

CytoScan™ Assay

SITE PREPARATION GUIDE

Manual Protocol

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Safety


Follow universal precautions in the laboratory. For waste disposal, follow federal, state, local, and within-country regulations.


Text alerts


Text alerts draw your attention to a particular piece of information. There are five types of text alerts: Note, Important, Caution, Warning, and Danger.

Note: Information that may be of interest or of help to a user but is not critical to the primary purpose of the text.

IMPORTANT! Information that is essential to the successful use of a product or the completion of a procedure and is not safety related.

 **CAUTION!** Alerts the user of hazards that, if not avoided, can cause minor or moderate personal bodily injury and/or damage to an instrument or loss of data.

 **WARNING!** Alerts the user to hazards that, if not avoided, can cause serious bodily injury or death, or produce potentially incorrect data that could cause harm to a patient.

 **DANGER!** Alerts the user to an imminent hazard that, if not avoided, will cause serious bodily injury or death, or will produce a result that could cause serious harm to a patient.

Contamination prevention

Proper laboratory practice is necessary because previously amplified PCR product is the most likely potential source of contamination.

- Set up the laboratory areas for a single-direction workflow from Pre-PCR to Post- PCR.
- Use dedicated equipment for each area (e.g., thermal cyclers, microfuges, pipettes and tips, ice buckets, etc.).
- Place all reagents and master stocks in use area. *Do not move equipment between Pre- and Post-PCR Rooms, e.g., ice buckets, pipettes, etc.*
- Place separate copy of assay procedure in Pre- and Post-PCR areas.
- Follow the laboratory standard procedure for re-entry to Pre-PCR Clean area from post PCR Area.
- Use filter tips for all pipetting steps.

Equipment in Pre-PCR Clean Area

- Freezer, -25 to -15°C
- Ice bucket with ice
- Laminar flow cabinet or PCR cabinet
- Microfuge
- Pipettes on stand
- Plate centrifuge
- Refrigerator
- Thermal cycler
- Vortexer

Equipment in Post-PCR Area

- Electrophoresis gel box
- Electrophoresis power supply
- Freezer, -25 to -15°C
- Gel imager
- GeneChip™ System 3000
 - Computer, monitor and keyboard
 - Fluidics station
 - Scanner
- GeneChip™ Hybridization Oven 645
- Ice bucket
- Magnetic stand
- Pipettes on stand
- Spectrophotometer, UV/VIS, single or multichannel
- Vortexer
- Microcentrifuge
- Microfuge
- Refrigerated plate centrifuge
- Refrigerator
- Thermal cycler(s)
- Vortexer with foam tube adaptor

Equipment and calibration

Set up, maintain, and calibrate all equipment according to the manufacturer's instructions.

Reagent handling and storage

- Store all reagents at the indicated temperatures and conditions (see [Chapter 3, Materials required](#))
- Do not store enzymes in a frost-free freezer
- Store reagents used for digestion, ligation, and PCR only in the Pre-PCR Clean Area

Thermal cyclers, 96-well plate, and adhesive seals

Use the thermal cyclers, 96-well plate, and adhesive films listed in [Chapter 3, "Materials required"](#).

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Laboratory Setup

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Before you begin

Read [Chapter 1, Introduction](#).

Configurations

Two setups are given following, one for two separate rooms and one for a single room with Pre- and Post-PCR separation. Always set up for a single-direction workflow.

Two separate rooms

This configuration greatly reduces the risk of sample contamination due to previously-amplified PCR products. These are the:

- Pre-PCR Clean Room
- Post-PCR Room

These rooms are set up to accommodate the steps in [Table 1](#).

Table 1 Assay workflow when two separate rooms are used

Room	Template (Genomic DNA)	PCR Product
Pre-PCR Clean Room Assay steps: – Genomic DNA preparation – Digestion – Ligation – PCR setup only	✓	⊘
Post-PCR Room Assay steps: – PCR thermal cycling and purification – Fragmentation – Labeling – Hybridization – Washing and staining – Scanning	⊘	✓

Pre-PCR Clean Room

The Pre-PCR Clean Room is a low-copy DNA template lab, and must be free of PCR product (amplicons). Setup and major pieces of dedicated equipment required are shown in [Figure 1](#). After entering the Post-PCR Room, do not re-enter the Pre-PCR Clean Room without first showering and changing into freshly laundered clothes.



