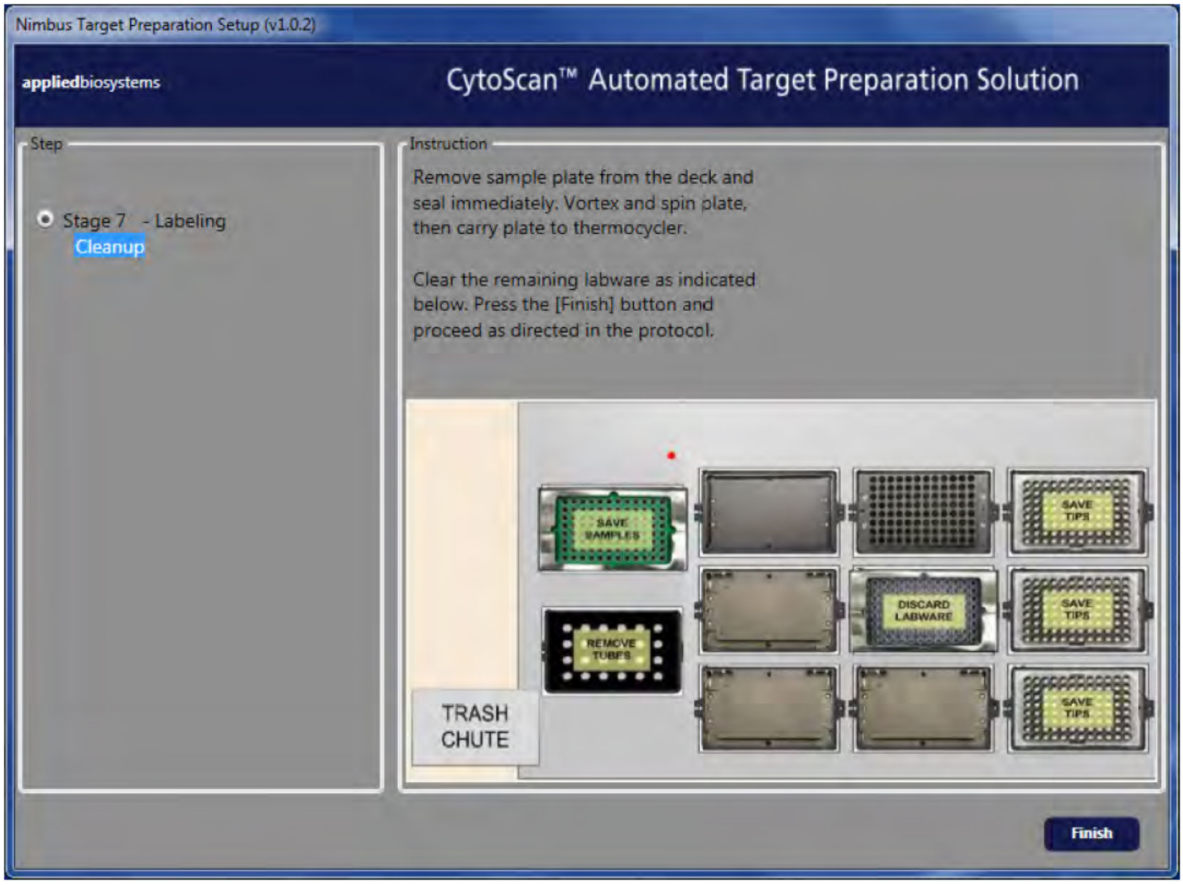
7. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen.



8. Click **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



9. Seal the sample plate tightly with a new seal.

10. Vortex at high speed for 1 second each in all corners and in the center according to the guidelines in "Seal, vortex, and centrifuge" on page 40, then centrifuge for 1 minute at 2,000 rpm.

11. Place the labeling plate in the preheated thermal cycler block, then run the **CytoScan Label** thermal cycler protocol. Total volume for Labeling = 70.5 µL

Temperature	Time
37°C	4 hours
95°C	15 minutes
4°C	Hold (OK to hold overnight)

12. When the **CytoScan Label** thermal cycler protocol finishes, leave the sample plate in the thermal cycler overnight or transfer to a chilled cooling block on ice.

13. Ensure that the plate is tightly sealed, then centrifuge at 2,000 rpm for 1 minute.

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

15. Do one of the following:
    - Proceed to "Stage 8A—Hybridization Mix" on page 303.
    - If not proceeding directly to the next stage, do one of the following:
      - Hold at 4°C on the thermal cycler overnight.
      - Freeze the samples at –20°C. The plate can be stored at –20°C for up to 10 days.
16. Click **Finish**, then click **Yes** to confirm.
    The method closes.

Stage 8A—Hybridization Mix

This stage uses NIMBUS™ Target Preparation Instrument with CytoScan™ assay-specific deck configuration. The instrument:

- Prepares the Hybridization Master Mix.
- Aliquots the Hybridization Master Mix into a distribution plate.
- Adds the Hybridization Master Mix to samples and performs mixing.

Note:

- Power on the INHECO™ Multi TEC Control to start cooling the block. The block takes about 20 minutes to reach 4°C.
- To proceed, click the ▶ arrow in the toolbar. Alternatively, double-click the **Start** icon on the desktop..

Materials required for Stage 8A—Hybridization Mix

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 55 Equipment and consumables required.

Quantity	Item
1	Applied Biosystems™ NIMBUS™ Target Preparation Instrument with the CytoScan™ assay-specific deck configuration
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1	Plate centrifuge
1	Mini centrifuge
1 rack	Conductive 300-µL filter tips in frames
1 rack	Conductive 1,000-µL filter tips in frames
1	Plates, 96-well, semi-skirted
1	PCR tube rack
1	Reservoir 4 ROW PYR PP 73 mL
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1	Ice bucket, filled with ice
1	Sample plate with labeled samples from Stage 7

Table 55 Equipment and consumables required. *(continued)*

Quantity	Item
1	Vortexer
2	PCR Tube Racks

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

Table 56 Reagents required.

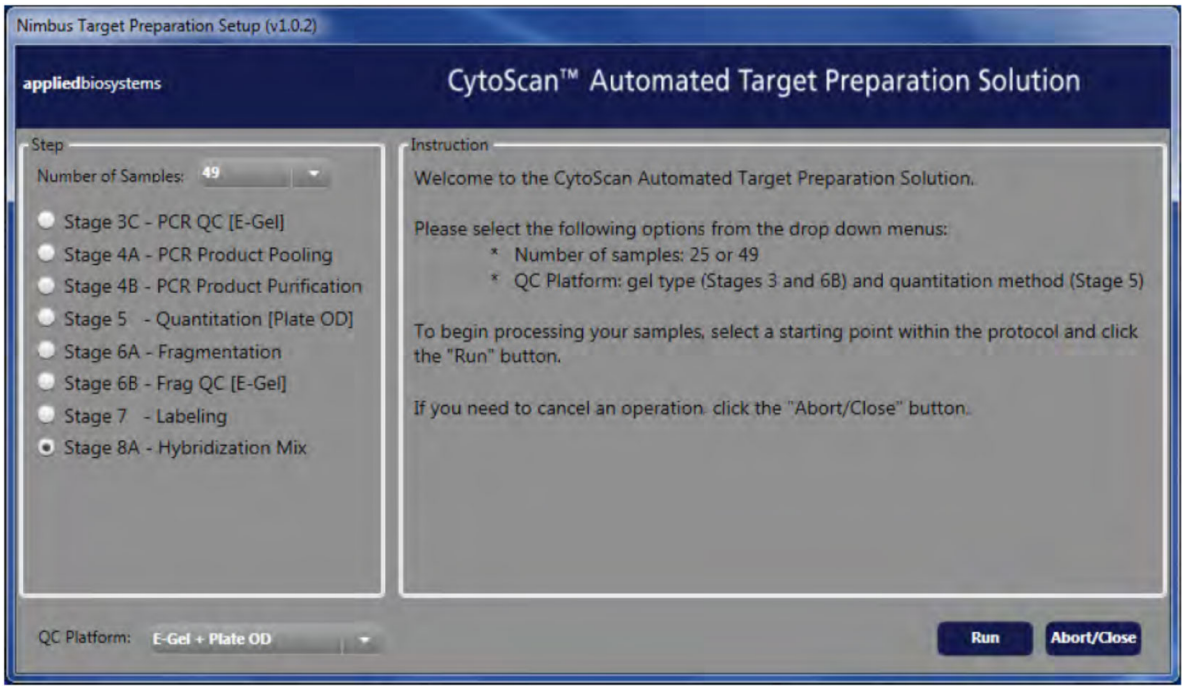
Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
2	Oligo Control Reagent	Module 3	902678
2	Hyb Buffer Part 1		901725
2	Hyb Buffer Part 2		901726
2	Hyb Buffer Part 3		901727
2	Hyb Buffer Part 4		901728

Prepare the reagents and consumables

1. Place a double cooling block on ice.
2. Prepare the samples.
   a. If the labeled samples from the previous stage are 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.
3. Thaw the following reagents at room temperature. When thawed, immediately place on the cooling block on ice.
   - Hyb Buffer Part 1
   - Hyb Buffer Part 2
   - Hyb Buffer Part 3
   - Hyb Buffer Part 4
   - Oligo Control Reagent
4. Vortex each reagent at high speed 3 times, 1 second each time.
5. Quickly centrifuge for 3 seconds, then place in the cooling block.

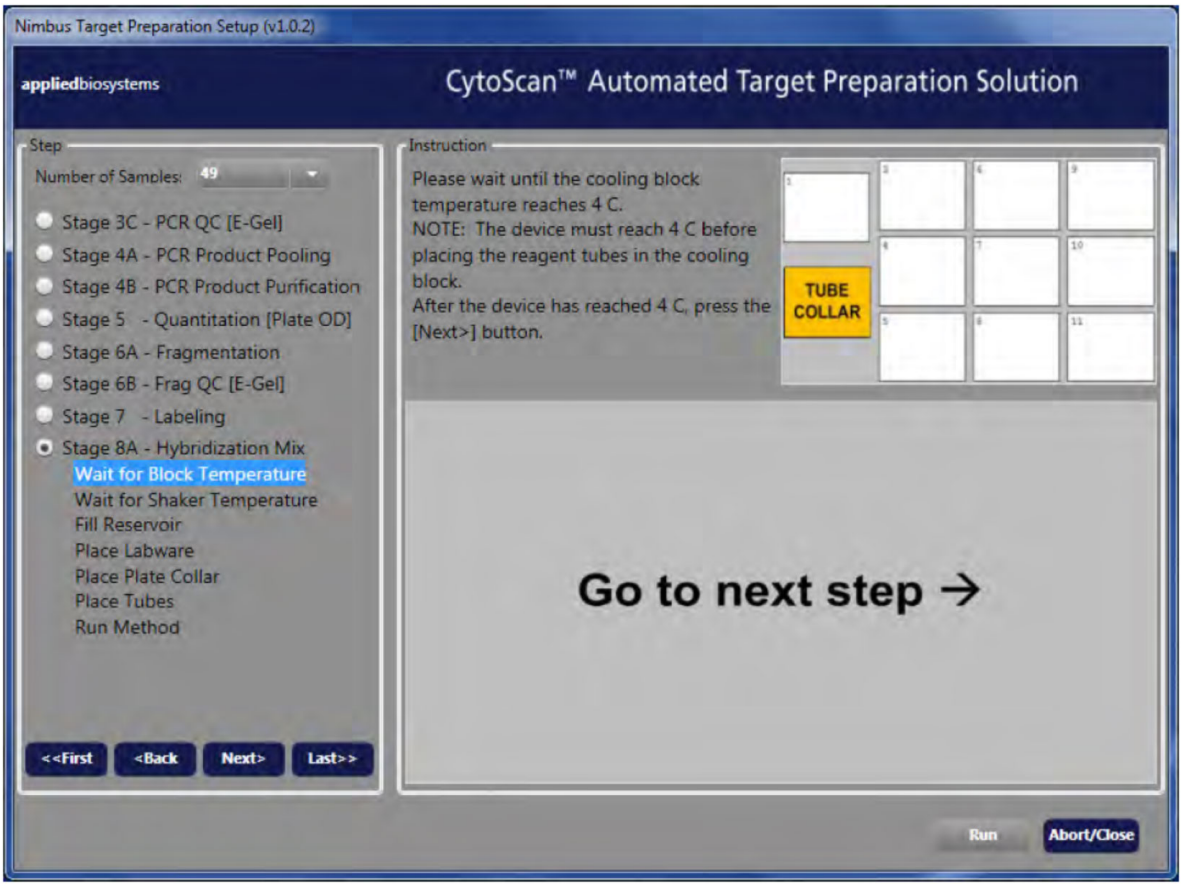
Run the Hybridization Mix method

1. On the **Welcome** screen, select the following options.
   - **Number of Samples**—49
   - **Stage 8A–Hybridization Mix**

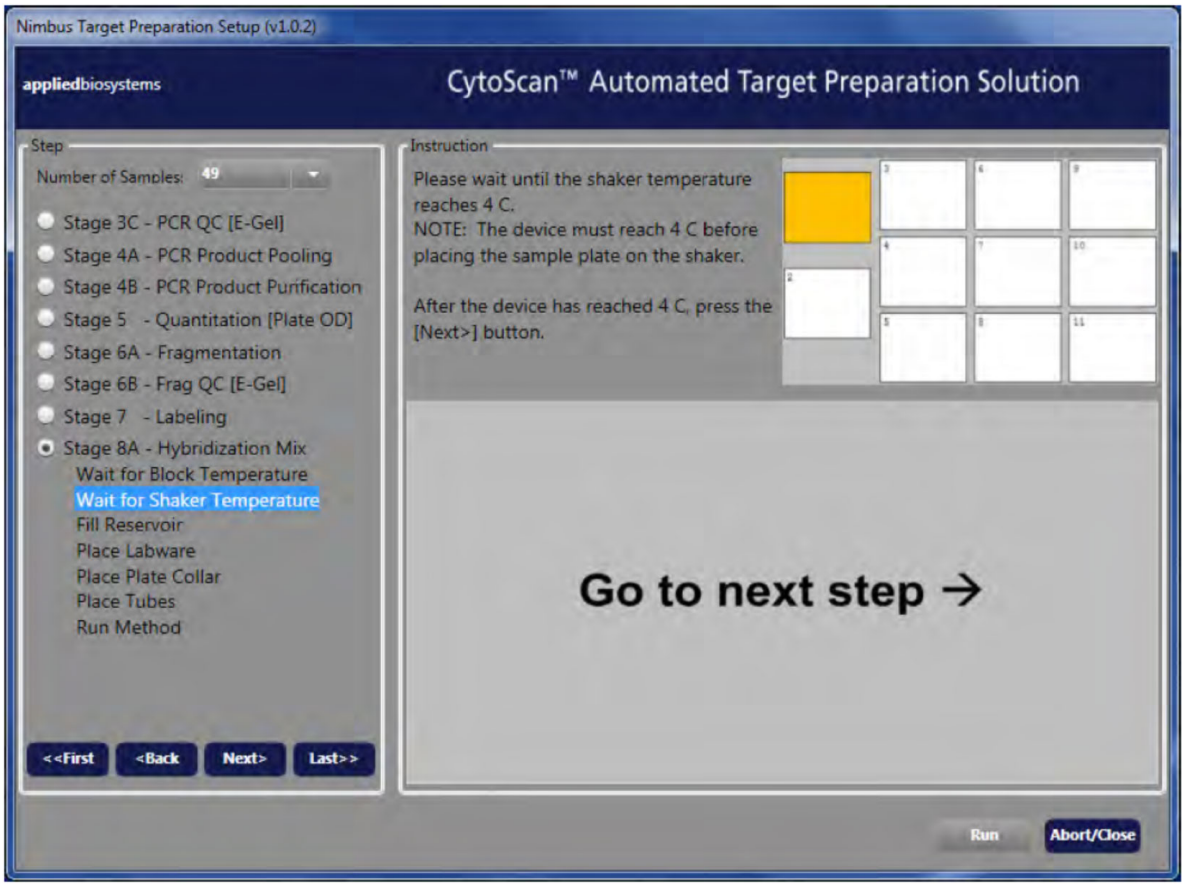


2. Click **Run** to start the method, then click **Yes** to confirm.

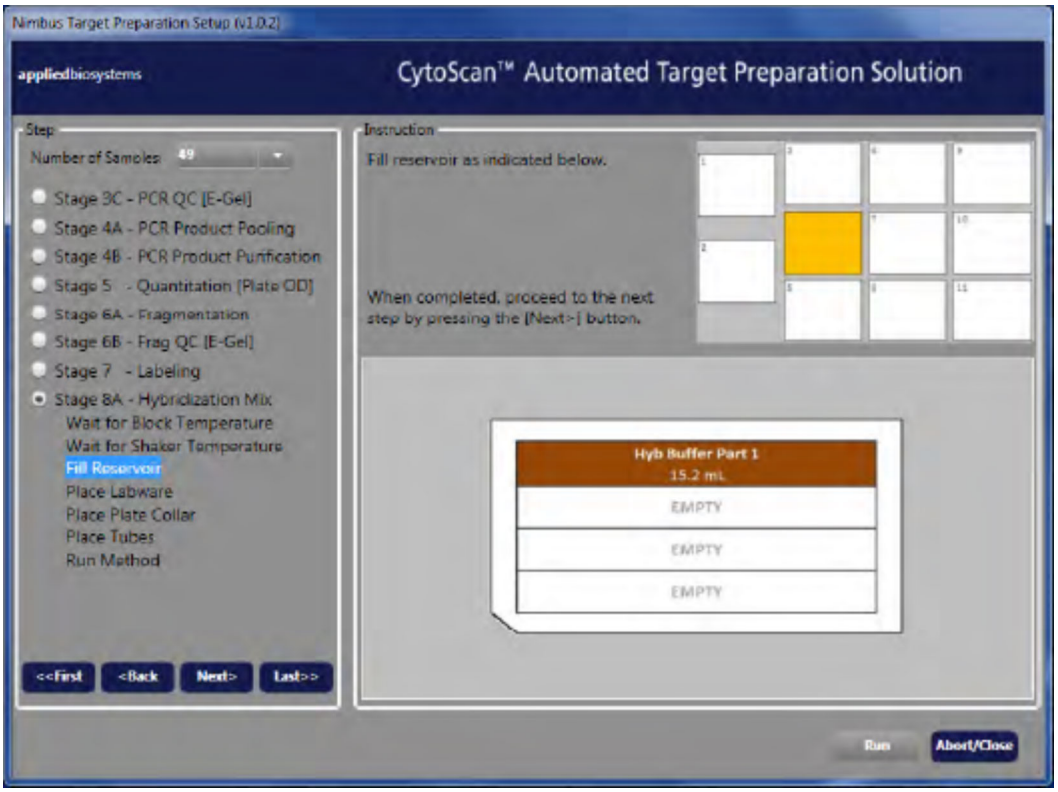
3. Wait until the cooling block temperature reaches 4°C, then click **Next**.



4. Wait until the shaker temperature reaches 4°C, then click **Next**.



5. Fill the reservoir as shown in the on-screen instructions, then click **Next**.

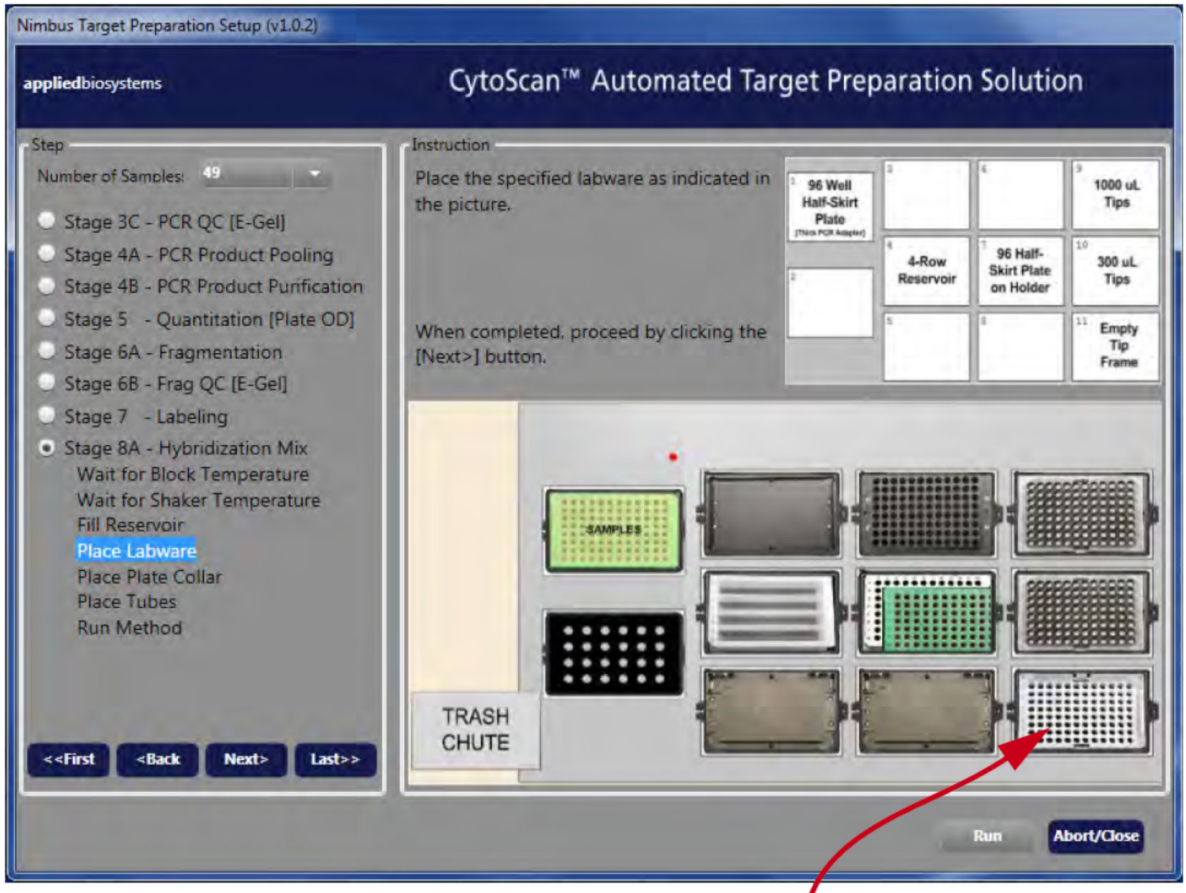


6. Set up labware on the NIMBUS™ Instrument deck as shown in the on-screen instructions, then click **Next**.

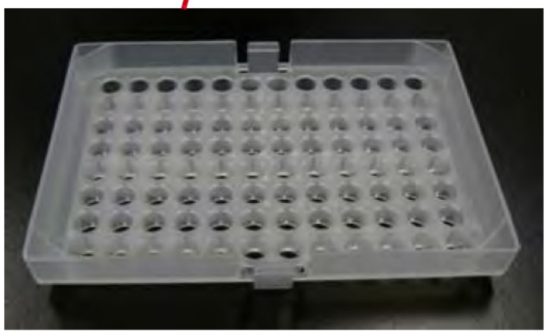
Note: Ensure that the sample plate is sitting flat on the adapter by pressing on all 4 corners of the plate.

