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OncoScan[™] CNV Plus Assay SITE PREPARATION GUIDE

for use with:

OncoScan™ CNV Plus Reagent Kit for Research OncoScan™ CNV Plus Array OncoScan™ CNV Plus Assay for Research

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OncoScan™ CNV Plus Reagent Kit for Research



Products manufactured at this site:

OncoScan™ CNV Plus Array

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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B00	2 April 2025	 Updated the reagent kit name from OncoScan™ CNV Plus Reagent Kit to OncoScan™ CNV Plus Reagent Kit for Research.
		 Updated the assay kit name from OncoScan™ CNV Plus Assay Kit to OncoScan™ CNV Plus Assay for Research.
		 Added the recommendation to use a compression pad that is compatible with the thermal cycler throughout the assay.
A.0	5 July 2023	Initial release in Thermo Fisher Scientific document control system. Supersedes legacy Affymetrix publication number 703175. Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty. Rewrite and restructure of document for clarity.

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OncoScan™ CNV Plus Assay

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Introduction to the assay

Obtaining genome wide copy number and loss of heterozygosity profiles from solid tumor samples is a significant challenge due to the difficulty of working with limited amounts of highly modified and degraded DNA derived from heterogeneous formalin-fixed, paraffin-embedded (FFPE) tumor samples. Traditional FFPE sample analysis techniques such as fluorescent *in situ* hybridization (FISH) are limited to locus specific, low-resolution copy number information. Next Generation sequencing approaches require target preparation methods that bias copy number determination. Deep coverage is also required to provide accurate copy number information from heterogeneous FFPE samples which may not be a practical option for most researchers.

The Applied Biosystems™ OncoScan™ CNV Plus Assay utilizes the Molecular Inversion Probe (MIP) assay technology. This technology performs well with highly degraded DNA, such as that derived from FFPE tumor samples, and with low amounts of DNA starting material. The assay can be performed in as little as 48 hours, which makes the assay a powerful solution for cancer research.

The OncoScan™ CNV Plus Assay can be run on existing Applied Biosystems™ microarray instruments. It provides whole-genome coverage with enriched SNP and CN content for over 900 cancer-related genes, plus the ability to detect frequently tested somatic mutations. The assay is capable of accurately identifying CN changes and allelic imbalances, including loss of heterozygosity (LOH), copy-neutral LOH (cnLOH), and chromothripsis across the entire genome. The OncoScan™ CNV Plus Assay is a complete and robust solution for analysis of FFPE solid tumor samples.

Assay workflow

This section provides an overview of the OncoScan™ CNV Plus Assay workflow. The assay typically takes 48 hours over 3 days to complete. It is performed in 2 areas, a Pre-PCR Room and a Post-PCR Room.

Day 1, Pre-PCR Room (PM)

Activities

The DNA sample plate is prepared. The assay begins with overnight annealing of the MIP probes.

- Preparation of non-amplified genomic DNA.
- · Annealing of the MIP probes to the DNA samples overnight.

Total time

2-3 hours

Overnight incubation time

16-18 hours

Day 2, Pre-PCR Room (AM)

Activities

The annealed DNA samples are processed through the 1st PCR reaction.

- Addition of Gap Fill Master Mix to the overnight annealed DNA sample.
- Divide each sample reaction into 2 wells on different rows. One well is the AT channel, the other is the GC channel (Channel Split).
- Gap Fill the annealed probe with AT or GC dNTP mixes.
- Exonuclease reaction to remove the unligated (non-gap filled) linear MIP probes.
- Cleavage enzyme reaction to linearize the gap-filled circular MIP probes.
- 1st PCR reaction to amplify the gap filled linearized MIP probes.

Total time

~2.5-3 hours

Day 2, Post-PCR Room (PM)

Activities

The amplified MIP products from the 1st PCR reaction are processed through the 2nd PCR reaction, HaelII digestion, and array hybridization. The arrays are hybridized overnight.

- 2nd PCR reaction to enrich the MIP products.
- HaellI digestion of the 2nd PCR product.
- QC Gels are run to determine the size distribution of the 1st PCR reaction, and the HaellI digested products.
- Preparation of hybridization cocktail with the HaellI digested product.
- Hybridization of samples onto the arrays.

Total time

~3.5-4.5 hours

Overnight hybridization time

16-18 hours

Day 3, Post-PCR Room (AM)

Activities

The hybridized arrays are processed to generate data for analysis.