Equipment Shown

1. Vortexer
2. Microfuge
3. Pipettes on stand
4. Ice bucket
5. Thermal cycler
6. Plate centrifuge
7. Freezer, -25 to -15°C
8. Refrigerator

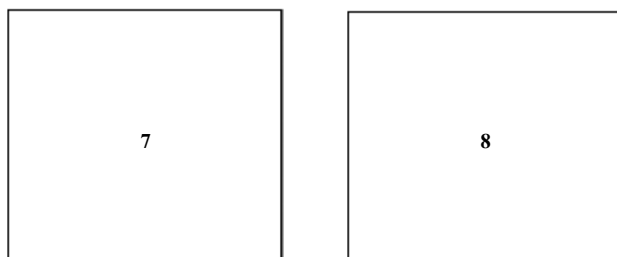
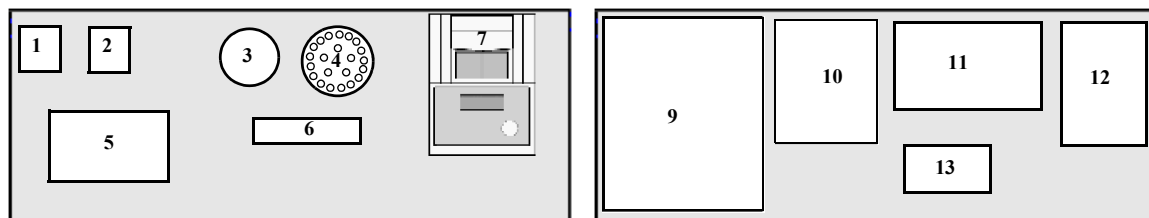


Figure 1 Pre-PCR Clean Room

Post-PCR Room

Setup and major pieces of dedicated equipment required are shown in Figure 2.



Equipment Shown

1. Vortexer
2. Microfuge
3. Pipettes on stand
4. Vortexer (with foam tube adaptor)
5. Ice bucket
6. Magnetic stand
7. Thermal cycler
8. GeneChip™ Hybridization Oven 450
9. Refrigerated plate centrifuge
10. Microcentrifuge
11. Spectrophotometer, UV/VIS, single or multichannel
12. Gel Imager
13. Electrophoresis gel box
14. Computer, monitor, keyboard
15. Fluidics Station
16. Scanner
17. Refrigerator
18. Freezer

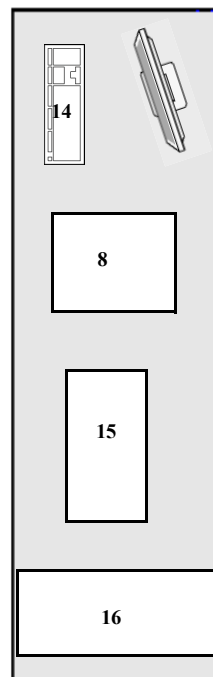
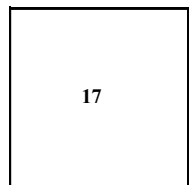
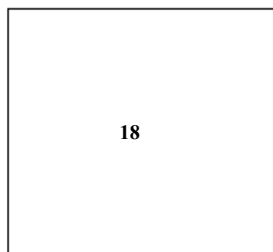


Figure 2 Post-PCR Room

One room with
single-direction
workflow

One room with two distinctly separated areas: *Pre-PCR Clean Area* and *Post-PCR Area*
(see Figure 3).