Place an empty tip frame on deck position 11.

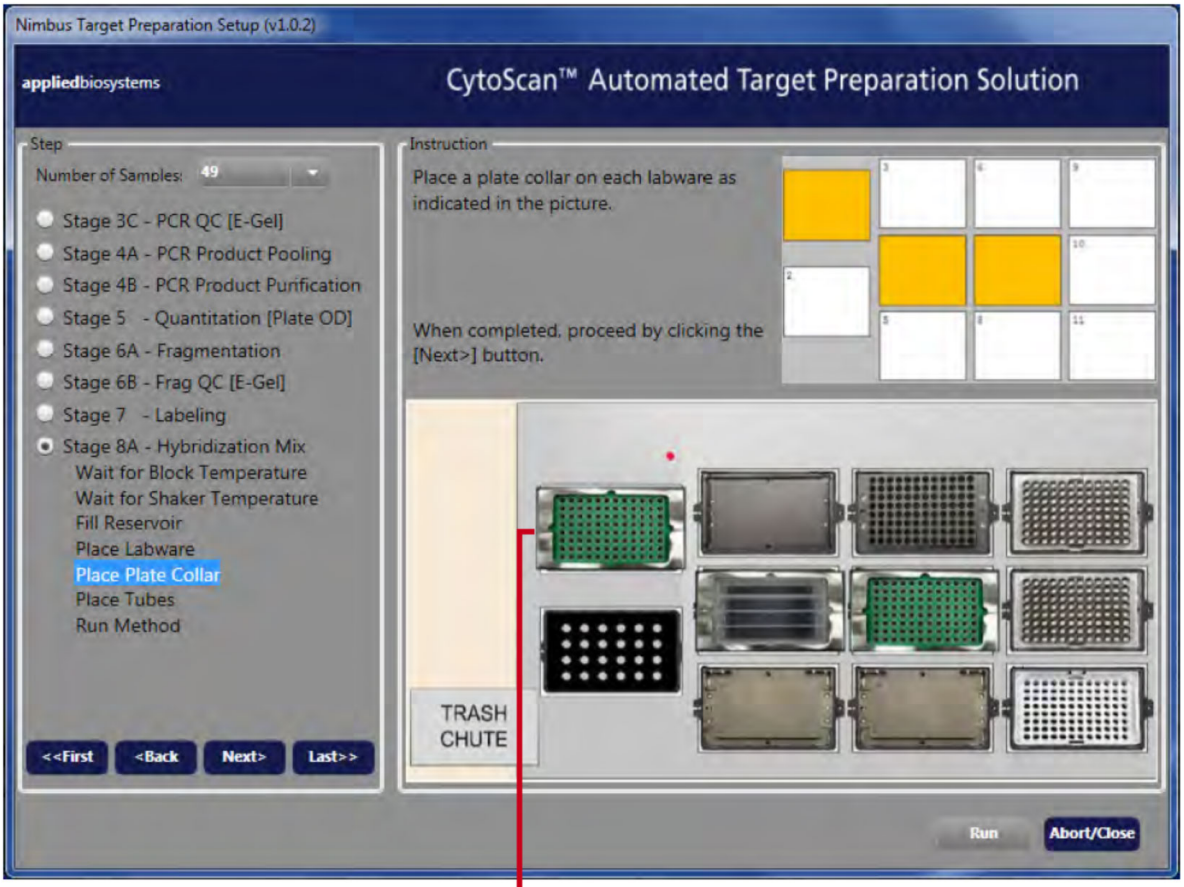
IMPORTANT! The deck images in the software user interface show the green Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641). The Bio-Rad™ HSS9601 (clear) or the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354 or 4483352) are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



Empty tip frame for position 11



7. Place a plate collar on each of the labware indicated in the on-screen instructions, then click **Next**. Labware at deck positions 1, 4, and 7 require a plate collar.



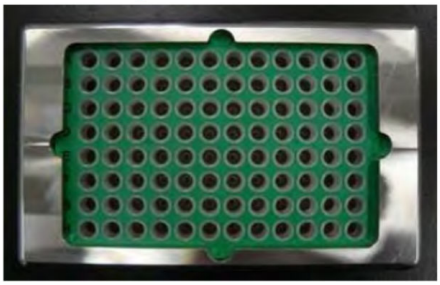
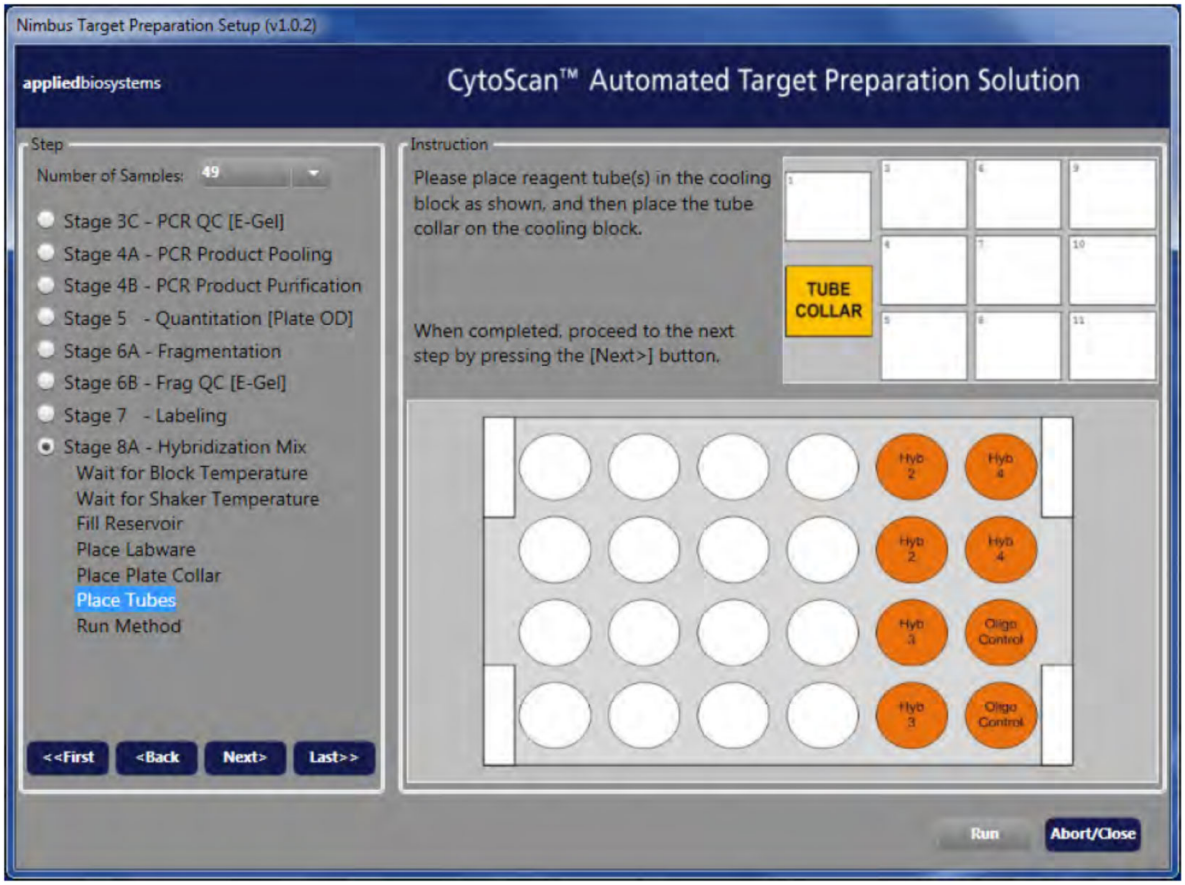
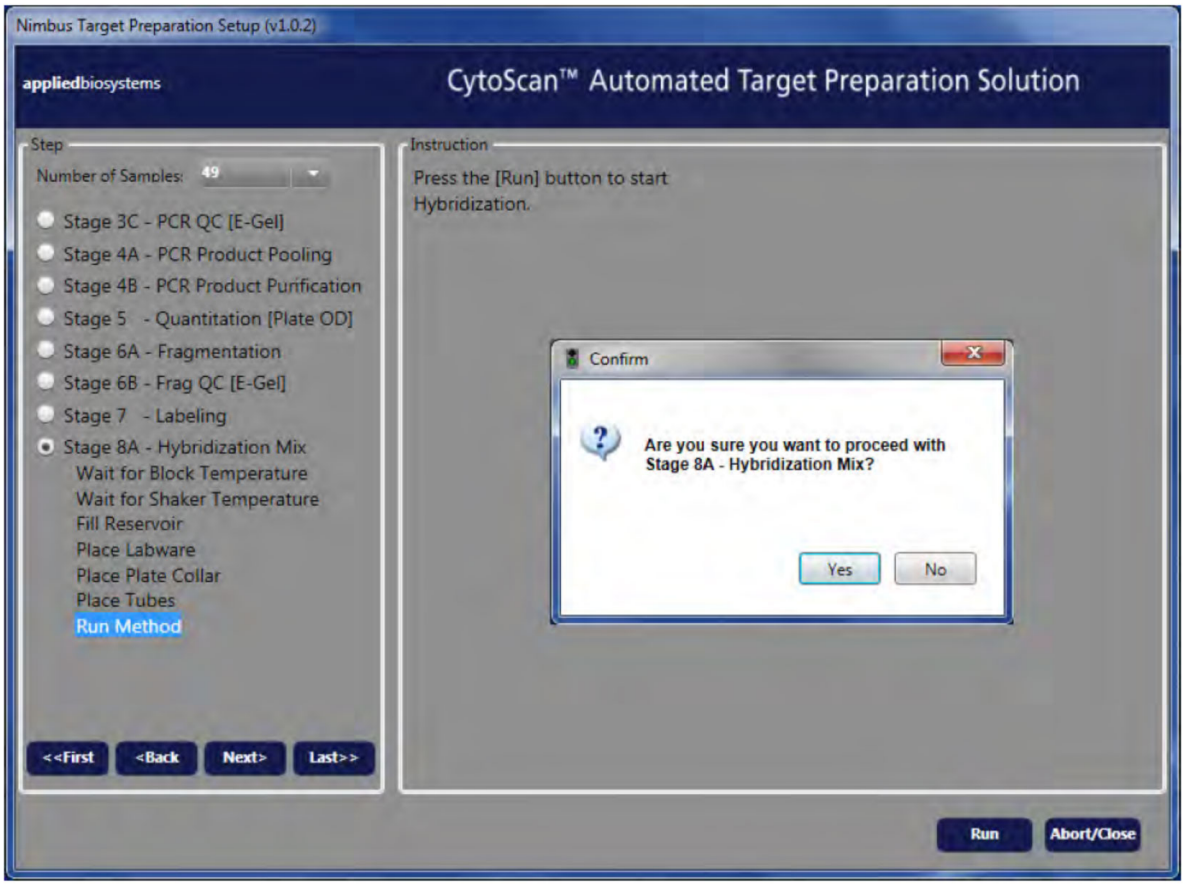


Plate collar on a sample plate

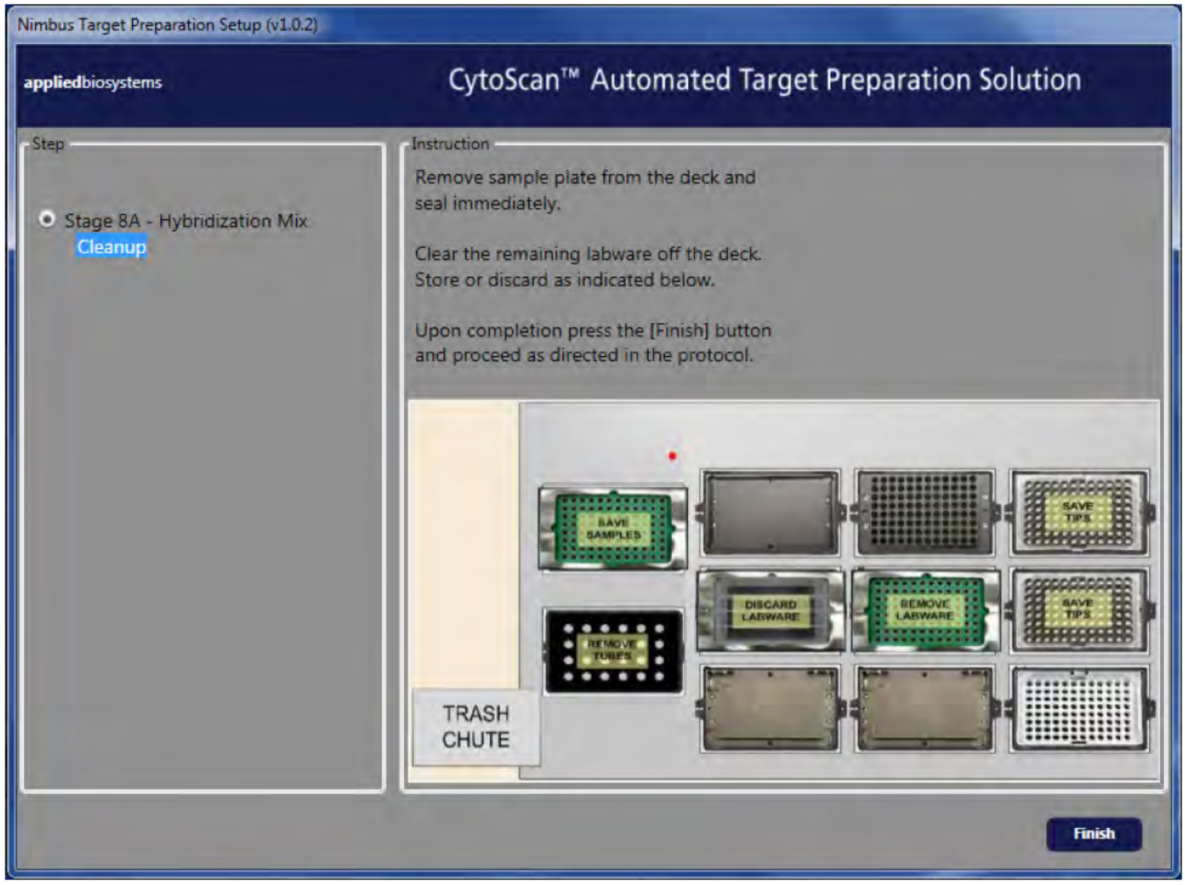
8. Place tubes on the NIMBUS™ Instrument deck as shown in the on-screen instructions. Place the tube collar on the CPAC as shown on the screen, then click **Next**.



9. **Run** to start the method, then click **Yes** to confirm.



The **Cleanup** screen appears after the method is finished.



10. Tightly seal the sample plate with a new seal. Confirm that the plate is well sealed.
11. Remove the labware from the NIMBUS™ Instrument deck as shown on the **Cleanup** screen.
12. Click **Finish**, then click **Yes** to confirm.
    The method closes.
13. Do one of the following:
    - Hold the plate on ice and proceed to “Stage 8B—Hybridization” on page 314.
    - Freeze the samples at –20°C. The plate can be stored at –20°C for up to 10 days.

Stage 8B—Hybridization

Materials required for Stage 8B—Hybridization

When you prepare the hybridization setup, leave the samples on the cooling block on ice.

IMPORTANT! Use only the thermal cyclers, 96-well plates, and adhesive film listed in "Thermal cyclers" on page 41.

Table 57 Equipment and consumables required.

Quantity	Item
As required	MicroAmp™ Clear Adhesive Film for 96-well plates
1 (Optional)	MicroAmp™ Adhesive Film Applicator
1 (Optional)	Plate centrifuge
1	Mini microcentrifuge
1	Cooling chamber, double block, chilled to 4°C placed on ice (do not freeze)
1 array per sample	CytoScan™ Array
1	GeneChip™ Hybridization Oven 645
1	Ice bucket, filled with ice
1	Sample plate with Hybridization Mix from Stage 8A
1	Pipette, single-channel P200
As needed	Pipette tips for P200 single-channel pipette
1	Thermal cycler
2 per array	Tough-Spots™ labels, 1/2" diameter
1	Vortexer
2	PCR Tube Racks

Important information about this stage

To ensure the best results, carefully read the following information before you start this stage of the assay. Becase this user guide is intended as an assay protocol document, there is no specific section on all of the various features and workflows available in the GeneChip™ Command Console™ software. To learn more about Command Console™, see the *GeneChip™ Command Console™ User Guide*.

Prepare the equipment

Verify that the GeneChip™ Hybridization Oven 645 is calibrated.

Service the hybridization oven at least once per year to ensure that operation is within specification.

1. Power on the thermal cycler to preheat the lid. Leave the block at room temperature.
2. Preheat the GeneChip™ Hybridization Oven 645.
   a. Power on the oven at least 1 hour before hybridization with the temperature set to 50°C.
   b. Set the rpm to 60.
   c. Turn the rotation on, then allow the oven to preheat for 1 hour before loading arrays.

Prepare the arrays and create a batch registration file

1. Unwrap the arrays, then place the arrays on the benchtop, septa-side up.
2. Mark the front and back of each array with a designation that identifies which sample is loaded onto each array.
3. Allow the arrays to warm to room temperature on the benchtop for 10 to 15 minutes.
   During this time, scan the barcode to use for batch registration.

Create a batch registration file

Use the GeneChip™ Command Console™ (GCC) to create a batch registration file to register a new sample.

IMPORTANT! Verify that you are running GCC v.3.2.2 or higher. If not, update your version of GCC to v.3.2.2 or the latest available.

---

1. From the GCC, launch the GCC Portal.
2. On the **Samples** tab, select **Batch Registration**.
   The **Batch Sample Registration** window opens.

3. In Step 1:
   a. Under **Create a Spreadsheet for**, enter the number of samples for which a spreadsheet needs to be created.
   b. In the **Project Set to** list, select **Default**.
   c. In the **Probe Array type set to** list, select the appropriate array type.
      - If using the CytoScan™ HD Array, select **CytoScan™ HD_Array**.
      - If using the CytoScan™ 750K Array, select **CytoScan™ 750K_Array**.
   d. Click **Download**.
      An Excel™ spreadsheet opens.
4. In Step 2:
   a. Name the experiment file using the following convention: .
      SampleName_PlateCoordinate_ExperimentDescriptionString_ArrayType_OperatorInitials_yyyymmdd
      The sample file name and the 'Array name' would be identical.
   b. Scan the corresponding barcodes for each sample name.

c. Save the Excel™ file in “Excel 97-2003 workbook” format.

5. In Step 3:
   a. Browse to the location of the batch registration file that was saved.
   b. Upload the batch registration file by clicking the tab to create new sample (ARR) files.
6. In the new window, click **Save** to save the new sample files.

Prepare the arrays

1. Place the arrays on a clean benchtop area designated for hybridization.

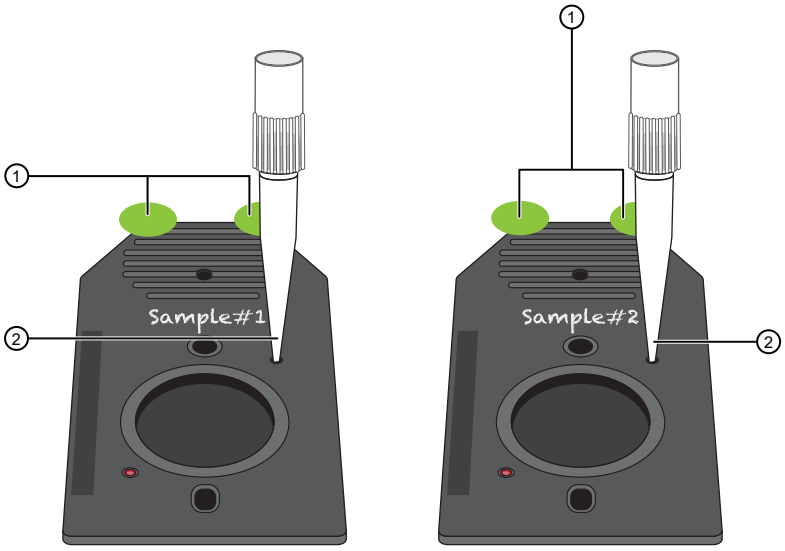
   IMPORTANT! To ensure that the data collected during scanning is associated with the correct sample, mark each array in a meaningful way. It is critical that you know which sample is loaded onto each array.

2. Unwrap the arrays and place on the benchtop, septa-side up.

   Allow the arrays to warm to room temperature on the benchtop for 10—15 minutes. During this time, scan the barcode for use in batch registration.

3. Mark the front and back of each array with a designation that identifies which sample is loaded onto each array.
4. Paste two 1/2” Tough-Spots™ labels on the top edge of the arrays for later use.
5. Insert a 200-µL pipette tip into the upper-right septum of each array.

   ① Tough-Spots™ label dots to cover the septa.
   ② 200-µL pipette tip to vent the array.



6. Vortex the plate at high speed for 1 second each in all corners and in the center. *Repeat* vortexing to ensure that the contents of the plate is well mixed, then centrifuge for 1 minute.

IMPORTANT! The volume in the hybridization plate is full. Vortex the plate to mix the sample and hybridization buffer well.

7. Place the plate onto the preheated thermal cycler, then run the **CytoScan Hybridization** thermal cycler protocol.

Temperature	Time
95°C	10 minutes
49°C	Hold

Load the samples onto arrays

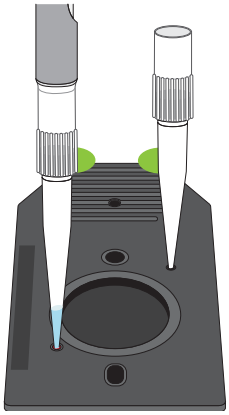
1. When the thermal cycler reaches 49°C, leave the samples at 49°C for at least 1 minute, then open the lid.

IMPORTANT! Load only 6–8 arrays at a time. Remove the seal from the hybridization plate for only 6–8 samples at a time.

2. If you are hybridizing more than 8 samples, cut and remove the seal from 6–8 samples at a time.

   Leave the remaining wells covered. Keeping these wells covered helps prevent cross-contamination and evaporation.

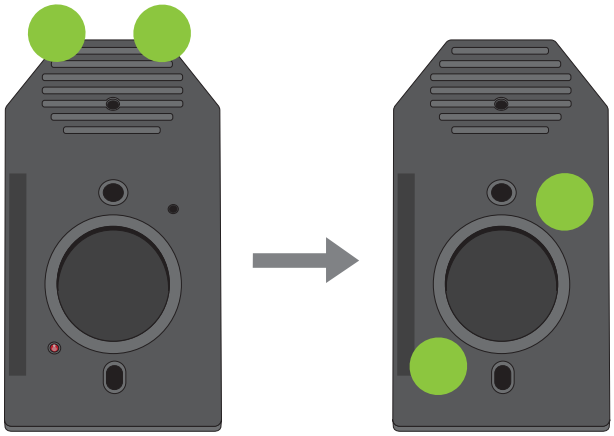
3. Using a P200 pipette, remove 200 µL of the first sample, then immediately inject it into an array.



IMPORTANT! The hybridization mix is viscous. Pipet slowly to ensure that all of the volume is loaded into the array.

4. Cover the septa on the array with the two 1/2” Tough-Spots™ labels that were previously placed on the top edge of the array.

   Press firmly on the labels so that they lay flat and to ensure a tight seal.



5. When 6–8 arrays are loaded and the septa are covered:
   a. Load the arrays into an oven tray evenly spaced.
   b. Immediately place the tray into the hybridization oven.

      Do not allow loaded arrays to sit at room temperature for more than about 1 minute. Ensure that the oven is balanced as the trays are loaded, and ensure that the trays are always rotating at 60 rpm.
6. Repeat this process until all samples are loaded onto arrays and are placed in the hybridization oven.

   Load all samples within 30 minutes.
7. Allow the arrays to rotate in the hybridization oven at 50°C and 60 rpm for 16–18 hours.

   IMPORTANT! This temperature is optimized for this product, and must be stringently followed.
8. For the next steps for processing the arrays, go to Chapter 7, “Wash, stain, and scan arrays”.

7 Wash, stain, and scan arrays

This chapter describes how to wash, stain, and scan the CytoScan™ Arrays. The instruments to use include the:

- GeneChip™ Fluidics Station 450 to wash and stain the arrays.
- GeneChip™ Scanner 3000 7G System to scan the arrays.

After the arrays are scanned, the array image (DAT file) is ready for analysis.

Equipment and consumables required

The following equipment and consumables are required for washing, staining and scanning arrays.

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

Item	Source
GeneChip™ Scanner 3000 7G System	00-0213
GeneChip™ Fluidics Station 450	00-0079
The instrument control application: GeneChip™ Command Console™ version 3.2.2 or higher	—
Eppendorf™ Safe-Lock™ Tubes, microcentrifuge tube, 1.5 mL, amber	Eppendorf™, 022363221
Eppendorf™ Safe-Lock™ Tubes, microcentrifuge tube, 1.5 mL, blue	Eppendorf™ 022363247
Eppendorf™ Safe-Lock™ Tubes, microcentrifuge tube, 1.5 mL, natural	Eppendorf™, 022363352
Pipets, (P-2, P-20, P-200, P-1000)	Rainin™ PIPETMAN™ (or equivalent)
Sterile-barrier pipette tips and nonbarrier pipette tips	**MLS**

(continued)

Item	Source
Tygon™ Tubing, 0.04” inner diameter	Cole-Parmer™, H-06418-04
USA Scientific™ Tough-Spots™ labels (3/8 inch)	NC9504463

Reagents required

The following reagents are required for washing and staining arrays. These reagents are recommendations, and have been tested and evaluated by Thermo Fisher Scientific.

Quantity	Reagent and cap color	CytoScan™ Reagent Kit	
		Module	Part No.
	Stain Buffer 1	Module 4	901751
	Stain Buffer 2		901752
	Array Holding Buffer		901733
	Wash A	Module 5	901680
	Wash B		901681

Fluidics station and scanner control software

Use the GeneChip™ Command Console™ to operate the fluidics station and the scanner. For more information on the GCC application, see the *GeneChip™ Command Console™ User Guide*.

Prime the fluidics station

Priming ensures that the lines of the fluidics station are filled with the appropriate buffers and that the fluidics station is ready to run fluidics station protocols.

Prime the fluidics station:

- When the fluidics station is first started.
- When wash solutions are changed.
- Before washing, if a shutdown has been performed.
- If the LCD window instructs you to prime.

The GeneChip™ Fluidics Station 450 is used to wash and stain the arrays; it is operated using GCC software.

1. Turn on the fluidics station.

2. Prime the fluidics station.
   - From Command Console™ application, start the ‘Launcher’.
   - From the Launcher, open the ‘GCC Fluidics Control’ application.
   - From the GCC Fluidics Control panel, select ‘PRIME_450’ script for the specific fluidics stations and the modules.

     IMPORTANT! Use GeneChip™ Wash A and Wash B buffers that are designated for only the CytoScan™ Assay Automated Workflow. These wash and stain buffers differ from GeneChip™ expression buffers.

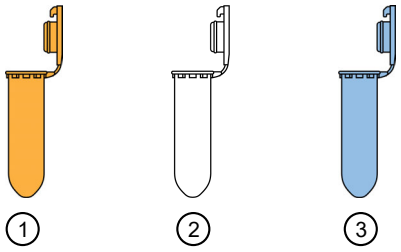
   - Intake buffer reservoir A: use Wash A.
   - Intake buffer reservoir B: use Wash B.

3. To initiate the fluidics script, click **Run** for each module or click **Run All**, for all the selected stations and modules.

Stage 9A—Wash and stain arrays

1. Briefly vortex the stain bottles before aliquoting the reagents.

2. Aliquot the following reagents into separate 1.5-mL microcentrifuge tubes for each array.



① Aliquot 500 µL Stain Buffer 1 into 1.5-mL microcentrifuge tubes (use amber color tubes because Stain Buffer 1 is light sensitive).
② Aliquot 500 µL Stain Buffer 2 into 1.5-mL microcentrifuge tubes (use clear/natural tubes).
③ Aliquot 800 µL Array Holding Buffer into 1.5-mL microcentrifuge tubes (use blue tubes).

3. Select a protocol from the GCC Fluidics Control Panel.
   a. If using the CytoScan™ HD Array, select **CytoScanHD_Array_450**.
   b. If using the CytoScan™ 750K Array, select **CytoScan750K_Array_450**.

4. Start the protocol, then follow the instructions in the LCD on the fluidics station.

   If you are unfamiliar with inserting and removing arrays from the fluidics station modules, see the appropriate fluidics station user guide or quick reference.

5. Eject the wash block to avoid sensor time out.

6. Remove any previously loaded empty vials.

7. When prompted to “Load vials 1-2-3”:
   a. Place 1 vial containing 500 µL Stain Buffer 1 in position 1.
   b. Place 1 vial containing 500 µL Stain Buffer 2 in position 2.
   c. Place 1 vial containing 800 µL Array Holding Buffer in position 3.
8. After 16 to18 hours of hybridization, remove no more than 8 arrays at a time from the oven. Remove the Tough-Spots™ labels from the arrays.

   IMPORTANT! After the arrays are removed from the hybridization oven, quickly load them onto the fluidics station. Delays during this step impact data quality.
9. Immediately insert the arrays into the designated modules of the fluidics station while the cartridge lever is in the Down or Eject position.
10. After the array is in loading position, load the array into fluidics station by moving the lever Up or into the Load position.
11. Wait for the command on the LCD screen, then press down on the needle lever to snap needles into position and to start the run.

    The fluidics protocol starts. The fluidics station dialog box at the workstation terminal and the LCD window show the status of the washing and staining steps.
12. When the wash and stain procedure is complete, remove the arrays from the fluidics station by first pressing down the cartridge lever to the Eject position.
13. Check the array window for bubbles or air pockets.
    a. If air bubbles are present, return the array to the fluidics station.
    b. Follow the instructions on the LCD panel of the fluidics station.
    c. Pull the lever up, then load to remove bubbles.
14. If air bubbles are still present after repeating the above process a few times, use the manual process.
    a. Insert a 200-µL pipette tip into the upper-right septum of the array.
    b. Using the pipette, remove half of the solution.
    c. Manually fill the array with Array Holding Buffer.
15. If the array has no bubble, it is ready for scanning. Proceed to “Stage 9B—Scan arrays” on page 324.

    If the arrays cannot be scanned promptly, store them at 4°C in the dark until ready for scanning. Perform the scan within 24 hours.

16. Pull up on the cartridge lever to engage the wash block. Remove the microcentrifuge vials containing stain, then replace with 3 empty vials as prompted.

17. When washing and staining are complete, shut down the fluidics station following the procedure in “Add arrays during an AutoLoader run” on page 325.

Stage 9B—Scan arrays

The GeneChip™ Scanner 3000 7G System is controlled by GeneChip™ Command Console™ software.

Prepare the scanner

Power on the scanner at least 10 minutes before use.

WARNING! The scanner uses a laser and is equipped with a safety interlock system. Defeating the interlock system can result in exposure to hazardous laser light.

Read and be familiar with the operation of the scanner before attempting to scan an array. See the *GeneChip™ Scanner 3000 7G Quick Reference*.

Prepare the arrays for scanning

1. If the arrays were stored at 4°C, allow them to warm to room temperature before scanning.

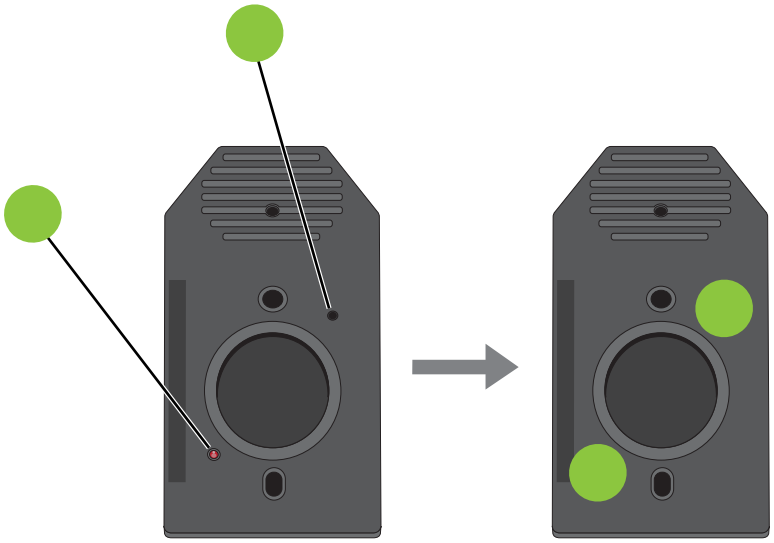
2. If necessary, clean the glass surface of the array with a nonabrasive towel or tissue before scanning.

   Do not use alcohol to clean the glass surface.

3. On the back of the array cartridge, clean excess fluid from around the septa.

4. Carefully cover both septa with Tough-Spots™ labels.

   Press firmly on the labels so that they lay flat and to ensure a tight seal. If the labels do not apply smoothly (for example, if you see bumps, bubbles, tears, or curled edges), do not attempt to smooth out the label. Remove the spot and apply a new spot.



Scan the array

If you are using the AutoLoader, see the AutoLoader user guide.

1. Open the GCC Scan Control application from the Launcher.
2. Load the arrays onto the AutoLoader of the scanner.
3. When all the arrays are loaded, click **Start** to initiate the scan.
4. Select the **arrays in carousel positions 1-4 at room temperature** checkbox. If the arrays are not at room temperature, do not select this option.

   The scanner waits 10 minutes before scanning starts to allow the arrays to reach room temperature.

   Only 1 scan per array is required. Pixel resolution and wavelength are preset and cannot be changed.

WARNING! The door is locked while the instrument is scanning. Do not attempt to open the door manually.

Add arrays during an AutoLoader run

1. Click the **Add Chips** icon .
2. In the **GeneChip Scanner** message, click **Add after Scan**.

   IMPORTANT! Do not use the **Add Now** feature. Use only the **Add after Scan** feature when working with CytoScan™ Arrays.

3. When the status on the scanner reads **AutoLoader Door Unlocked**, open the scanner, then add the arrays.

4. Close the scanner.
5. When the following message is displayed, click **OK**.
6. After you click **OK**, click **Resume**.
7. If any arrays in the carousel are to be rescanned, select the **Allow rescans** option.

Shutting down the fluidics station

1. Gently lift up the cartridge lever to engage (close) the washblock.

   After removing an array from the holder, the LCD window displays the message **ENGAGE WASHBLOCK**. The instrument automatically performs a Cleanout procedure. The LCD window indicates the progress of this procedure.

2. When **REMOVE VIALS** is displayed in the LCD, remove the vials.

   The **REMOVE VIALS** message indicates that the cleanout procedure is complete.

3. If no other processing is to be performed, place the wash lines into a bottle filled with deionized water.
4. Using GCC, choose the **Shutdown_450** protocol for all modules.
5. Run the protocol for all modules.

   The Shutdown protocol is critical to instrument reliability. See the instrument user guide for more information.

6. When the protocol is complete, power down the instrument.
7. Empty the waste bottle.

IMPORTANT! To maintain the cleanliness of the fluidics station and obtain the highest quality image and data possible, a weekly bleach protocol is highly recommended. See “Fluidics station Bleach protocol” on page 367.

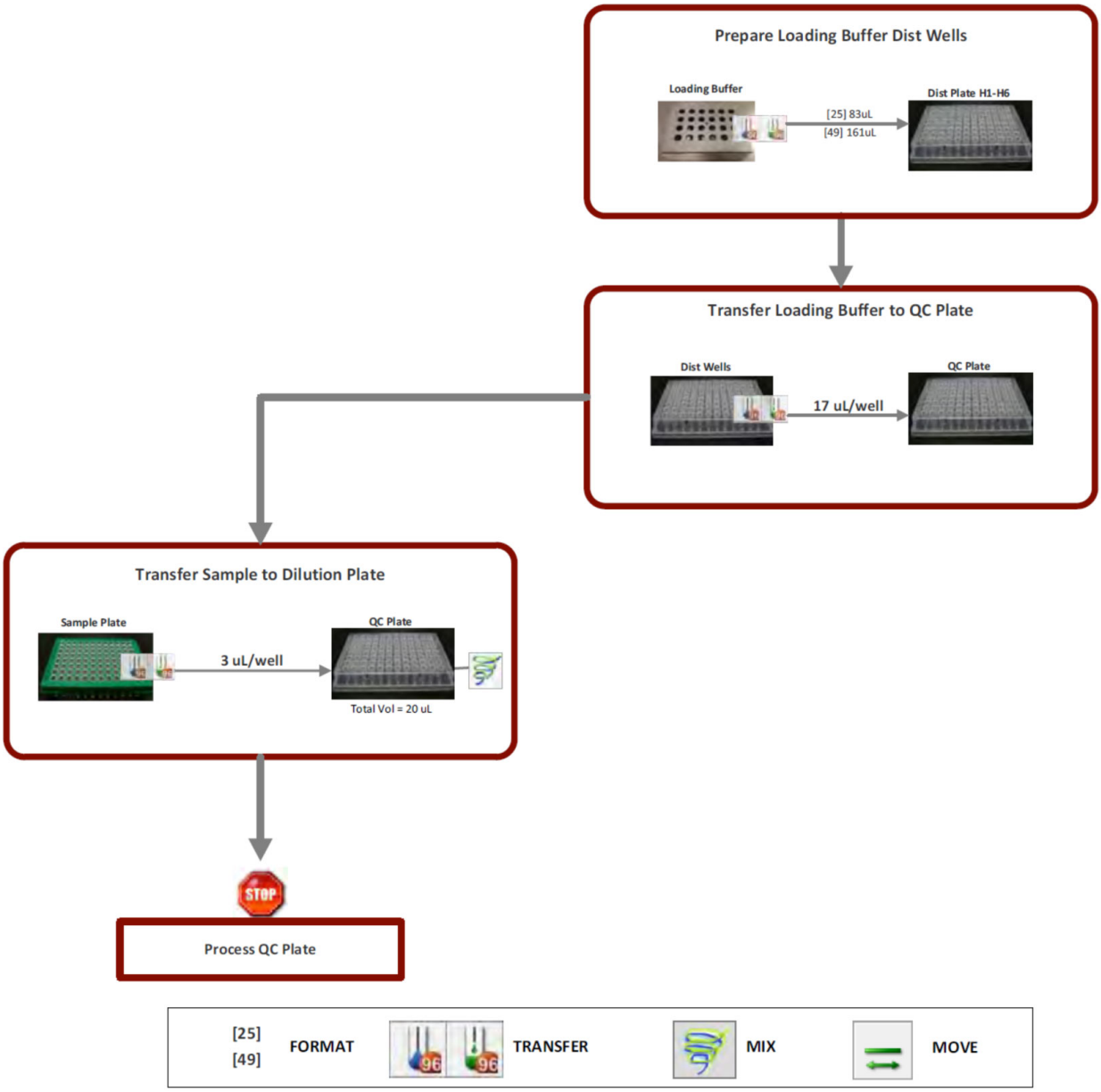
A

CytoScan™ Assay workflow diagrams for the NIMBUS™ Instrument

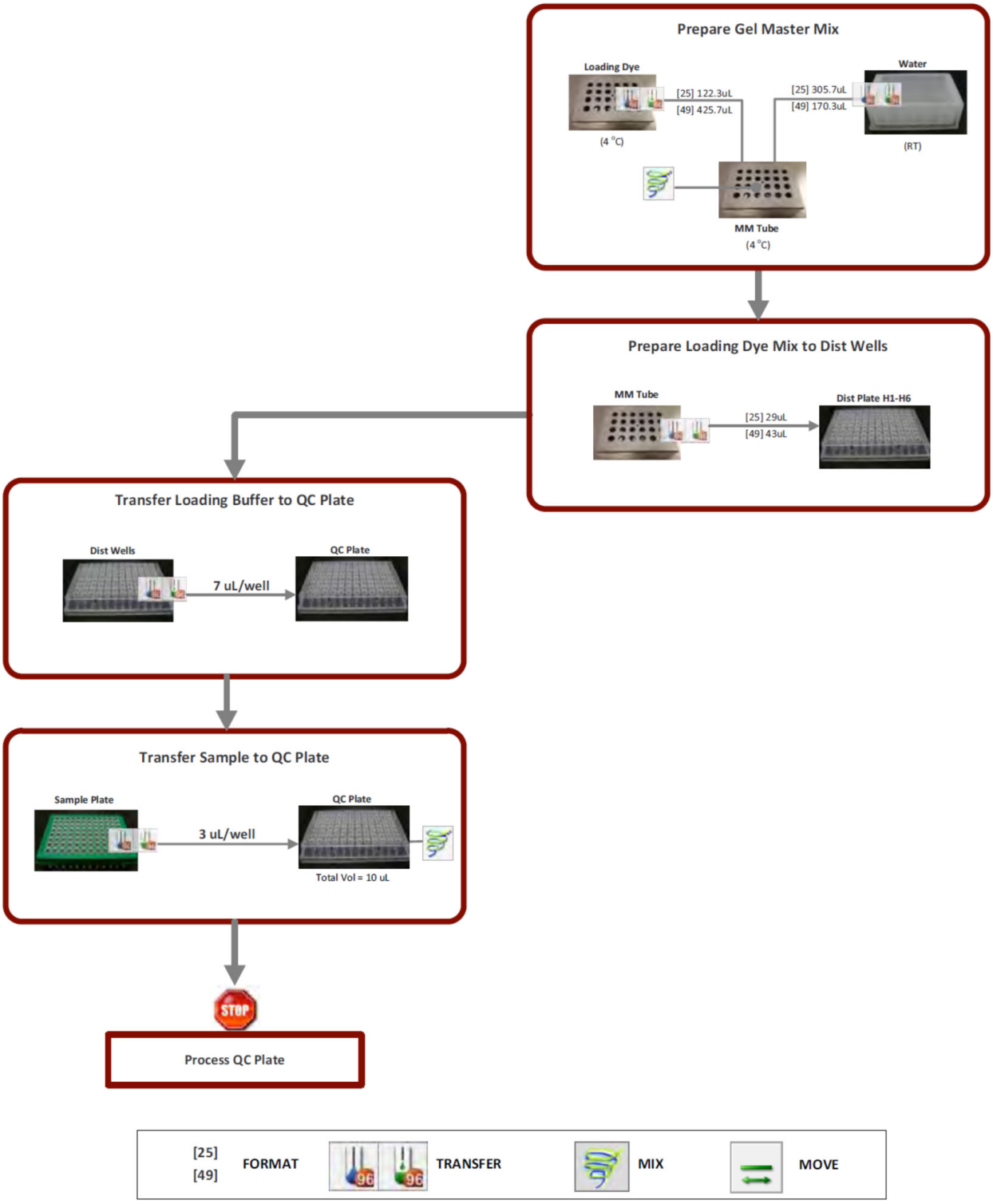
NIMBUS™ Instrument run times for the CytoScan™ Assay