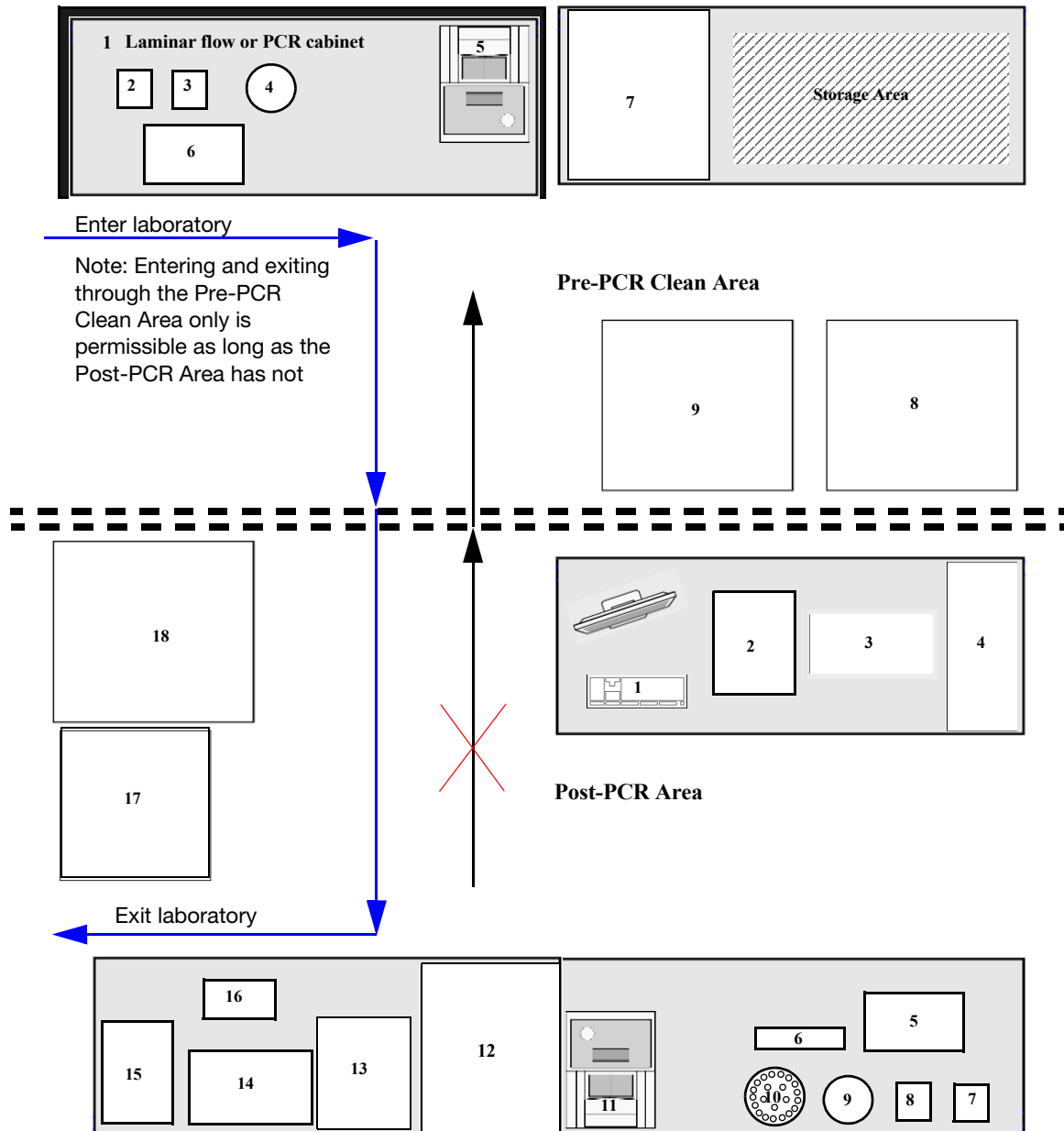


Figure 3 One room configuration

Equipment in Pre-PCR Clean Area

Setup and major pieces of dedicated equipment required are shown in [Figure 3](#).

1. Laminar flow cabinet or PCR cabinet
2. Vortexer
3. Microfuge
4. Pipettes on stand
5. Ice bucket with ice
6. Thermal cycler
7. Plate centrifuge
8. Freezer, -25 to -15°C
9. Refrigerator

Equipment in Post-PCR Area

Setup and major pieces of dedicated equipment required are shown in [Figure 3](#).