Samples	Stage 3C	Stage 4A	Stage 4B	Stage 5	Stage 6A	Stage 6B	Stage 7	Stage 8A
25	7:17	3:05	1:36:13	7:08	8:56	10:27	9:20	15:28
49	10:17	3:04	1:42:39	8:13	9:34	15:22	11:41	18:05

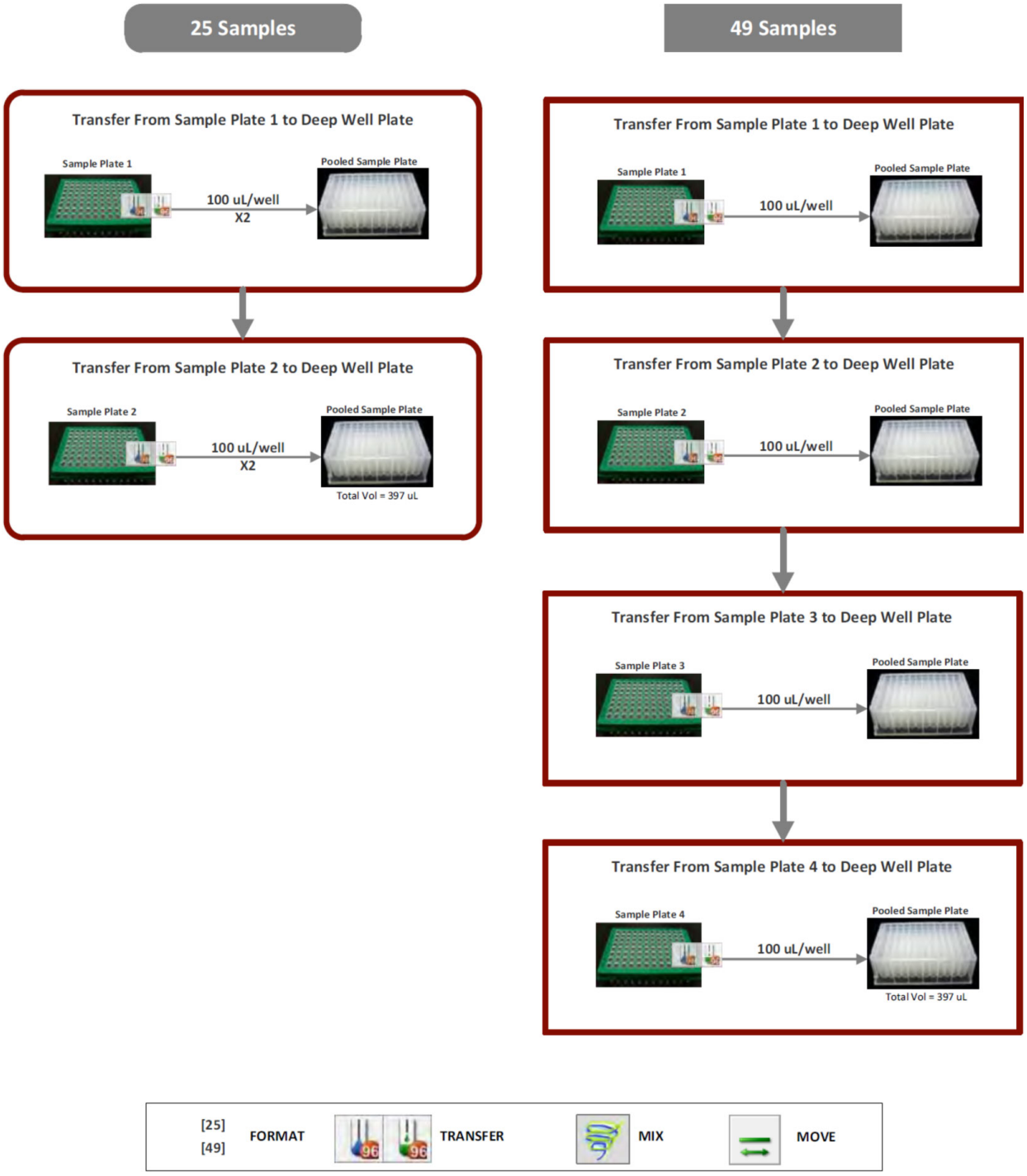
Workflow for Stage 3C—PCR product check (E-Gel™ Agarose Gel)



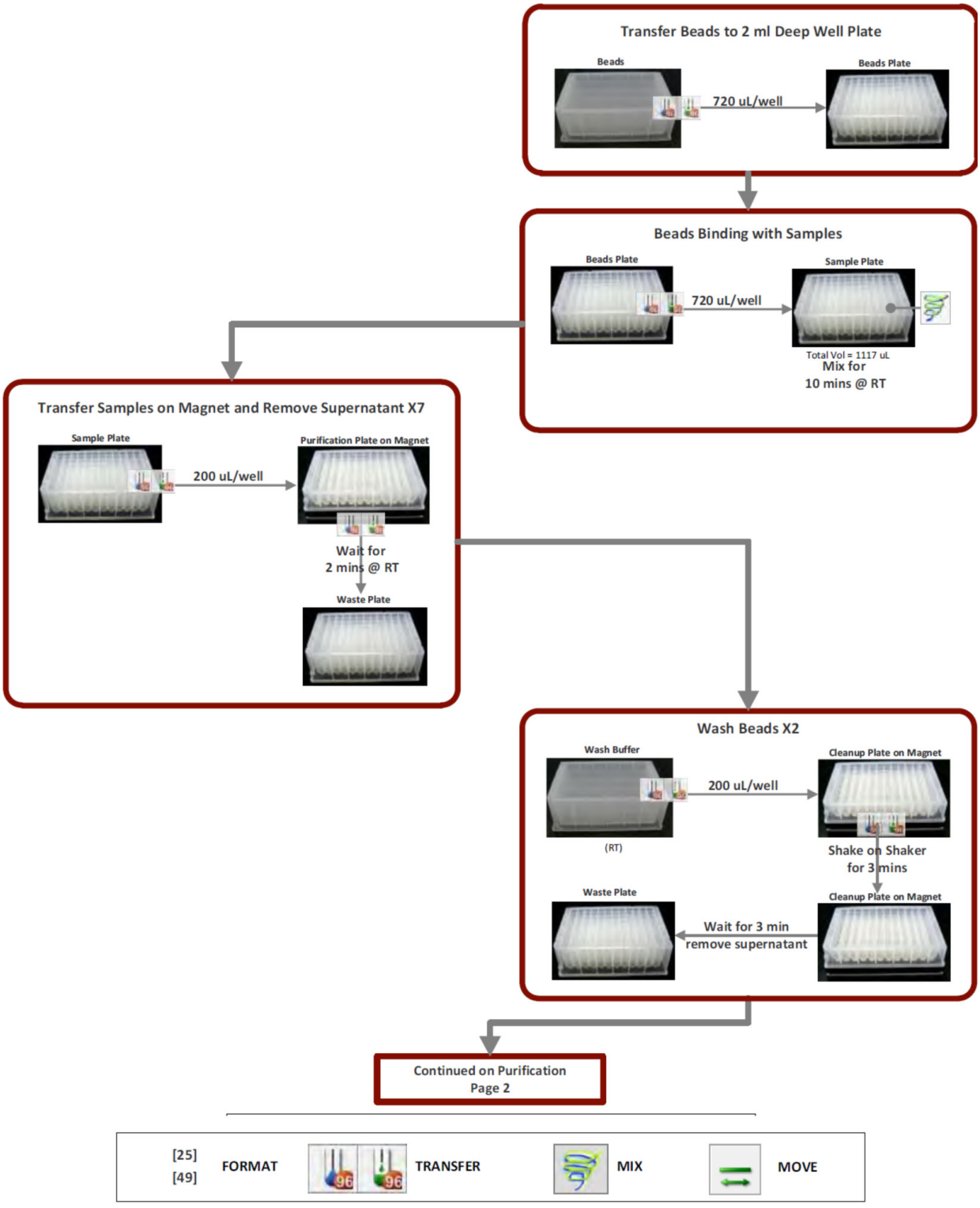
Workflow for Stage 3C—PCR product check (TBE gel)

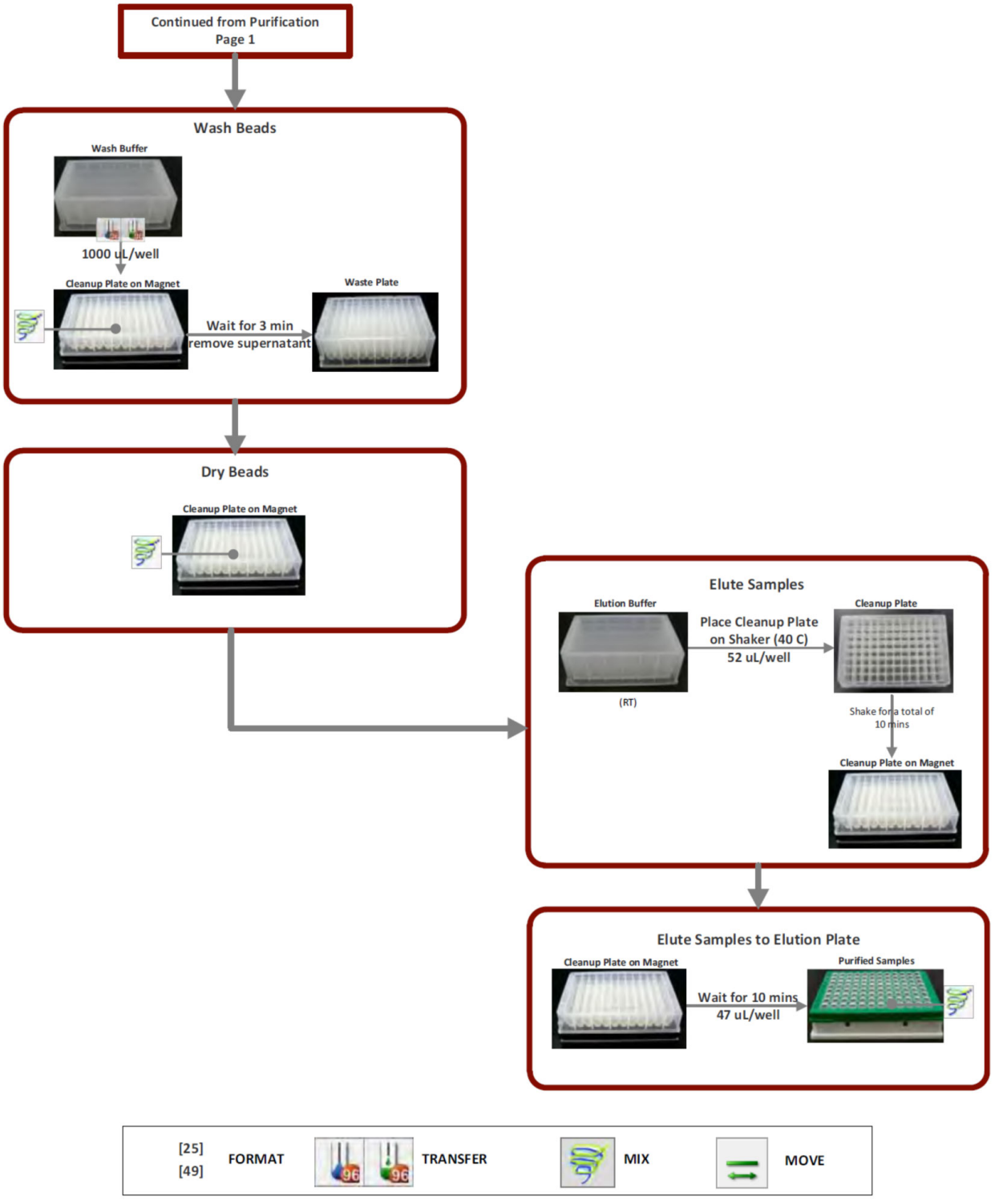


Workflow for Stage 4A—PCR product pooling

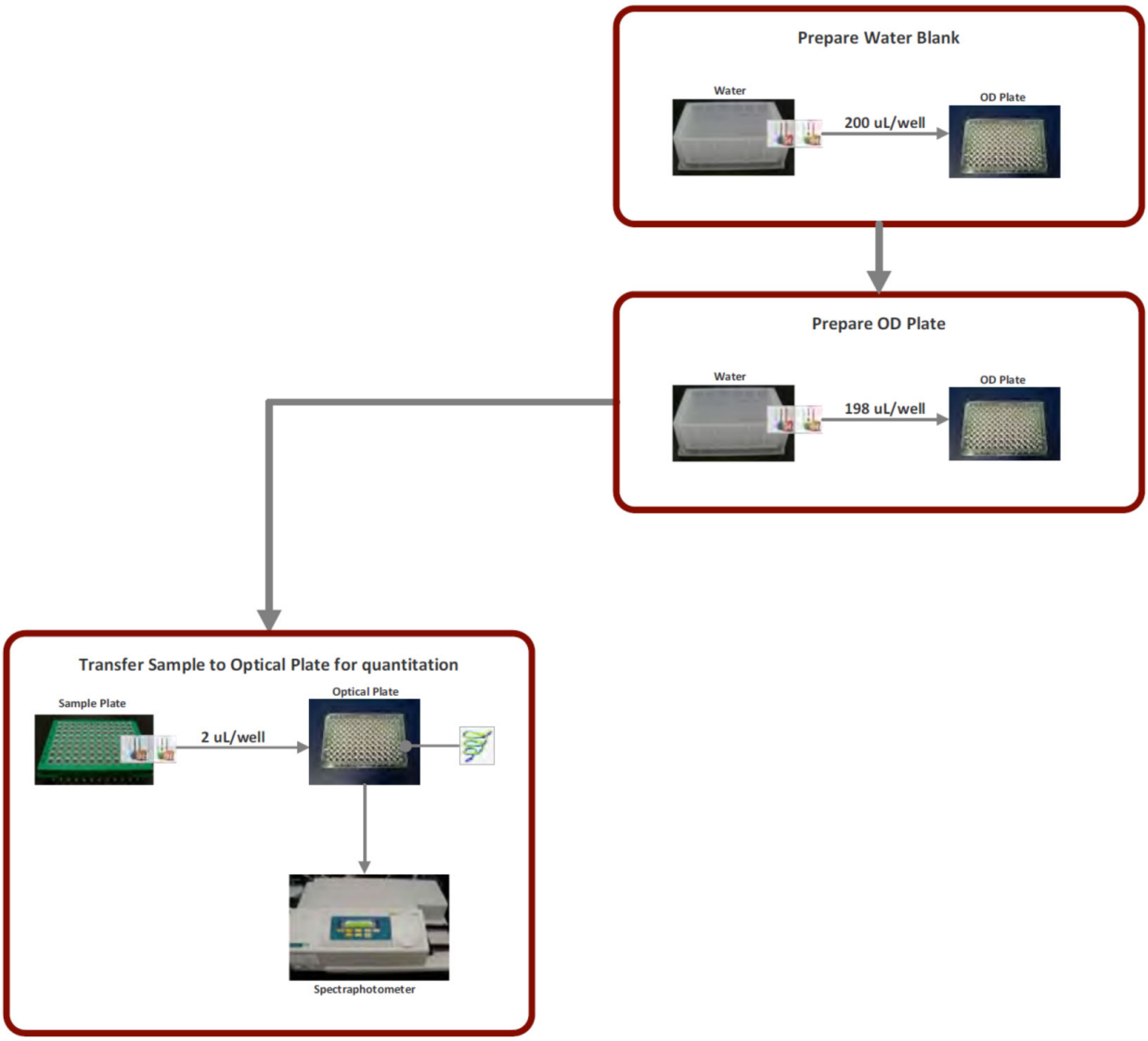


Workflow for Stage 4B—PCR product purification

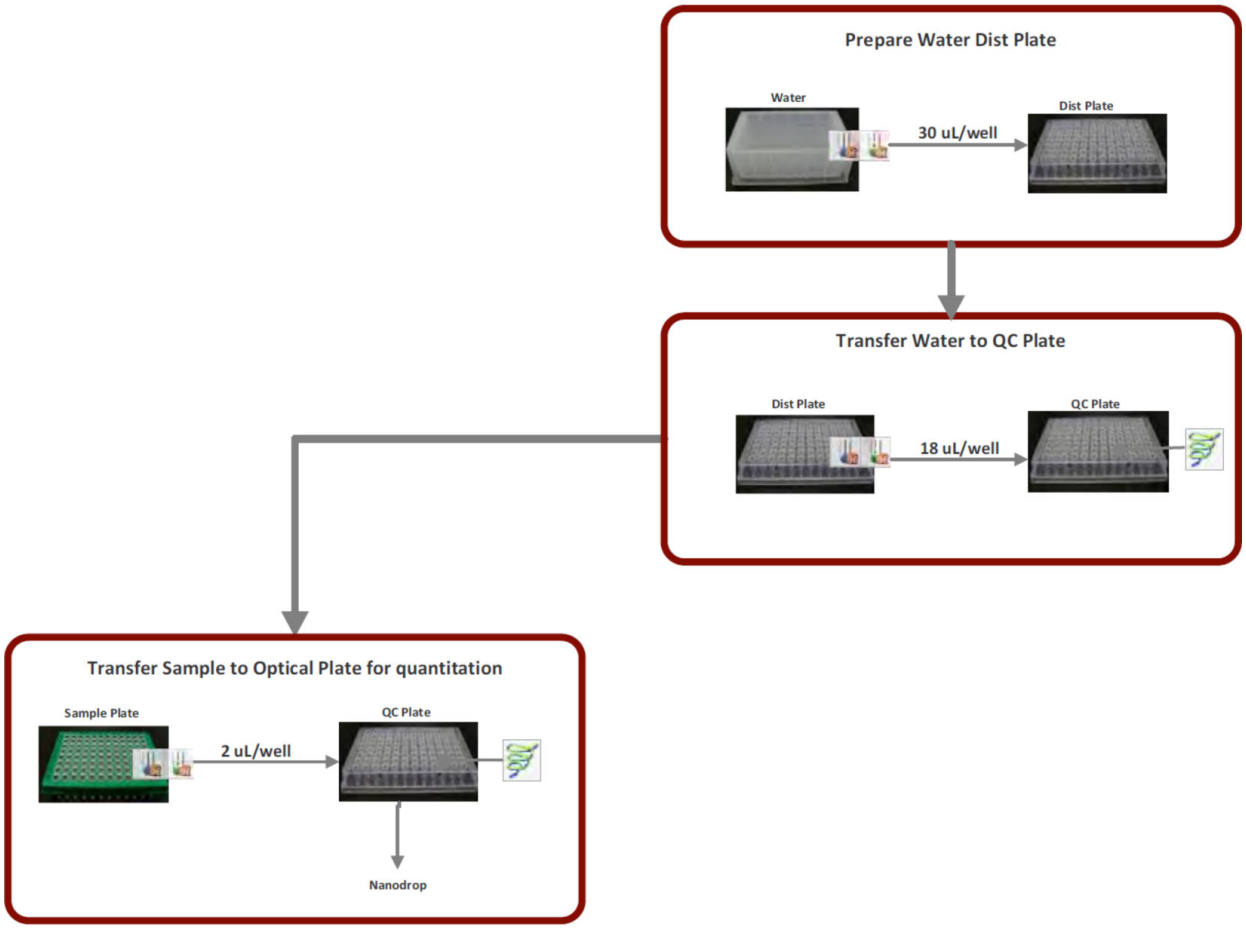



Continued from Purification
Page 1
Wash Beads
Wash Buffer
1000 uL/well
Cleanup Plate on Magnet
Waste Plate
Wait for 3 min
remove supernatant
Dry Beads
Cleanup Plate on Magnet
Elute Samples
Elution Buffer
(RT)
Place Cleanup Plate
on Shaker (40 C)
52 uL/well
Cleanup Plate
Shake for a total of
10 mins
Cleanup Plate on Magnet
Elute Samples to Elution Plate
Cleanup Plate on Magnet
Wait for 10 mins
47 uL/well
Purified Samples
[25]
[49]
FORMAT
TRANSFER
MIX
MOVE

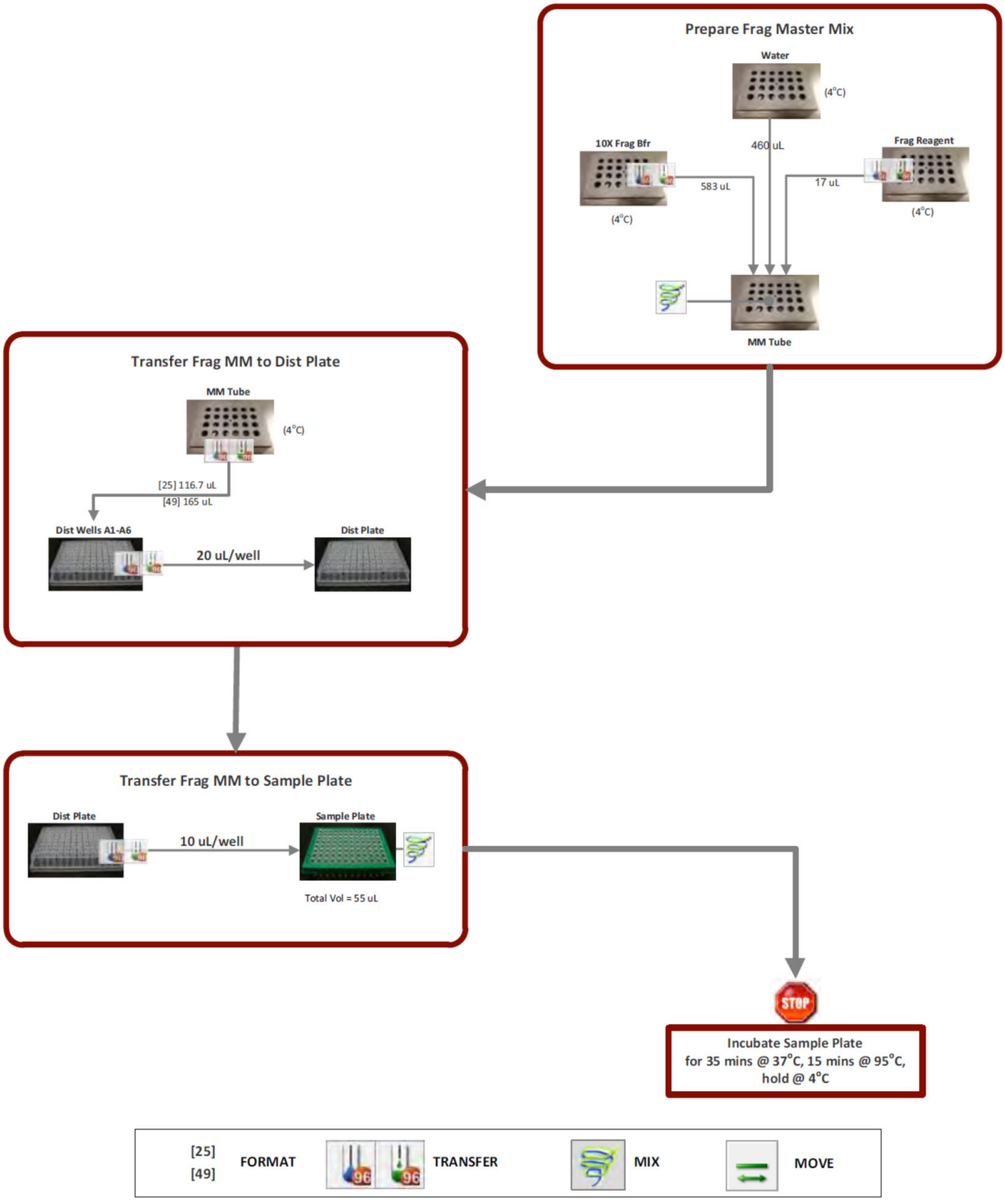
Workflow for Stage 5—Quantitation (Plate OD)



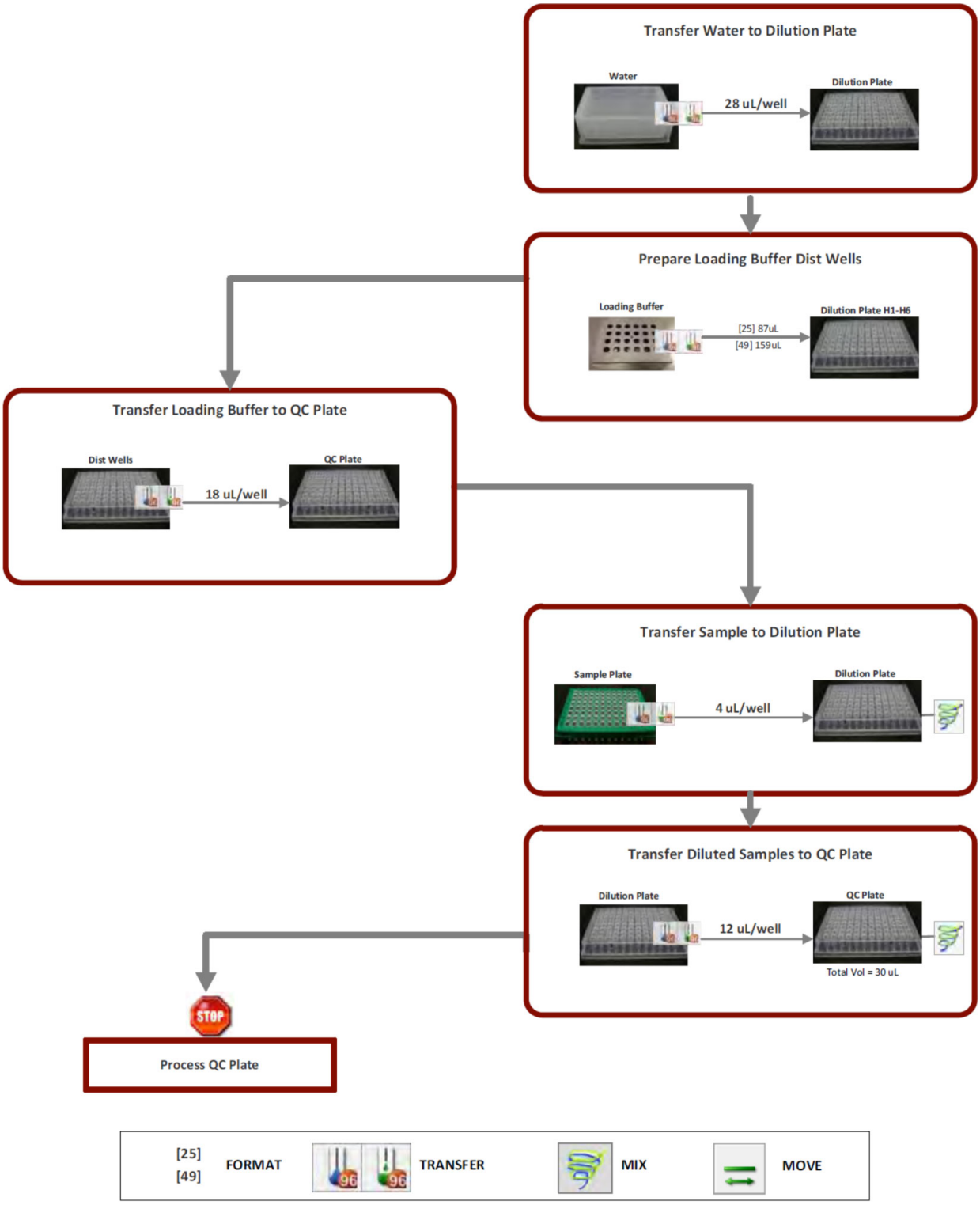
Workflow for Stage 5—Quantitation (NanoDrop™ Spectrophotometer)



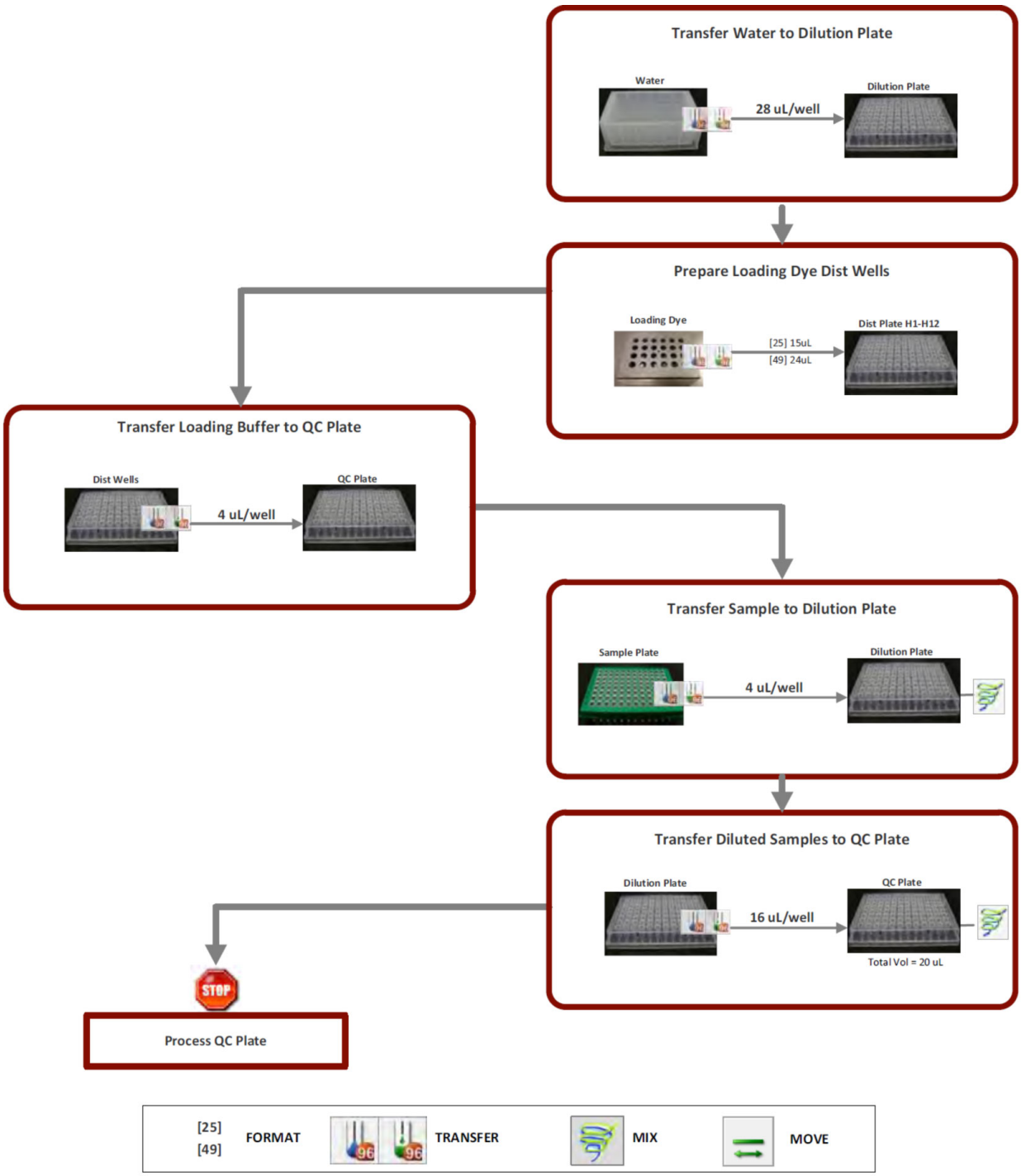
Workflow for Stage 6A—Fragmentation



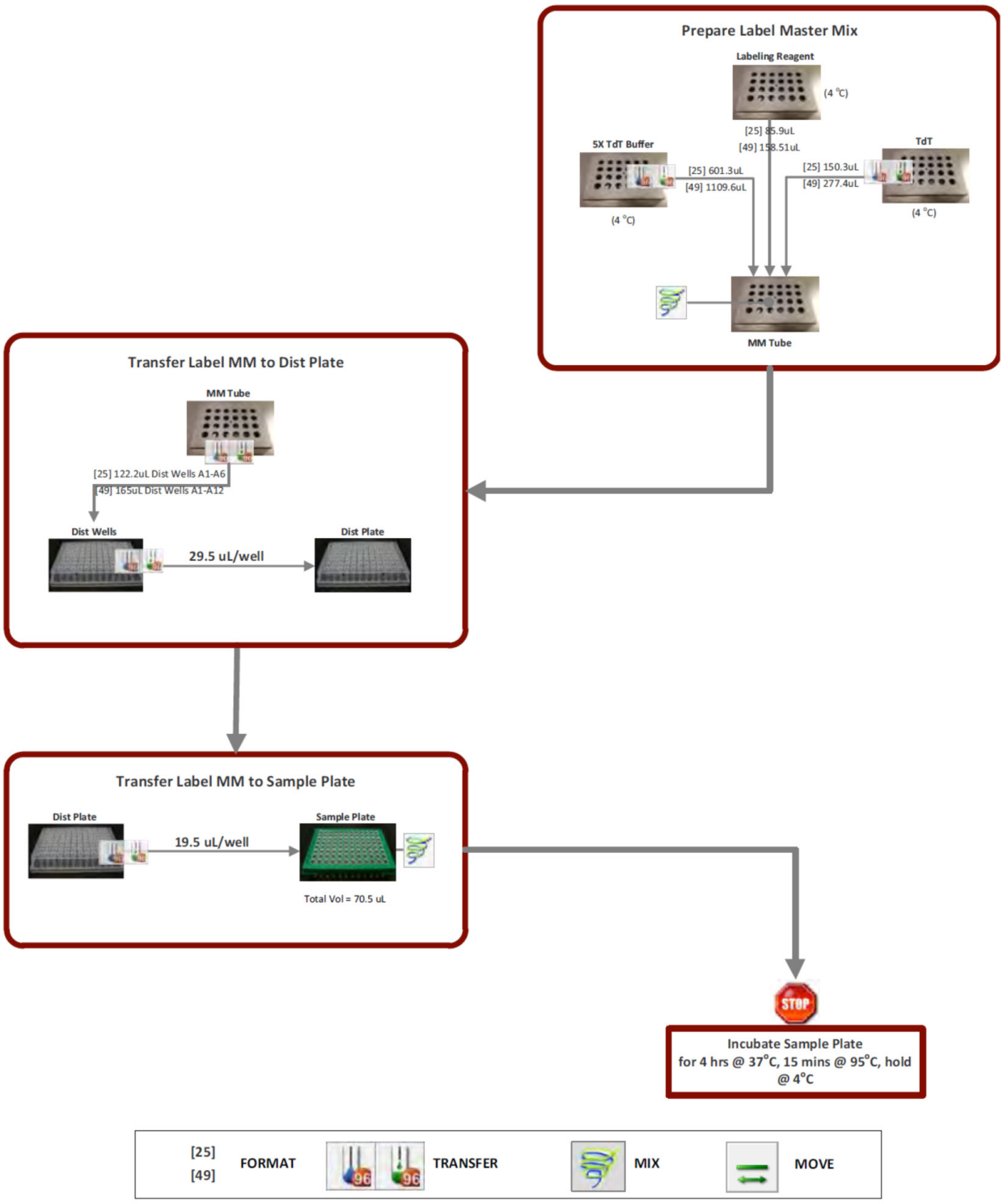
Workflow for Stage 6B—Fragmentation QC (E-Gel™ Agarose Gel)



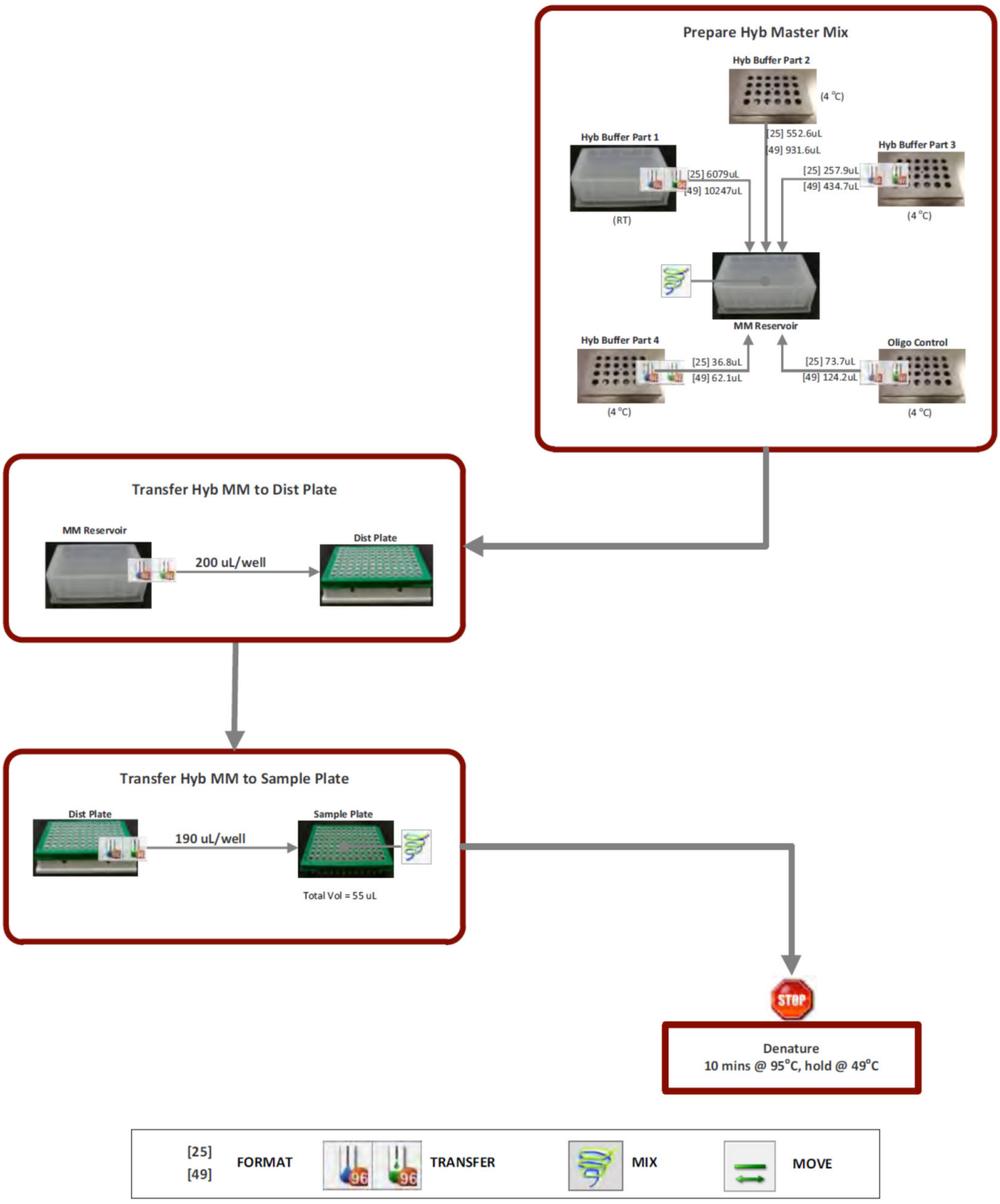
Workflow for Stage 6B—Fragmentation QC (TBE Gel)



Workflow for Stage 7—Labeling



Stage 8A—Hybridization Mix



B

Troubleshooting

General assay performance recommendations

- Carefully read and follow the protocol as written.
- As with any assay using PCR, the CytoScan™ Assay Automated Workflow has an inherent risk of contamination with PCR product from previous reactions. We strongly recommend 2 separate work areas be used to minimize the risk of cross-contamination during the assay procedure. It is essential to adhere to workflow recommendations. Set up PCR reactions in only the Pre-PCR Clean Area. Personnel should not reenter the Pre-PCR Clean Area after exposure to PCR products without first showering and changing into clean clothes.
- The CytoScan™ Assay Automated Workflow has been validated using the specified reagents and suppliers. Substitution of reagents and taking shortcuts are not recommended because your results could be suboptimal. For example, always use nuclease-free water and PCR reagents from Clontech™.
- Plan ahead to ensure that the reagents and equipment required, including pipettes, are in the correct work area. Ensuring that the proper equipment is available in the proper laboratory areas makes the workflow easier, and helps reduce the risk of sample contamination.
- Pay particular attention to the storage and handling of reagents. Proper storage and handling is particularly important for enzymes such as DNA Ligase and the Fragmentation Reagent. Both of these enzymes are sensitive to temperatures exceeding –20°C.

  To prevent loss of enzyme activity:
  - Store the enzymes in a cooler placed in a –20°C freezer to preserve activity. When taking out enzymes for reaction setup, always use a cooler chilled to –20°C.
  - Take care when pipetting enzymes stored in glycerol, which is viscous. Do not store enzymes at –80°C.
- The PCR reaction for this assay has been validated using the specified thermal cyclers. We highly recommend that your PCR thermal cyclers be calibrated regularly. Take care when programming your thermal cycler and use the recommended 96-well plate.

- It is essential to run gels to monitor both the PCR and the fragmentation reactions.

  For the PCR reaction, individual PCR products are run on a gel. Product (bands) should be visible in the 150- to 2,000-bp size range. See “Stage 3C—PCR product check” on page 83.

  Following fragmentation, run your samples on a gel. Successful fragmentation is verified by the presence of a majority of the distribution between 25 and 125 bp. See “Stage 6B—Fragmentation QC” on page 142. Alternatively, analyze the fragmented samples using the Agilent™ 2100 Bioanalyzer™ Instrument.
- Always run positive and negative controls in parallel with each group of samples.