1. Vortexer
2. Microfuge
3. Pipettes on stand
4. Vortexer (with foam tube adaptor)
5. Ice bucket
6. Magnetic stand
7. Thermal cycler
8. GeneChip™ Hybridization Oven 450
9. Refrigerated plate centrifuge
10. Microcentrifuge
11. Spectrophotometer, UV/VIS, single or multichannel
12. Gel imager
13. Electrophoresis gel box
14. Computer, monitor, keyboard
15. Fluidics station
16. Scanner
17. Refrigerator
18. Freezer, -25 to -15°C

GeneChip™ System 3000 installation

The system consists of the GeneChip Fluidics Station, scanner, barcode reader, computer, monitor, and keyboard. Install in the Post-PCR Room/Area on a bench that is free of possible vibration.

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Materials required

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From Thermo Fisher Scientific

Equipment and software required

Table 2 Thermo Fisher Scientific equipment and software required

✓	Item	Qty	Cat. No.
Equipment			
<input type="checkbox"/>	GeneChip™ System 3000 – GeneChip™ Scanner 3000 with AutoLoader – GeneChip™ Fluidics Station 450 – Workstation	1	00-0210
<input type="checkbox"/>	GeneChip™ Hybridization Oven 645		00-0331
<input type="checkbox"/>	Tubing, Silicone peristaltic for Fluidics Station 450		400110
Software			
<input type="checkbox"/>	Applied Biosystems™ GeneChip Command Console Software		Version 3.2.2 or higher
<input type="checkbox"/>	Chromosome Analysis Suite (ChAS 2.1 or higher)		901394

Optional equipment

Table 3 Optional Thermo Fisher Scientific equipment

✓	Item	Part Number
<input type="checkbox"/>	GeneChip™ System 3000Dx v.2 (running AMDS v1.1 or higher)	00-0334

CytoScan™ Reagent Kit

Table 4 CytoScan™ Assay Kit–24 Reaction Kit components (Cat. No. 901808)

Cap color	Component	Part No.	Storage
CytoScan™ Module 1: Pre-Lab Restriction and Ligation		904004	
●	Nsp I	901718	–25°C to –15°C
●	Nsp I Buffer	901719	
●	100X BSA	901720	
●	Adaptor, Nsp I	902702	
●	DNA Ligase buffer	901722	
●	DNA Ligase	901723	
○	REF DNA 103 (50 ng/μL)	900421	
○	PCR Primer	902674	
CytoScan™ Module 2: Pre-Lab TE Buffer and Water		904001	
●	Low EDTA TE Buffer	902979	2°C to 8°C
●	Water, Nuclease-Free	902976	
CytoScan™ Module 3: Post-Lab Fragmentation, Labeling and Hybridization		904002	
●	Fragmentation Reagent	902428	–25°C to –15°C
●	Fragmentation Buffer	903001	
●	TdT Enzyme	902675	
●	TdT Buffer	902676	
●	DNA Labeling Reagent	902677	
●	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	2°C to 8°C
●	Stain Buffer 2	901752	
●	Array Holding Buffer	901733	
○	Purification Beads	901807	
○	Water, Nuclease-free	901781	
CytoScan™ Module 5: Post-Lab Elution Buffer and Purification Wash Buffer		903000	15°C to 30°C
○	Elution Buffer	901738	
○	Purification Wash Buffer	901372	
○	Wash A	901680	15°C to 30°C
○	Wash B	901681	

Array and reagent kit bundles

Table 5 Applied Biosystems array and reagent bundles available

Bundle name	Items	Cat. No.
CytoScan™ HD Kit Plus 24	CytoScan HD Arrays+ CytoScan Reagent Kit + CytoScan Amplification Kit, sufficient for 24 reactions	905824
CytoScan HD Kit Plus 96	CytoScan HD Arrays + CytoScan reagent kit + CytoScan Amplification Kit, sufficient for 96 reactions	905896
CytoScan™ 750K Kit Plus 24	CytoScan 750K Arrays+ CytoScan Reagent Kit + CytoScan Amplification Kit, sufficient for 24 reactions	905924
CytoScan 750K Plus 96	CytoScan 750K Arrays+ CytoScan Reagent Kit + CytoScan Amplification Kit, sufficient for 96 reactions	905996
CytoScan™ HD Kit	CytoScan HD Arrays + CytoScan Reagent Kit sufficient for 24 reactions	901835
CytoScan™ 750K Kit	CytoScan 750K Arrays + CytoScan Reagent Kit sufficient for 24 reactions	901859