  The absence of bands on your PCR gel for the negative control confirms that no previously amplified PCR product has contaminated your samples. Use genomic DNA from the CytoScan™ Reagent Kit as a positive control. These controls are effective troubleshooting tools that help you confirm the successful completion of each stage of the assay.
- Oligonucleotide controls are included in the reagent kit. These controls are added to the target samples before hybridization and act to confirm successful hybridization, washing, staining, and scanning of the array.
- Regularly calibrate all single-channel and multichannel pipettes.
- Confirm that your spectrophotometer is accurately calibrated, and ensure that the OD measurement is within the linear range of the instrument according to the manufacturer’s recommendations.
- Hybridization oven temperature is critical to the performance of the assay. Use only the GeneChip™ Hybridization Oven 645. Service your hybridization ovens at least once a year to ensure that they are operating within specification.

Troubleshooting CytoScan™ assay performance

Table 58 PCR Gel QC step.

Problem	Likely Cause	Solution
Faint or no PCR product visible on gel. Both samples and positive control affected.	Failed restriction digest or adapter ligation.	• Repeat the assay from the beginning with genomic DNA control after reviewing best practices, ensuring that all equipment is correctly calibrated, and reagents are handled and stored properly. • If available, include ligated material from a previous successful experiment as a positive control for the PCR step. If it fails again, repeat with fresh reagents.
		Ensure that the ligation buffer is thoroughly resuspended before use.
		Ensure that the reaction plates are sealed tightly in all steps.

Table 58 PCR Gel QC step. *(continued)*

Problem	Likely Cause	Solution
Faint or no PCR product visible on gel. Both samples and positive control affected.	Non-optimal PCR conditions.	• Use only calibrated thermal cyclers. • Verify that the PCR programs are entered correctly.
		Check the PCR reagents. Use only those reagents recommended by Thermo Fisher Scientific.
		• Verify pipette calibration and function. • Repeat PCR from the remaining digestion/ligation material, if available. • Otherwise, restart from the beginning.
		Take care with preparation of master mixes. Ensure accurate pipetting and thorough mixing.
		• Use the recommended 96-well PCR plates and plate seals. • Ensure that the plates are sealed tightly in all steps.
	Ligation reaction not diluted or diluted ligation reaction not mixed properly before PCR.	Ensure that the ligation reaction is correctly diluted with the water provided in the kit, then mix properly before proceeding with PCR.
Faint or no PCR product visible on the gel. Samples are affected but positive control is OK.	Insufficient or degraded genomic DNA.	• Use the starting amount of 250 ng genomic DNA. • Confirm the concentration using a calibrated spectrophotometer. • Confirm that the genomic DNA sample meets quality and integrity guidelines. See Chapter 4, “Genomic DNA general requirements”.
	Sample DNA contains enzymatic or chemical inhibitors. Nsp I can be inhibited by high concentrations of salts.	Ensure that genomic DNA is extracted using one of the recommended procedures. See Chapter 4, “Genomic DNA general requirements”.
Wrong size distribution of PCR product.	Mispipetting of PCR primer volume in the master mix.	• Verify pipette calibration and function. • Repeat PCR from the remaining digestion/ligation material, if available. • Otherwise, restart from the beginning.
	Mispipetting of Taq polymerase in the master mix.	

Table 58 PCR Gel QC step. *(continued)*

Problem	Likely Cause	Solution
PCR product evident in the negative control.	Reagents or equipment contaminated with ligated product or amplified product.	• Always use filter tips. • Clean the Pre-PCR Clean Area and equipment thoroughly using 10% bleach. • Decontaminate the pipettes following manufacturer's recommendation. • Retrain personnel on prelab best practices. • Repeat the assay using fresh reagents and sample.
	Non-optimal PCR conditions.	• Use only calibrated thermal cyclers. • Verify that the PCR programs are entered correctly.
		Check the PCR reagents. Use only those reagents recommended by Thermo Fisher Scientific.
		• Verify pipette calibration and function. • Repeat PCR from the remaining digestion/ligation material, if available. • Otherwise, restart from the beginning.
		Take care with preparation of master mixes. Ensure accurate pipetting and thorough mixing.
		• Use the recommended 96-well PCR plates and plate seals. • Ensure that the plates are sealed tightly in all steps.
	Ligation reaction not diluted or diluted ligation reaction not mixed properly before PCR.	Ensure that the ligation reaction is correctly diluted with the water provided in the kit, then mix properly before proceeding with PCR.
Faint or no PCR product visible on the gel. Samples are affected but positive control is OK.	Insufficient or degraded genomic DNA.	• Use the starting amount of 250 ng genomic DNA. • Confirm the concentration using a calibrated spectrophotometer. • Confirm that the genomic DNA sample meets the quality and integrity guidelines. See Chapter 4, "Genomic DNA general requirements".

Table 58 PCR Gel QC step. *(continued)*

Problem	Likely Cause	Solution
Faint or no PCR product visible on the gel. Samples are affected but positive control is OK.	Sample DNA contains enzymatic or chemical inhibitors. Nsp I can be inhibited by high concentrations of salts.	Ensure that genomic DNA is extracted using one of the recommended procedures. See Chapter 4, “Genomic DNA general requirements”.
Wrong size distribution of PCR product.	Mispipetting of PCR primer volume in the master mix.	• Verify pipette calibration and function. • Repeat PCR from the remaining digestion/ligation material, if available. • Otherwise, restart from the beginning.
	Mispipetting of Taq polymerase in the master mix.	

Table 59 Devices or instruments.

Problem	Likely cause	Solution
Incorrect thermal cycler protocol.	Operator error.	Restart the run with a new CytoScan™ Reagent Kit, and correct program.
CPAC or Thermoshake is not responding or reach the correct temperature.	Connection problem.	Ensure that the devices are correctly set up.
Incorrect shaking.	Device out of calibration.	Contact Thermo Fisher Scientific for service.
Thermoshake unable to regulate temperature.	Low cooling liquid level.	Refill the cooling liquid reservoir regularly.
Instrument stalls in the middle of a method.	Hardware error.	Contact Thermo Fisher Scientific for service.
Error in detecting liquid.	Misplaced reagent.	Restart run with correct deck setup.
Incorrect alignment of instrument with consumable.	Incorrect pedestal configuration.	Restart run with correct deck setup.
	Incorrect placement of consumables.	Restart run with correct deck setup.
Mis-picked tip.	Missing component during run.	Restart run with correct deck setup.
Failure to pick or place plate.	Damaged gripper assembly.	Contact Thermo Fisher Scientific for service.
	Incorrect instrument set up.	Restart run with correct deck setup.
Instrument fails to resume from pause.	Software bug.	Continue the process manually.

Table 59 Devices or instruments. *(continued)*

Problem	Likely cause	Solution
Instrument control software stops working.	Power interruption.	Continue the process manually.
Automated method problem.	Instrument error. Software error. Incorrect deck setup.	Consult the quick reference card to see if the problem can be corrected with the correct deck setup or retrying the step. If not, abort the method and eject tips into the waste chute, if possible. Find the location in the workflow with the log file and workflow guide, then continue the process manually.

Table 60 CytoScan™ Automation workflow recovery.

CytoScan™ automation method–assay step	Automation substep	Recoverable?	Recovery process
Stage 3C—PCR product check (E-Gel™ Agarose Gel)	Transfer loading buffer to distribution wells.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer loading buffer to QC plate.	Yes	
	Prepare single well in distribution plate.	Yes	
	Format tip rack.	Yes	
	Transfer samples to QC plate.	No	
Stage 3C—PCR product check (TBE Gel)	Prepare loading dye mix.	Yes	Abort the method and eject tips into the waste chute, if possible. Manually prepare mix and continue the process.
	Transfer QC reagent to distribution wells.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer QC reagent to QC plate.	Yes	
	Prepare single well in distribution plate.	Yes	
	Format tip rack.	Yes	
	Transfer samples to QC plate.	No	

Table 60 CytoScan Automation workflow recovery. *(continued)*

CytoScan™ automation method–assay step	Automation substep	Recoverable?	Recovery process
Stage 4A—PCR product pooling	Format tip rack.	Yes	Abort the method and eject tips into the waste chute if possible. Continue the process manually.
	Pool samples.	No	
Stage 4B—PCR product purification	Transfer Binding Beads to empty plate.	Yes	Abort the method and eject tips into the waste chute if possible. Continue the process manually.
	Add Binding Beads to samples.	Maybe, if samples are not affected.	
	Bead binding.	Maybe, if samples are not affected.	Abort the method and eject tips into the waste chute if possible. Continue the process manually.
	Remove bead supernatant.	Maybe, if samples are not affected.	
	Return F1000 tips to source and move 300-µL tips.	Yes	
	Move plate to shaker.	Maybe, if samples are not affected.	
	Add 200 µL of wash buffer to beads.	Maybe, if samples are not affected.	
	Shake beads for 2 minutes.	Maybe, if samples are not affected.	
	Move plate to magnet.	Maybe, if samples are not affected.	
	Reformat tips.	Yes	
	Remove supernatant.	Maybe, if samples are not affected.	
	Move plate to shaker.	Maybe, if samples are not affected.	
	Add 200 µL of wash buffer to beads.	Maybe, if samples are not affected.	

Table 60 CytoScan Automation workflow recovery. *(continued)*

CytoScan™ automation method–assay step	Automation substep	Recoverable?	Recovery process
Stage 4B—PCR product purification	Shake beads for 2 minutes.	Maybe, if samples are not affected.	Abort the method and eject tips into the waste chute if possible. Continue the process manually.
	Move to magnet and remove supernatant.	Maybe, if samples are not affected.	
	Add 1,000 µL wash buffer to beads.	Maybe, if samples are not affected.	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Remove wash supernatant.	Maybe, if samples are not affected.	
	Dry beads.	Maybe, if samples are not affected.	
	Add elution buffer to beads.	Maybe, if samples are not affected.	
	Shake beads for 10 minutes.	Maybe, if samples are not affected.	
	Move to magnet for 10 minutes.	Maybe, if samples are not affected.	
	Transfer samples to Bio-Rad™ plates.	Maybe, if samples are not affected.	
Stage 5—Quantitation (NanoDrop™ Spectrophotometer)	Format tip rack.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Prepare water distribution plate.	Yes	
	Prepare single well in distribution plate.	Yes	
	Transfer water to NanoDrop™ Spectrophotometer plate.	Yes	
	Transfer samples to NanoDrop™ Spectrophotometer plate.	No	Restart the stage.
Stage 5—Quantitation (Plate OD)	Format tip rack.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.

Table 60 CytoScan Automation workflow recovery. *(continued)*

CytoScan™ automation method–assay step	Automation substep	Recoverable?	Recovery process
Stage 5—Quantitation (Plate OD)	Prepare water blanks.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer water to OD plate.	Yes	
	Format tip rack.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Prepare water blanks.	No	
	Transfer water to OD plate.	No	
	Format tip rack.	No	
	Transfer samples to UV plate.	No	
Stage 6A—Fragmentation	Transfer water.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer Fragmentation Buffer.	No	None. Restart the stage.
	Return F1000 tips.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Format tip rack.	Yes	
	Load tips.	Yes	
	Transfer Fragmentation Reagent.	Reagents lost.	Restart the stage manually.
	Mix Fragmentation Master Mix.	Reagents lost.	
	Transfer Fragmentation Master Mix to distribution wells.	Reagents lost.	
	Prepare Fragmentation Master Mix distribution plate.	Reagents lost.	
	Prepare single well in distribution plate.	Reagents lost.	Restart the stage manually.
	Beginning of transfer of Fragmentation Master Mix (reagents only).	Reagents lost.	

Table 60 CytoScan Automation workflow recovery. *(continued)*

CytoScan™ automation method–assay step	Automation substep	Recoverable?	Recovery process
Stage 6A—Fragmentation	Transfer Fragmentation Master Mix to samples.	Sample lost.	Run gel/QC. Manual—new kit.
Stage 6B—Fragmentation QC (TBE gel)	Prepare water distribution plate.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Prepare single well in distribution plate.	Yes	
	Transfer 5X loading dye to distribution wells (4% TBE gel).	Yes	
	Transfer 5X loading dye to QC plate.	Yes	
	Prepare single well in distribution plate.	Yes	
	Format tip rack.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer water to dilution plate.	Yes	
	Transfer samples to dilution plate.	No	Restart the stage.
	Transfer diluted samples to QC plate.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
Stage 7—Labeling	Transfer TdT Buffer	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Format tip rack.	Yes	
	Transfer Labeling Reagent.	No	Restart the stage.
	Transfer TdT.	No	
	Mix Labeling Master Mix.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer Labeling Master Mix to distribution wells.	Yes	
	Load tips.	Yes	
	Prepare Labeling Master Mix distribution plate.	Yes	

Table 60 CytoScan Automation workflow recovery. *(continued)*

CytoScan™ automation method–assay step	Automation substep	Recoverable?	Recovery process
Stage 7—Labeling	Prepare single well in distribution plate.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer Labeling Master Mix to samples.	No	Restart the stage.
Stage 8A—Hybridization Mix	Transfer Hyb Buffer Part 1.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer Hyb Buffer Part 2.	No	Restart the stage.
	Transfer Hyb Buffer Part 3.	No	
	Format tip rack.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Transfer Hyb Buffer Part 4.	No	Restart the stage.
	Transfer Oligo Control Reagent.	No	
	Remove partial tip row.	Yes	Abort the method and eject tips into the waste chute, if possible. Continue the process manually.
	Mix Hybridization Master Mix.	Yes	
	Return partial tip row.	Yes	
	Prepare Hybridization Master Mix distribution plate.	Yes	
	Prepare single well in distribution plate.	Yes	
	Transfer Hybridization Master Mix to samples.	No	Restart the stage.

Table 61 Purification yield QC step.

Problem	Likely cause	Solution
Low eluate volume (<47 µL)	Insufficient volume due to pipetting error or pipet out of calibration.	Verify pipette calibration. Ensure that 52 µL of Elution Buffer is added to the Purification Beads for elution, and the tubes are centrifuged before placing on the magnet.
Low yields (the average purification yield of 7 or more samples is <3.0 µg/µL or individual yield is <2.5 µg/µL)	Loss of sample before purification.	If the yield is not adequate, repeat the assay.
	Possible problems with input genomic DNA.	• Use the recommended collection and purification procedures to avoid carryover of inhibitors such as heme, EDTA, and so on. • Use the starting amount of 250 ng genomic DNA. • Confirm the concentration using a calibrated spectrophotometer. • Confirm that the genomic DNA sample meets the quality and integrity guidelines provided in Chapter 4, “Genomic DNA general requirements”.
	Purification Wash Buffer was prepared incorrectly.	Verify that the correct volume of absolute ethanol was added to the Purification Wash Buffer before use.
	Inadequate mixing of Purification Beads and PCR reactions during binding.	Take care to completely mix the PCR reactions and the Purification Beads during sample binding.
	Inadequate bead washing before elution.	Repeat purification with attention towards complete removal of the binding eluate before the bead wash.
	Excess Elution Buffer added to beads.	Verify pipette calibration and function.
	Incorrect buffer was used for elution.	Verify that the Elution Buffer was used during the elution step and not the Purification Wash Buffer.
	Purification Beads were over dried.	Do not dry Purification Beads longer than the recommended time.
	The eluted DNA plate was inadequately vortexed before taking an aliquot for an OD reading.	Eluted DNA can be heterogeneous. Repeat the dilution followed by an OD reading, making sure to vortex the eluted DNA and the OD plate thoroughly at each step.
	PCR reaction volume was inaccurate.	Repeat the assay and confirm that the PCR reaction is set up correctly.

Table 61 Purification yield QC step. *(continued)*

Problem	Likely cause	Solution
High yields (>4.5 µg/µL)	Too little Elution Buffer added to the Purification Beads.	Verify pipette calibration and function. Ensure that 52 µL of Elution Buffer is added to the Purification Beads for elution.
	Eluted DNA plate inadequately vortexed before OD reading is taken.	Eluted DNA can be heterogeneous. Repeat the dilution followed by OD reading, being sure to vortex the eluted DNA and the OD plate thoroughly at each step.
	Instruments or pipettes out of calibration or incorrectly set.	Verify instrument and pipette calibration and settings during operation.
	Incorrect yield calculation formula in the software template.	Verify the formula used to calculate the yields from a given OD measurement.
$OD_{260/280}$ ratio is not between 1.8 and 2.0	PCR product not adequately washed.	Ensure that proper volume of absolute ethanol is added to the Purification Wash Buffer, and follow the procedure provided in Chapter 5, "CytoScan™ Assay Automated Workflow for 25 samples".
	Error made while taking the OD readings.	Retake the OD following the instructions provided in Chapter 5, "CytoScan™ Assay Automated Workflow for 25 samples".
OD_{320} measurement is >0.1	Purification beads carried over into purified samples.	Centrifuge the sample for 5 minutes. Place on the MagnaRack™ and pipette out the eluate. Retake the OD measurement.
	Scratches or dust particles on the OD plate.	Ensure that the bottom surface of the OD plate is clean and free of scratches.
	Air bubbles are present in the diluted DNA in the OD plate.	Vortex the OD plate, centrifuge the plate again following the guidelines provided in Chapter 5, "CytoScan™ Assay Automated Workflow for 25 samples", then retake the OD measurement.

Table 62 Fragmentation QC step (gel or Bioanalyzer™).

Problem	Likely cause	Solution
Over fragmentation: Majority of fragmented sample appears <50 bp on an E-Gel™ *48* Agarose Gels, 4%.	Excess Fragmentation Reagent was added during preparation of the Fragmentation Master Mix.	• Carefully observe the pipette tip and the shaft during pipetting of the fragmentation reagent. Touch the tip to the inside of the vial to help remove any droplets of enzyme clinging to the exterior of the tip. • Ensure that pipettes are calibrated.

Table 62 Fragmentation QC step (gel or Bioanalyzer). *(continued)*

Problem	Likely cause	Solution
Over fragmentation: Majority of fragmented sample appears <50 bp on an E-Gel™ *48* Agarose Gels, 4%.	Purified samples or assembled reactions were allowed to warm to room temperature during reaction assembly or before incubation.	Ensure that the plate centrifuge is completely chilled to 4°C before centrifuging the assembled fragmentation plate.
		Keep the master mix, samples, and reaction components on ice or in a cooling block at all times during master mix assembly and dispensing of the master mix to the samples.
		Verify that the fragmentation reaction temperature and incubation time are correctly programmed on the thermal cycler, and that the fragmentation mix is made correctly according to the guidelines.
		Ensure that the master mix tube and strip tubes are prechilled before reaction setup.
	Incorrect fragmentation reagent unit was used to prepare the master mix.	Verify the unit activity on the label of the Fragmentation Reagent tube and formulate the master mix appropriately.
Under fragmentation: PCR product is still visible in 150—2,000 bp size region on an E-Gel™ *48* Agarose Gels, 4%.	Improper storage or handling of the Fragmentation Reagent.	• Always store the Fragmentation Reagent at –20°C. • Handle the Fragmentation Reagent as minimally as possible, holding the vial at the cap rather than the center. Return the Fragmentation Reagent to the cooler as soon as the reagent has been dispensed. We recommend storing the Fragmentation Reagent at –20°C inside a cooler to preserve its activity. • Do not over vortex the Fragmentation Reagent.
	• Insufficient Fragmentation Reagent or Fragmentation Buffer was added during assembly of the Fragmentation Master Mix. • Improper mixing of the Fragmentation Master Mix.	Verify pipette calibration and function. Take care when preparing the master mix to ensure accurate pipetting and thorough mixing.

Table 62 Fragmentation QC step (gel or Bioanalyzer). *(continued)*

Problem	Likely cause	Solution
Under fragmentation: PCR product is still visible in 150—2,000 bp size region on an E-Gel™ *48* Agarose Gels, 4%.	The Fragmentation Master Mix was not made fresh or was allowed to warm to room temperature before use.	• Always keep the Fragmentation Master Mix on ice to preserve activity. • Work quickly during reaction assembly. • Do not save or reuse a previously assembled Fragmentation Master Mix.
	Samples were frozen during fragmentation reaction assembly or centrifugation.	• Ensure that cold blocks are not chilled to –20°C as sample freezing can occur. • Before centrifugation, ensure that the interior of the chilled plate centrifuge is not lower than 2°C.
	Incorrect Fragmentation Reagent units were used to prepare the master mix.	Verify the unit activity on the label of the Fragmentation Reagent tube and formulate the master mix appropriately.
Under fragmentation: PCR product is still visible in 150—2,000 bp size region on a E-Gel™ *48* Agarose Gels, 4%.	Thermal cycler was not programmed correctly or is out of calibration.	• Confirm that the ragmentation reaction temperature and incubation time are correctly programmed on the thermal cycler. • Confirm that the Fragmentation Master Mix is made correctly according to the guidelines. • Verify that the thermal cycler is within calibration tolerance limits.

Table 63 CEL file generation.

Problem	Likely cause	Solution
.CEL file is not generated	Signal from the corner checkerboards is absent.	Verify that the Oligo Control Reagent was added to the Hybridization Master Mix during assembly. The Oligo Control Reagent must be present during hybridization to ensure proper grid alignment.
	Signal from corner checkerboards is dim.	• Verify that the correct amount of the Oligo Control Reagent was added to the Hybridization Master Mix during assembly. • Ensure that GeneChip™ Hybridization Oven 645 is calibrated and set to the correct temperature. • Ensure that the Hybridization Master Mix was correctly assembled and added at the correct volume to the fragmented samples. • Confirm that Stain Buffer 1 and Stain Buffer 2 are placed in the correct order on the fluidics station. Stain Buffer 1 is light sensitive. Always store Stain Buffer 1 in the dark when not in use. • Use only those staining reagents provided by Thermo Fisher Scientific.
	Bright hybridization artifact(s) obscure gridding oligo locations on the array.	Try to manually align the grid. See the *GeneChip™ Command Console™ User Guide* for instructions. If manual grid alignment fails to produce a .CEL file, repeat the experiment.