Other equipment

Table 6 GeneChip™ Hybridization Oven 645

✓	Item	Specifications
<input type="checkbox"/>	Input Voltage	100–120 VAC, 5A maximum or 220–240 VAC, 2.5A maximum 50–60 Hz
<input type="checkbox"/>	Rotisserie Rotation Speed	10–80 RPM, programmable to 1 RPM
<input type="checkbox"/>	Oven Temperature Set Point Programmable Range	30–70°C, programmable to 0.1°C
<input type="checkbox"/>	Time to Temperature	30 minutes from ambient to 60°C
<input type="checkbox"/>	Oven Temperature Accuracy	±1.0°C from 35–60°C
<input type="checkbox"/>	Communications (optional)	Nine-pin RS-232 port, 9600 Baud rate Monitors and reports oven temperature, rotisserie rotation rate, and oven status

From other suppliers

Reagents required but not provided.

Table 7 Reagents from other suppliers, required

✓	Item
<input type="checkbox"/>	Bleach, Sodium Hypochlorite prepared from a concentrate solution without additives at final working concentration of 0.615% (v/v)
<input type="checkbox"/>	PCR gel DNA ladder: 50 to 2000 bp
<input type="checkbox"/>	Fragmentation gel DNA ladder: 25 to 2600 bp
<input type="checkbox"/>	Ethanol, absolute
<input type="checkbox"/>	Loading buffer solution
<input type="checkbox"/>	TBE buffer 5X

Consumables required but not provided

Table 8 Consumables from other suppliers, required

✓	Item
<input type="checkbox"/>	Adhesive films, clear, PCR-certified, 96-well plates
<input type="checkbox"/>	Adhesive label dot, 1/2-inch and 3/8-inch
<input type="checkbox"/>	Agarose gel, 2%
<input type="checkbox"/>	Agarose gel, 4%
<input type="checkbox"/>	Microcentrifuge tubes, nuclease-free, sterile, 1.5 mL polypropylene
<input type="checkbox"/>	Microcentrifuge tubes, nuclease-free, sterile, 2.0 mL polypropylene
<input type="checkbox"/>	Microcentrifuge tubes, nuclease-free, sterile, non-stick, 1.5 mL amber polypropylene
<input type="checkbox"/>	Microcentrifuge tubes, nuclease-free, sterile, non-stick, 1.5 mL blue polypropylene
<input type="checkbox"/>	Microcentrifuge tubes, nuclease-free, sterile, non-stick, 1.5 mL natural polypropylene
<input type="checkbox"/>	Microcentrifuge tubes, nuclease-free, sterile, non-stick, 50 mL polypropylene microcentrifuge tubes
<input type="checkbox"/>	Pipette tips with aerosol barriers, 20 μ L, 200 μ L, and 1000 μ L
<input type="checkbox"/>	Plate, OD for UV, 96-well (required only if using microplate spectrophotometer)
<input type="checkbox"/>	Plates, unskirted PCR with a maximum volume of 330 μ L
<input type="checkbox"/>	Reagent reservoir, 25 mL
<input type="checkbox"/>	Tube strips, nuclease-free, sterile, 8-well, 0.2 mL polypropylene

Pre-PCR Clean Area equipment required but not provided

Table 9 Pre-PCR Clean Room equipment, required

✓	Item
<input type="checkbox"/>	If assay is to be performed in one room: <ul style="list-style-type: none"> • Laminar Flow Cabinet, 6 foot or <ul style="list-style-type: none"> • PCR Cabinet
<input type="checkbox"/>	Benchtop Cooler, with the capacity to hold 8 to 32 tubes (1.5 mL) and ability to maintain temperature below -15°C for 2 hours.
<input type="checkbox"/>	Centrifuge, plate, multipurpose, 330 μL capacity
<input type="checkbox"/>	Cooling chamber, double-block, with the capacity to hold 96 well plates with a maximum volume capacity of 330 μL
<input type="checkbox"/>	Freezer, -25 to -15°C ; deep freeze; manual defrost; 17 cu ft
<input type="checkbox"/>	Microfuge (for tubes and strip tubes)
<input type="checkbox"/>	Pipettors: <ul style="list-style-type: none"> • 12-channel, 2–20 μL • 12-channel, 20–200 μL • single-channel, 100–1000 μL • single-channel, 2–20 μL • single-channel, 20–200 μL
<input type="checkbox"/>	Rectangular Ice Tray Large – 9L (16 x 13 in, 41 x 33cm)
<input type="checkbox"/>	Storage Racks, Tube, 96-well
<input type="checkbox"/>	Thermal cycler: capable of holding 200 μL volume and 96-well plate; heat block capable of holding temperature of 4–99.9 $^{\circ}\text{C}$; temperature accuracy of $\pm 0.25^{\circ}\text{C}$ (at 35–99.9 $^{\circ}\text{C}$); average heating and cooling rate of 2.6 $^{\circ}\text{C}$ per second; thermal uniformity of $\pm 0.5^{\circ}\text{C}$.
<input type="checkbox"/>	Vortexer, 60 Hz, 75 W, 600–3200 RPM

Post-PCR Room equipment required but not provided

Table 10 Post-PCR Room equipment required

✓	Item
<input type="checkbox"/>	Adhesive film applicator (hard plastic)
<input type="checkbox"/>	Anti-vibration pad, used with vortexer to prevent movement during operation
<input type="checkbox"/>	Cooler, benchtop, with capacity to hold 8 to 32 tubes (1.5 mL) and maintain temperature below -15°C for 2 hours
<input type="checkbox"/>	Cooling chamber, double-block, with capacity to hold 96-well plates with a maximum volume capacity of 330 μL
<input type="checkbox"/>	Electrophoresis supplies
<input type="checkbox"/>	Freezer, -25 to -15°C ; deep freeze; manual defrost; 17 cu ft
<input type="checkbox"/>	Gel imager
<input type="checkbox"/>	Magnetic rack with magnet on the side and capable of holding 8–12 tubes of 1.5–2-mL capacity
<input type="checkbox"/>	Microcentrifuge, non-refrigerated with capacity to hold 24 tubes and maximum rotation speed of 16,200 xg
<input type="checkbox"/>	Microfuge (for tubes and strip tubes)
<input type="checkbox"/>	Microtube foam insert
<input type="checkbox"/>	Pipettors: <ul style="list-style-type: none"> • 12-channel, 100–1200 μL • 12-channel, 2–20 μL • 12-channel, 20–200 μL • single-channel, 100–1000 μL • single-channel, 2–20 μL • single-channel, 20–200 μL
<input type="checkbox"/>	Plate centrifuge, refrigerated multipurpose, plate carriers for 4 x 96-well assay plates
<input type="checkbox"/>	Platform Head, 6-inch, for microtube foam insert
<input type="checkbox"/>	Rectangular ice tray, large - 9L (16 x 13 in; 41 x 33 cm)
<input type="checkbox"/>	Refrigerator, 2 – 8°C , 6 cu ft
<input type="checkbox"/>	Spectrophotometer, UV/VIS, single or multichannel
<input type="checkbox"/>	Storage racks, tube, 96-well
<input type="checkbox"/>	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 $\pm 0.25^{\circ}\text{C}$ (at 35 – 99.9°C); average heating and cooling rate of 2.6°C per second; thermal uniformity of $\pm 0.5^{\circ}\text{C}$.
<input type="checkbox"/>	Vortexer, 60 Hz, 75 W, 600–3200 RPM

Thermal cycler programs

Before you begin processing samples, enter and save these programs into the appropriate thermal cycler(s).