Table 64 Data QC failure.

Problem	Likely Cause	Solution
Low or failing SNPQC	• Cross-contamination between samples in a plate. • Contaminated reagents, equipment, or input DNA.	• Repeat the assay using a control sample of known integrity, such as Genomic DNA. • Review and follow best practices. – Ensure a tight plate seal at every step. – Use fresh filter tips at each pipetting step. – Use caution when pooling PCR product. • If the problem persists, use fresh reagents and fresh input DNA. • Decontaminate the Pre-PCR Clean Area and equipment, if needed.
		Process only 6 to 8 arrays at a time. When processing arrays for washing, it is important to work quickly because delays in this step impact data quality.
		Perform all steps after removal of arrays from the oven to the time the washing begins with minimal delays.

Table 64 Data QC failure. *(continued)*

Problem	Likely Cause	Solution
Low or failing SNPQC	Over or under fragmentation of the PCR product	See above.
	Hybridization oven out of calibration or oven model is not compatible with this assay.	• Use only the GeneChip™ Hybridization Oven 645 for this assay. • Have the oven serviced.
Elevated or failing MAPD	Assay drift due to variation in assay execution.	• Recalibrate pipettes to ensure accurate delivery of reagent volumes. • If the problem persists, consider operator retraining or review by an Thermo Fisher Scientific Field Applications Scientist. • Review Chapter 3, “Guidelines for use”.
	Over fragmentation	See above.
	Degraded starting material.	• Perform a QC gel of input DNAs to evaluate samples for degradation. • Ensure that the DNA samples are of high quality. For example, run in a E-Gel™ *48* Agarose Gel, 1% to E-Gel™ *48* Agarose Gels, 2%, and compare to the genomic DNA control provided in CytoScan™ Reagent Kit.
	Reference is inappropriate for the sample.	Use only the recommended sample types (peripheral blood and cell line DNA).
High waviness-SD	Degraded genomic DNA.	Confirm that the genomic DNA sample meets the quality and integrity guidelines in Chapter 4, “Genomic DNA general requirements”.
	Incompatible sample type.	Use only cell-line or blood-derived genomic DNA.
	Incompatible genomic DNA extraction method used.	Use only the recommended extraction methods. See Chapter 4, “Genomic DNA general requirements”.
	Sample-specific effect.	See the *Chromosome Analysis Suite User Guide*.
High MAPD with low SNPQC	Error during washing the array.	• Ensure that the Wash A and B lines of the fluidics station are placed in the correct wash buffers during priming and array washing. • Ensure that the fluidics stations are maintained according to the guidelines in the *GeneChip™ Fluidics Station 450 User Guide for GCC*.

Thermo Fisher Scientific instruments

Under any of the following conditions, unplug the instrument from the power source and contact Thermo Fisher Scientific Technical Support:

- When the power cord is damaged or frayed.
- If any liquid has penetrated the instrument.
- If the instrument does not perform to specifications after service or calibration.

Ensure that you have the model and serial number available when calling Thermo Fisher Scientific Technical Support.

NIMBUS™ Target Preparation Instrument

If a hardware problem is encountered while running the CytoScan™ Automated Target Preparation Solution on the Applied Biosystems™ NIMBUS™ Target Preparation Instrument, do the following:

- See the *Microlab NIMBUS™ User Guide*, Pub. No. 62965-01
- For information on recovering a run, contact Thermo Fisher Scientific. Gather the following information beforehand:
  - Your Instrument Model Number: 66454-01
  - NIMBUS™ Configuration: Open NIMBUS™ 96 with gripper (9+2) deck
    a. Serial Number
    b. Software version
    c. Trace files:
       Extension: *.trc
       Windows™ 7 Path: `C:\Program Files (x86)\Hamilton Company\LogFiles`
       Windows™ 10 Path: `C:\Program Files (x86)\Hamilton\LogFiles`
    d. ComLinkLogfile:
       Look for the date stamp
       Windows™ 7 Path: `C:\Program Files (x86)\Hamilton Company\Bin\LogFiles`
       Windows™ 10 Path: `C:\Program Files (x86)\Hamilton\Bin\LogFiles`

C

Analyzing sample fragmentation using the Agilent™ 2100 Bioanalyzer™ Instrument

1. Thaw the fragmentation aliquot prepared in Step 10 on page 151 of "Stage 6B—Fragmentation QC" on page 142 at room temperature. Mix sample by vortexing. Use 1 µL per sample as input volume for the Agilent™ 2100 Bioanalyzer™ Instrument.

2. Use the Agilent™ DNA 1000 Kit (Cat. No. 5067-1504).

   See the user guide for instructions on sample preparation and sample analysis on the Agilent™ 2100 Bioanalyzer™ Instrument.

3. Evaluate the DNA fragmentation distribution using the sample elution profile shown in the electropherogram (Figure 36).

4. To evaluate each profile, the 15 bp control peak should be identified and assigned correctly (tallest peak, elution time around 43 seconds).

   Correctly fragmented DNA samples have profiles that stretch out below and above 15 bp. Under- or over-fragmentation is indicated by profiles where the majority of the area under the curve lies either above or below 15 bp, respectively.

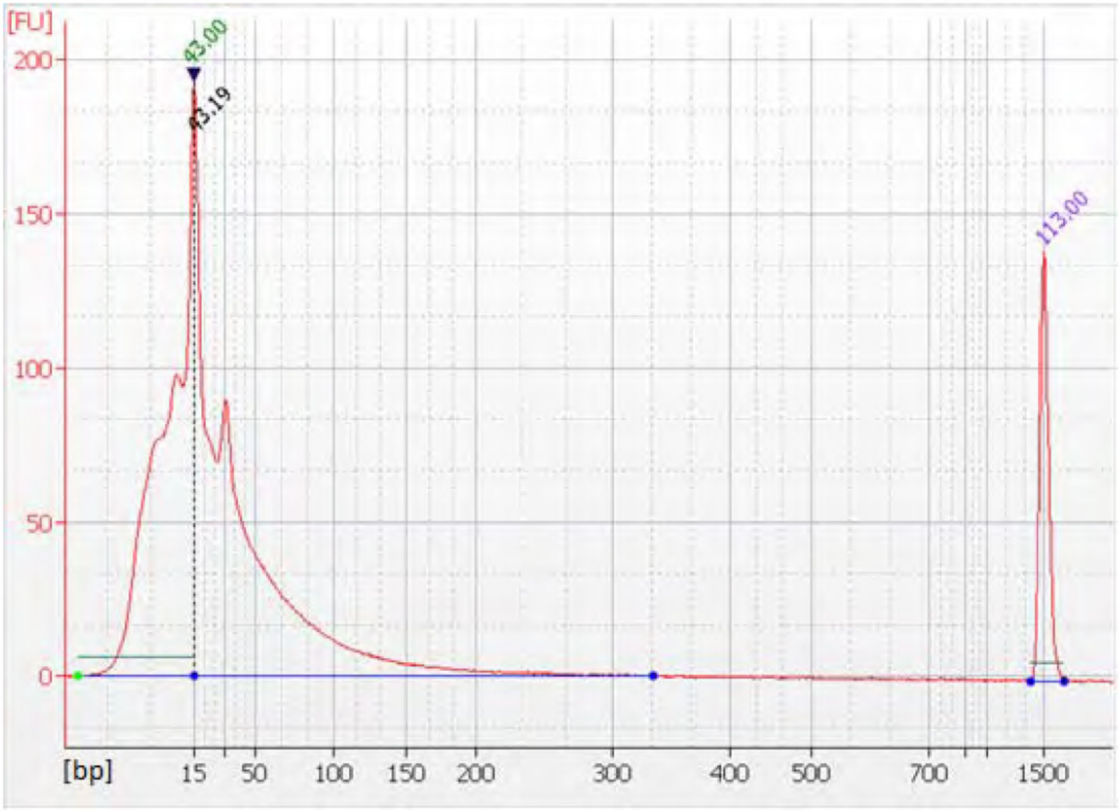


Figure 36 Example of acorrectly fragmented DNA sample.

D Equipment care and maintenance

Routine care for the Applied Biosystems™ NIMBUS™ Target Preparation Instrument

Regular care and cleaning of the NIMBUS™ Target Preparation Instrument is recommended to ensure successful runs, avoid stoppage during a run, and avoid premature damage to the equipment.

O-ring care

The O-rings on the head of the NIMBUS™ Target Preparation Instrument allow the instrument to pick up and manipulate the pipette tips that are loaded on the deck. When the head is left in a squeezed position for an extended amount of time, the O-rings wear out and the head is stressed.

WARNING! Do Not leave tips on the head or leave the head in a squeezed position for extended lengths of time. This practice can lead to premature O-ring wear and poor system performance.

On successfully completing a method, the head is normally parked with the O-rings in a relaxed position. If a method is interrupted or stopped, it is possible that the head can stand still with the O-rings in the squeezed position (Figure 37). For instances when the O-rings remain in the squeezed position, try initializing the instrument.

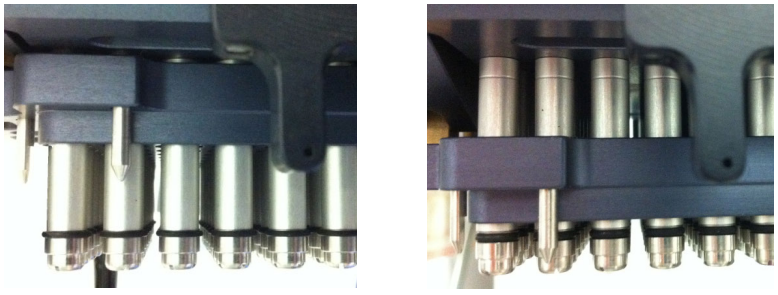


Figure 37 O-rings shown in a squeezed and relaxed state.

① "Squeezed" state.
② O-rings relaxed.

To help preserve the longevity of the O-rings, remember the following:

- Never power off the instrument with any tips that are loaded on the head.
- Never leave the instrument with the head in the squeezed position.
- Initializing the instrument usually resolves the head remaining in the squeezed position leaving the head in a relaxed position.

Tip isolator

The tip isolator is the frame that is fastened in deck position 11. For several steps in the method, a square deepwell plate is placed under the tip isolator frame. During a run, tips that are placed in this position are designated for stamping reagents from one plate to another. During the deck cleanup step of a method, the used tips can accidentally touch against the inner dividing walls of the tip isolator frame. Cross-contamination can occur when new tips are placed in deck position 11 for subsequent methods or runs.

Clean the tip isolator

We recommend cleaning the inner dividing walls of the tip isolator frame after every run.

1. Spray a laboratory tissue with 70% ethanol.
2. Wipe the surfaces of the inner dividing walls of the tip isolator frame.

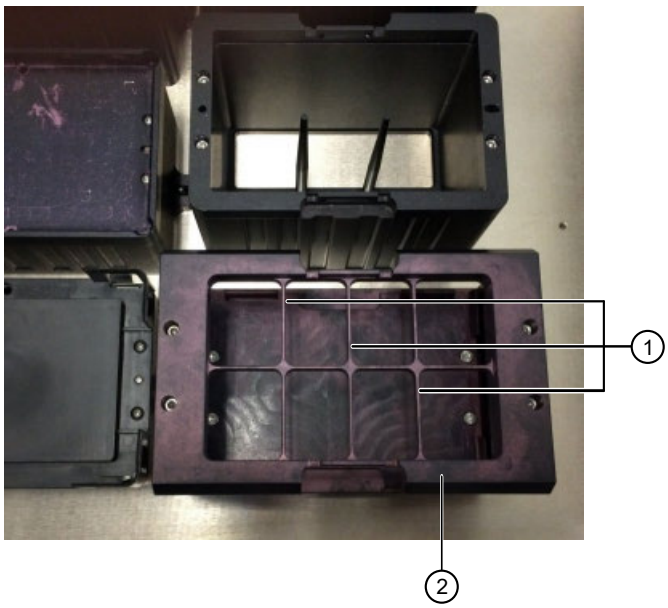


Figure 38 Tip Isolator on NIMBUS™ Target Preparation Instrument deck.

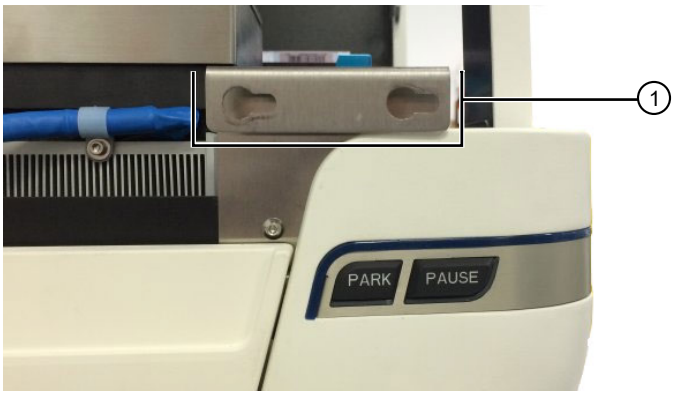
① Inner dividing walls of the Tip Isolator frame.
② Tip Isolator frame in deck position 11.

Trash chute

Assemble the trash chute

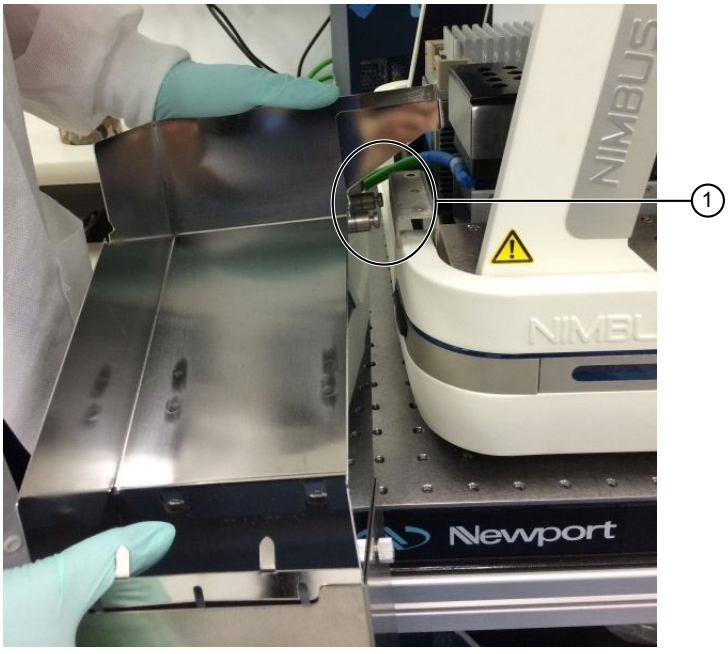
This section provides instructions for the proper assembly of the trash chute and trash chute cover to the NIMBUS™ Instrument. Use these instructions to attach properly or remove the trash chute if needed for cleaning or other purposes. To remove the trash chute, follow these instructions in reverse.

1. Find the trash chute mounting bracket on the left side of the NIMBUS™ Instrument.



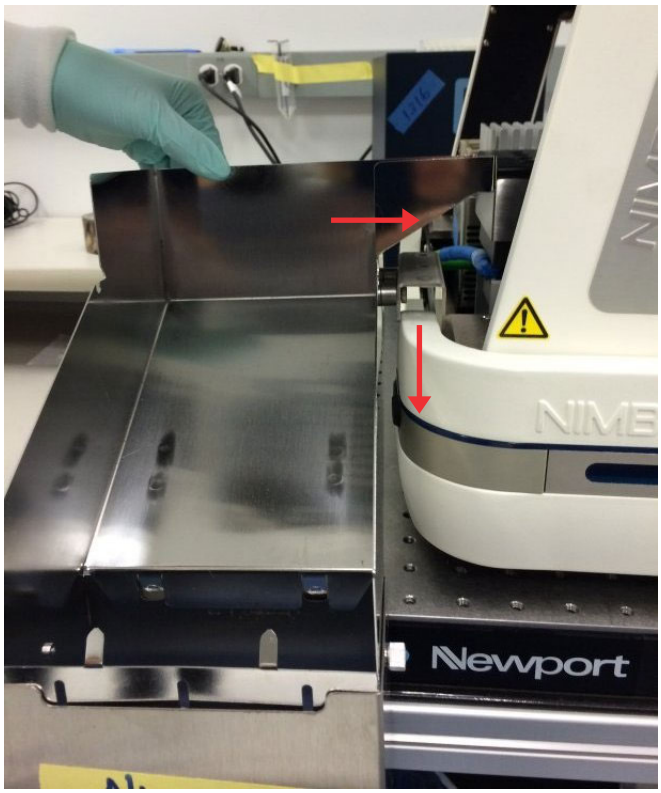
① Trash chute mounting bracket

2. Hold the trash chute with both hands, then align the 2 securing pins on the right side of the trash chute with the 2 holes in the trash chute mounting bracket.

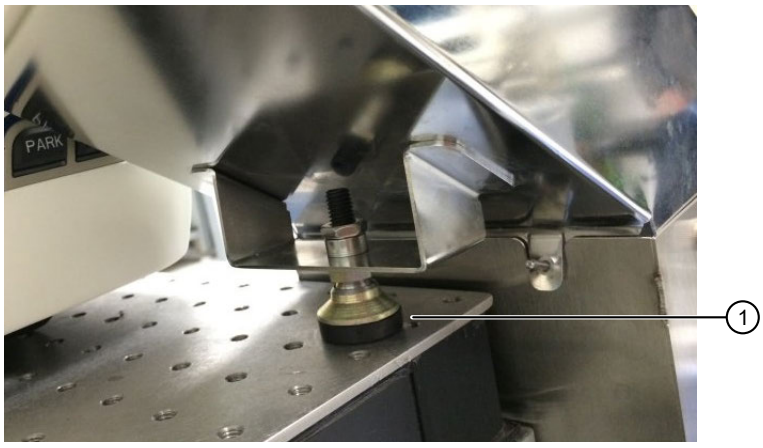


① Align the securing pins with holes in trash chute mounting bracket.

3. Insert the securing pins into the holes in the trash chute mounting bracket, then pull the trash chute forward toward the front of the NIMBUS™ Instrument. This action holds the trash chute in place.



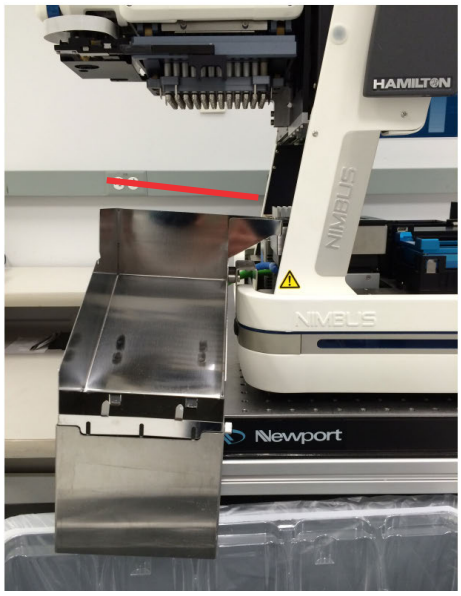
4. After successfully attaching the trash chute to the NIMBUS™ Instrument, turn the leveling foot, on the underside of the trash chute, to support, then level the trash chute.

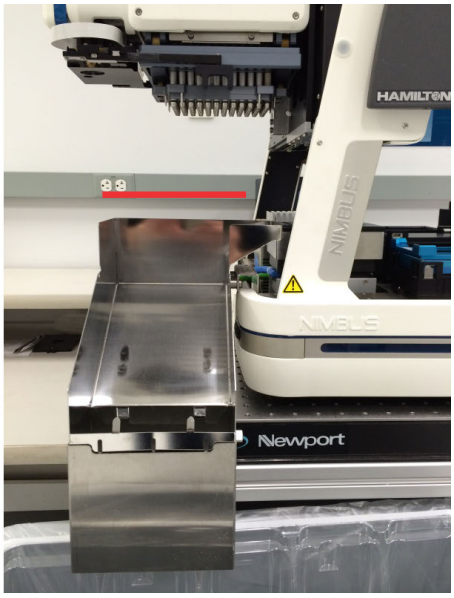


① Leveling foot

5. Ensure that the trash chute is level.

IMPORTANT! Ensure that the trash chute is level. Trash chutes that are not level result in problems with disposal of tips, plates, or other materials from the deck.

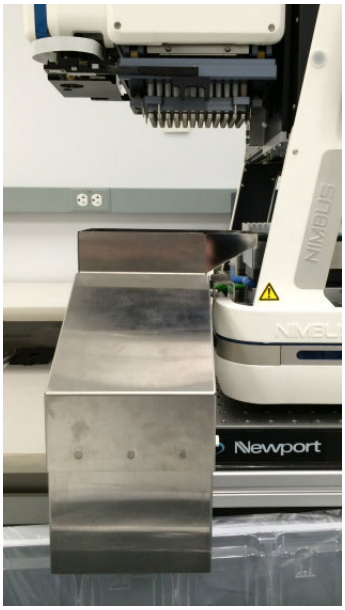




① ②

① Example of a trash chute that is not level.
② Example of a trash chute that is properly attached and leveled.

6. After the trash chute has been installed, then leveled, attach the trash chute cover.



Clean the trash chute

As you run the assay and complete the various methods, the NIMBUS™ Instrument discards all used tips and a 1.2-mL square plate down the trash chute. As this happens, it is common for the trash chute to develop a sticky buildup from the liquids that remained on the disposed materials. A trash chute with heavy buildup can result in disposed plates and tips to get lodged or stuck. It also increases the likelihood for tips to bounce off the chute and onto the deck as they are ejected from the head. When a blockage in the chute occurs, disposed materials can no longer freely fall onto the waste container. Instead, disposed materials build up in the chute and eventually spill over onto the deck or the track of the robotic arm.