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 programs listed in [Table 11](#) and [Table 12](#) are used in this protocol. Enter and store these programs on the appropriate thermal cycler in the Pre-PCR Clean Area and the Post-PCR Area.

Pre- and Post-PCR programs

Table 11 Pre-PCR clean room

Number of thermal cyclers required	Program name
1	CytoScan Digest
	CytoScan Ligate

Table 12 Post-PCR room

Number of thermal cyclers required	Program name
1	CytoScan PCR Assay
	CytoScan Assay Fragment
	CytoScan Assay Label
	CytoScan Assay Hyb

Program your thermal cyclers

CytoScan Digest

Table 13 CytoScan Digest thermal cycler program

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

CytoScan Ligase

Table 14 CytoScan Ligase thermal cycler program

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

CytoScan PCR

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

- Ramp speed: Max
- Volume: 100 µL

Table 15 CytoScan PCR thermal cycler program

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 (for up to 24 hrs)	

CytoScan Fragment

Table 16 CytoScan Fragment thermal cycler program

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

CytoScan Label

Table 17 CytoScan Label thermal cycler program

Temperature	Time
37°C	4 hr
95°C	15 minutes
4°C	∞

CytoScan Hyb

Table 18 CytoScan Hyb thermal cycler program

Temperature	Time
95°C	10 minutes
49°C	∞



Consumables required from other suppliers

Table 19 Consumables required from other suppliers

✓	Item	Vendor	Cat. No.
<input type="checkbox"/>	MicroAmp Clear Adhesive Film for 96-well plates	Applied Biosystems	4306311
<input type="checkbox"/>	Pipette tips, 20 µL filter tips	Rainin	GP-L10F
<input type="checkbox"/>	Pipette tips, 200 µL filter tips	Rainin	GP-L200F
<input type="checkbox"/>	Pipette tips, 1000 µL filter tips	Rainin	GP-L1000F
<input type="checkbox"/>	Plates, 96-well unskirted PCR	Bio-Rad	MLP-9601
<input type="checkbox"/>	Plate, OD for UV spec, 96-well (required only if using microplate spectrophotometer)	E & K Scientific	EK-25801
<input type="checkbox"/>	Reagent Reservoir, 25 mL	Diversified Biotech	RESE-3000
<input type="checkbox"/>	TBE Gel, 4%, BMA Reliant precast	Lonza Group LTD	54929
<input type="checkbox"/>	TBE Gel, 2%, BMA Reliant precast	Lonza Group LTD	54939
<input type="checkbox"/>	TBE for electrophoresis	Any vendor or house made	
<input type="checkbox"/>	TrackIt™ 25 bp DNA Ladder	Life Technologies	10488-022
<input type="checkbox"/>	Tough-Spots, 1/2"	Diversified Biotech	Spot 2200
<input type="checkbox"/>	Tough-Spots, 3/8"	USA Scientific	9185-0000
<input type="checkbox"/>	Tube, Safe-Lock Tube 1.5 mL, Amber	Eppendorf	022363221
<input type="checkbox"/>	Tube, Safe-Lock Tube 1.5 mL, Blue	Eppendorf	022363247
<input type="checkbox"/>	Tube, Safe-Lock Tube 1.5 mL, Natural	Eppendorf	022363204
<input type="checkbox"/>	Tube, centrifuge 50 mL	VWR	93000-036
<input type="checkbox"/>	Tube, centrifuge 15 mL	VWR	21008-103
<input type="checkbox"/>	Tube strips, 8-well, 0.2 mL	VWR	20170-004

Symbols

Symbol/ Label	Statement/Meaning
REF	Part/Catalog Number
LOT	Lot Number

Symbol/ Label	Statement/Meaning
	Expiration Date YYYY-MM Kit will expire on the last day of the month.
	Temperature Limitation

For support visit [thermofisher.com/support](https://www.thermofisher.com/support) or email techsupport@lifetech.com
[thermofisher.com](https://www.thermofisher.com)

27 January 2023

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