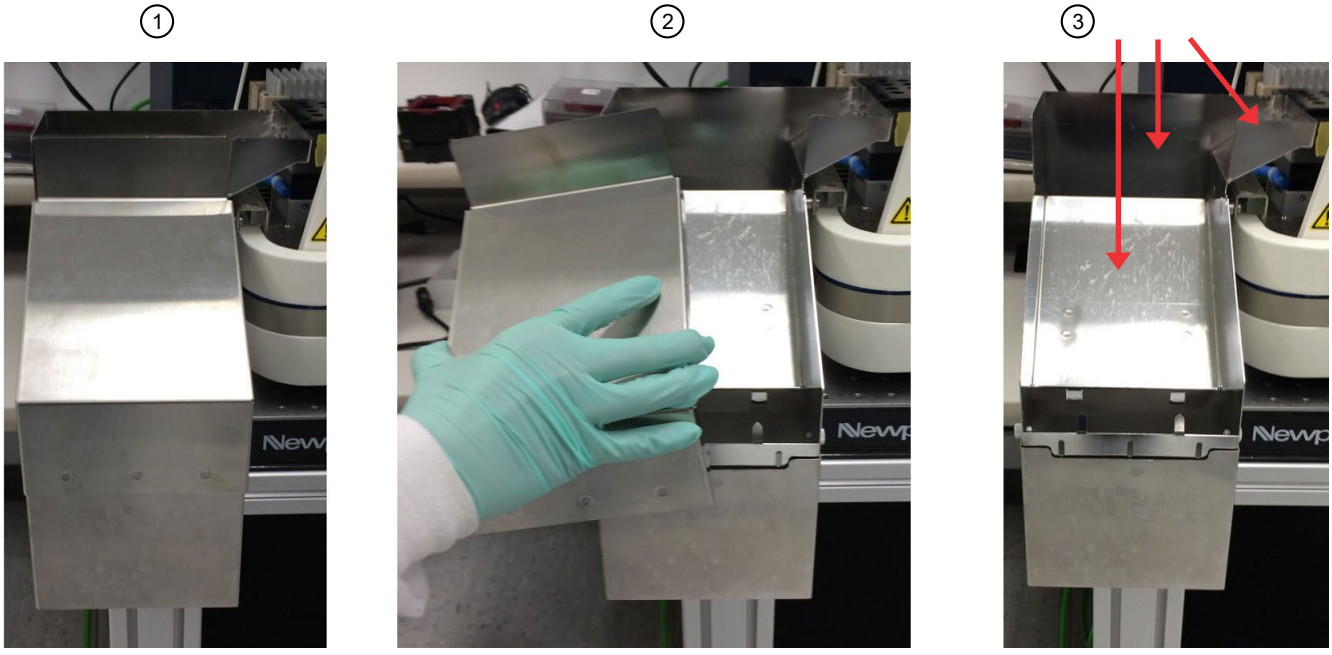
To avoid problems with items getting stuck in the trash chute, we recommend a light cleaning after each stage of the assay. A deep cleaning of the trash chute is recommended periodically to remove accumulated build-up on the trash chute that was not successfully removed with the recommended light cleaning procedure.

Perform a light cleaning

1. Remove the trash chute cover.
2. Clean the inner surface of the trash chute by wiping down the area with ultra-pure water and laboratory wipes. Follow this cleaning with a 70% ethanol wipe-down to disinfect the surface.

   Note: Do not use bleach to clean trash chute or trash chute cover.

3. Replace cover.



① Trash chute with cover.
② Remove cover.
③ Surfaces of the trash chute to clean.

Perform a deep cleaning of the trash chute

1. Remove the trash chute cover.
2. Remove the trash chute from the NIMBUS™ Instrument by first sliding the chute towards the back of the instrument, then pulling it away from the machine. If needed, review detailed instructions. (See “Assemble the trash chute” on page 362.)

   Note: Do not use bleach to clean trash chute or trash chute cover.
3. In a warm bath of soapy DI water, clean the entire trash chute and trash chute cover removing any buildup present.
4. Rinse with DI water. Wipe down with 70% ethanol.
5. Dry the trash chute and trash chute cover.
6. Reconnect the trash chute to the NIMBUS™ Instrument, as instructed in “Assemble the trash chute” on page 362.

Thermoshake device maintenance

The INHECO™ Thermoshake device must be maintained with periodic checking and refilling of cooling fluids to ensure proper function and avoid damages. See the INHECO™ user guide for detailed instructions.

Additional cooling fluid can be ordered from INHECO™ (Cat. No. 3800053).

GeneChip™ Fluidics Station 450 care and maintenance

General fluidics station care

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

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

Fluidics station Bleach protocol

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

Run a bleach cycle

We recommend the use of dedicated bottles for bleach and DI water to avoid carryover, or cross-contamination, from the bleach protocol. For your convenience, we offer the following additional bottles.

Table 65 Recommended bottles.

Description	Source
Media Bottle, SQ, 500 mL	400118
Media Bottle, SQ, 1000 mL	400119

1. Disengage the washblock for each module by pressing down on the cartridge lever. Remove any probe array cartridge.

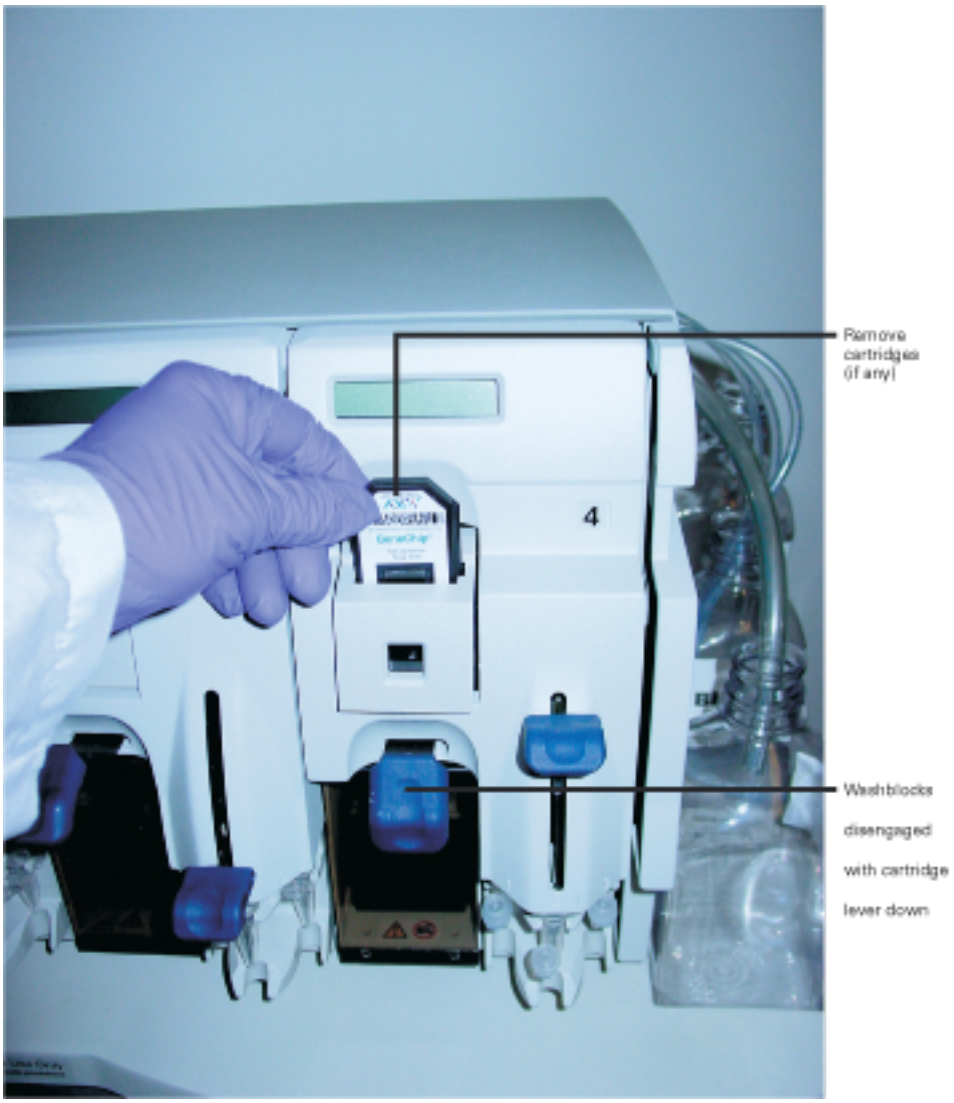


Figure 39 Disengaged washblocks showing cartridge levers in the down position.

2. In a 1-liter plastic or glass graduated cylinder, combine 43.75 mL of commercial bleach (such as a commercial bleach that is 6.15% sodium hypochlorite) with 456.25 mL of DI H_2O, then mix well. The final volume is 500 mL of 0.525% sodium hypochlorite.

IMPORTANT!

- Prepare beach solution fresh at the time of use.
- Each fluidics station with 4 modules requires 500 mL of 0.525% sodium hypochlorite solution.

3. Pour the solution into a 500 mL plastic bottle, then place the plastic bottle on the fluidics station.

4. Perform the bleach cycle.
   a. Place on the fluidics station an empty 1-liter waste bottle, a 500 mL bottle of bleach, and a 1-liter bottle of DI water.

      The bleach protocol requires about 1 liter of DI water.

   b. Insert the waste line into the waste bottle.

   c. Immerse all 3 wash and water lines into the bleach solution.

      IMPORTANT! Do NOT immerse the waste line into the bleach.

5. Open the instrument control software (GCC).

6. In the **Fluidics Station** window, choose the current bleach protocol for each module, then, select the **Check/Uncheck All Stations and Modules** option.

7. Run the protocol for all modules.

   The fluidics station does not start until the needle lever is pressed down. The temperature ramps up to 50°C.

Figure 40 Needle levers in the down position

8. Follow the prompts on each LCD. Load empty 1.5 mL vials onto each module if not already done so.

9. Press down on each of the needle levers to start the bleach protocol (Figure 40).
   The fluidics station starts the protocol, emptying the lines and performing the cleaning cycles using bleach solution. After about 30 minutes, the LCD prompts you when the bleach cycle is over and the rinse cycle is about to start.

Run a rinse cycle

When the bleach cycle has finished, the second part of the protocol is a rinse step. This step is essential to remove all traces of bleach from the system. Failure to complete this step can result in damaged arrays.

1. Follow the prompts on the LCD for each module. Lift up on the needle levers and remove the bleach vials. Load clean, empty vials onto each module.

2. Remove the 3 wash and water lines from the bleach bottle, then transfer them to the DI water bottle.

   Immerse the 3 wash and water lines in the DI water bottle. There is no need to be concerned about the bleach remaining in the lines at this step.

Figure 41 Cleaning the wash and water lines.

3. Press down on the needle levers to start the rinse cycle.

   The fluidics station empties the lines and rinses the needles.

   When the rinse is completed (after about 1 hour), the fluidics station brings the temperature back to 25°C and drains the lines with air. The LCD display reads ***CLEANING DONE***.

4. Discard the vials used for the bleach protocol.

5. After completing the bleach protocol, follow the suggestions for storage of the GeneChip™ Fluidics Station 450..

Table 66 Storage suggestions for the GeneChip™ Fluidics Station 450.

If:	Then do this:
Planning to use the system immediately	After running the bleach protocol, remove the DI water supply used in the rinse phase, then install the appropriate reagents for use in the next staining and washing protocol (including fresh DI water). Perform a prime protocol without loading the probe arrays. **WARNING!** Failure to run a prime protocol can result in irreparable damage to the loaded hybridized probe arrays.
Not planning to use the system immediately	Because the system is already well purged with water, there is no need to run an additional shutdown protocol. Remove the old DI water bottle, then replace it with a fresh bottle.
Not planning to use the system for an extended period of time (longer than one week)	Remove the DI water, then perform a “dry” protocol shutdown. This removes most of the water from the system and prevents unwanted microbial growth in the supply lines. Also, remove the pump tubing from the peristaltic pump rollers.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Symbols on this instrument

In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.

(continued)

Symbol and description
CAUTION! Risk of electrical shock.
CAUTION! Hot surface.
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Location of safety labels

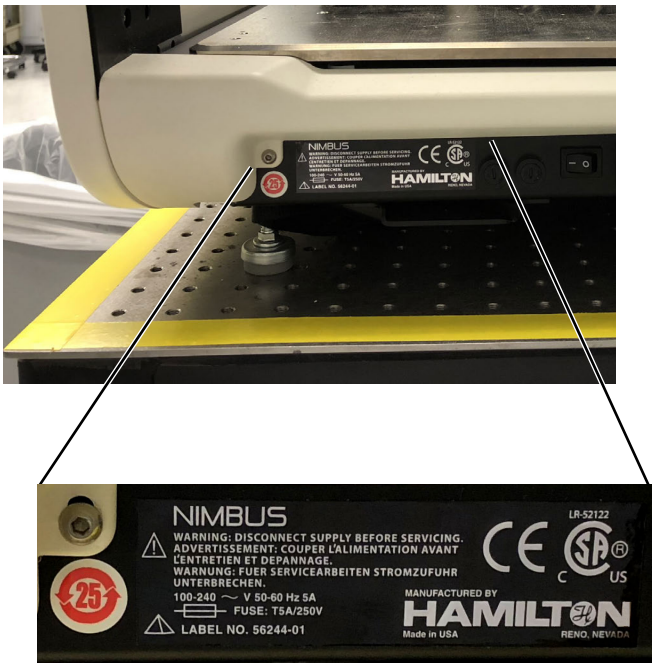
Label and location


Figure 42 Warning label on the right side of the unit to the left of the power switch.

(continued)

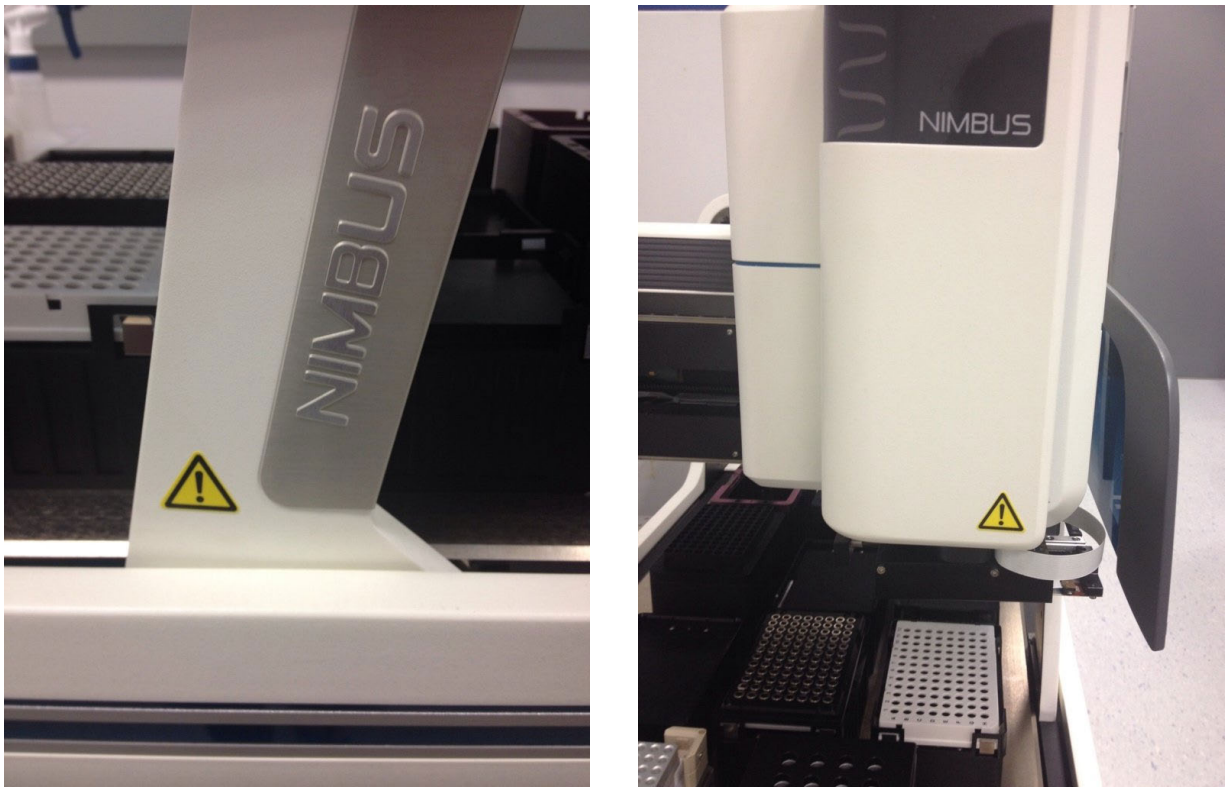
Label and location


Figure 43 Pinch points.

① Gantry arm
② NIMBUS™ Instrument head

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)

(continued)

Symbols and descriptions	
⎓	Direct current
~	Alternating current
≂	Both direct and alternating current

Conformity symbols

Conformity mark	Description
C SP US	Indicates conformity with safety requirements for Canada and U.S.A.
25	Indicates conformity with China RoHS requirements.
CE	Indicates conformity with European Union requirements.
	Indicates conformity with the WEEE Directive 2012/19/EU. ⚠ **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

If covers are removed, do not use the instrument. Contact Technical Support.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Voltage Selector Switch. Before installing the instrument, verify that the voltage selector switch is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures: • Reorient or relocate the receiving antenna. • Increase the separation between the equipment and receiver. • Connect the equipment into an outlet on a circuit different from that to which the receiver is connected. • Consult the dealer or an experienced radio/TV technician for help.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

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

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

Biological hazard safety

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

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020 **www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf**
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs) **www.who.int/publications/i/item/9789240011311**

F

Documentation and support

Related documentation

Document	Publication number	Description
CytoScan™ Assay Automated Workflow on Applied Biosystems™ NIMBUS™ Site Preparation Guide	MAN0025399	Provides guidance on reagents, instruments, and supplies required to run the CytoScan™ Assay Automated Workflow on the Applied Biosystems™ NIMBUS™.
CytoScan™ Assay Automated Workflow on Applied Biosystems™ NIMBUS™—25 Samples Quick Reference	MAN0017988	An abbreviated reference for the target preparation step of the CytoScan™ Assay Automated Workflow on the Applied Biosystems™ NIMBUS™ for 25 samples. This quick reference document is for experienced users.
CytoScan™ Assay Automated Workflow on Applied Biosystems™ NIMBUS™—49 Samples Quick Reference	MAN0017987	An abbreviated reference for the target preparation step of the CytoScan™ Assay Automated Workflow on the Applied Biosystems™ NIMBUS™ for 49 samples. This quick reference document is for experienced users.
Recommended Alternative Microarray Consumables Quick Reference	MAN0019853	A quick reference document identifying recommended alternative replacement consumables for use in microarray assays.
Analysis software		
Chromosome Analysis Suite User Guide	MAN0027798	Provides instructions for using the Chromosome Analysis Suite Software (ChAS) for in-depth CN result exploration. The software enables cytogenetic analysis to view and summarize chromosomal aberrations, including copy number gain or loss, loss of heterozygosity segments, or variant data, across the genome.
Instruments and operating software documents		
MICROLAB™ NIMBUS™ User Guide	Hamilton™ Company, 62965-01	The Hamilton™ Company user guide for the NIMBUS™ Instrument. This document is shipped in the deck hardware kit.

(continued)

Document	Publication number	Description
GeneChip™ Data Collection Software (GCDC) User Guide	MAN0026726	Provides instructions for using GeneChip™ Data Collection Software (GCDC) to control GeneChip™ instrument systems. GCDC provides an intuitive user interface for instrument control in processing GeneChip™ cartridge arrays.
GeneChip™ Command Console™ (GCC) User Guide	MAN0027771	Provides instructions for using GeneChip™ Command Console™ (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.
GeneChip™ System 3000 Instrument Operation and Use	MAN0028330	Provides instructions for running the GeneChip™ System 3000 instrumentation using the GCDC software.
GeneChip™ System 3000 Site Preparation Guide	MAN0029136	Provides the information to prepare your facility and personnel for equipment installation, laboratory set up, training on and use of the GeneChip™ System 3000.
GeneChip™ Fluidics Station 450 User Guide for GCC	08-0295	Provides instructions for using the GeneChip™ Fluidics Station 450 to wash and stain GeneChip™ cartridge arrays.
GeneChip™ Fluidics Station 450 for GCC Quick Reference	08-0294	An abbreviated set of instructions for the experienced user for using the GeneChip™ Fluidics Station 450 to wash and stain GeneChip™ cartridge arrays.
GeneChip™ Scanner 3000 7G with AutoLoader Quick Reference	08-0181	An abbreviated set of instructions for the experienced user for using the GeneChip™ Scanner 3000 7G System with Workstation and AutoLoader to scan next-generation higher-density arrays.
GeneChip™ Scanner 3000 7G Quick Reference	08-0182	An abbreviated set of instructions for the experienced user for using the GeneChip™ Scanner 3000 7G to scan next-generation higher-density arrays.
GeneChip™ Hybridization Oven 645i User Guide	MAN0027670	Provides instructions for using the GeneChip™ Hybridization Oven 645i for hybridization of up to 64 GeneChip™ cartridge arrays at one time.
GeneChip™ Hybridization Oven 645 User's Guide	08-0255	Provides instructions for using the GeneChip™ Hybridization Oven 645 for hybridization of up to 64 GeneChip™ cartridge arrays at one time.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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

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

Limited product warranty

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