- Array wash and stain in the GeneChip[™] Fluidics Station 450.
- Array Scan in the GeneChip[™] Scanner 3000 7G.

Total time

1.5 hours on the GeneChip™ Fluidics Station 450 and 7 minutes per array in the GeneChip™ Scanner 3000 7G (2 arrays per sample).

Day 3, Post-PCR Room (PM)

The CEL files are available for data analysis and interpretation using the Applied Biosystems™ Chromosome Analysis Suite (ChAS) software.

Optional assay stopping points

- The **DNA Sample Plate** can be frozen if the assay cannot be started.
- The 1st PCR Plate can be frozen if the assay cannot be continued.
- HAE Plate can be frozen at the end of the HaellI reaction if the assay cannot be continued. (Do not
 add hybridization cocktail to the HAE Plate.)

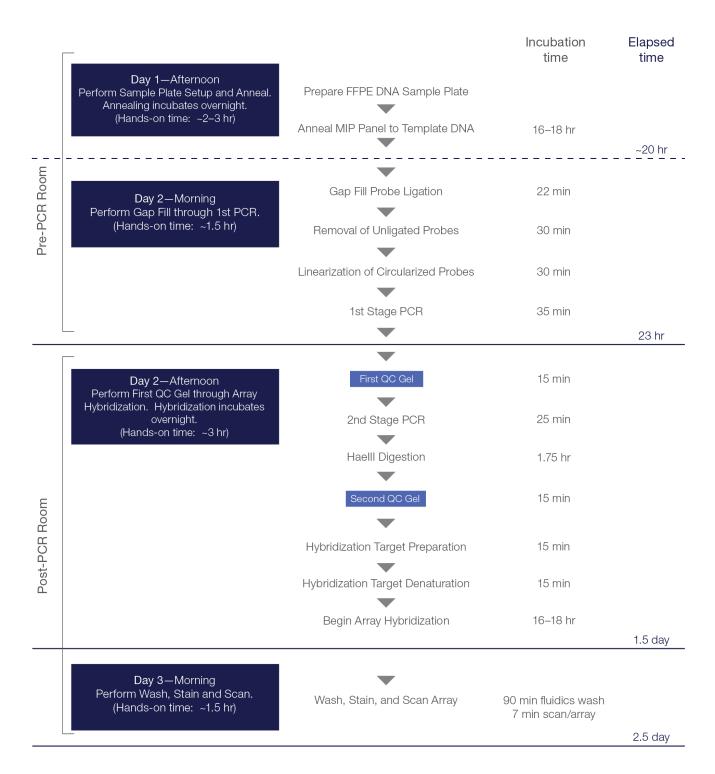


Figure 1 OncoScan™ CNV Plus Assay workflow.

Sample run configuration

This user guide provides workflows to process:

- 7-sample protocol: 5 test samples, 1 positive control, 1 negative control
- 9-sample protocol: 7 test samples, 1 positive control, 1 negative control
- 13-sample protocol: 11 test samples, 1 positive control, 1 negative control
- 25-sample protocol: 23 test samples, 1 positive control, 1 negative control

Note: The negative control is processed through the assay and used for the 2 in-process QC gels. It is not hybridized onto arrays.

Table 1 Detail of the samples and arrays required based on the number of reactions processed per run.

Total number of reactions per run	Number of test samples per run	Positive control sample per run	Negative control per run	Number of runs per assay kit	Total number of samples hybridized per run (excludes negative control)	Number of arrays required per run	Total number of arrays needed for all runs
7	5	1	1	4	6	12	48
9	7	1	1	3	8	16	48
13	11	1	1	2	12	24	48
25	23	1	1	1	24	48	48



Laboratory requirements and recommendations

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This section describes requirements and recommendations for facilities and equipment needed to perform the OncoScan™ CNV Plus Assay protocol. It is essential to set up the lab to prevent contamination, use equipment that meets specifications, purchase the proper consumables, and use the required reagents. Deviating from this user guide is not recommended as results can be suboptimal.

Proper laboratory setup is necessary to prevent previously amplified product from being a source of contamination. It is strongly recommended to set up 2 separate work areas: a Pre-PCR Room and a Post-PCR Room. The first half of the assay is performed in the Pre-PCR Room (Anneal through 1st PCR) and the second half will take place in the Post-PCR Room (2nd PCR through Array scanning). It is essential to perform assay steps in the appropriate work area and maintain a single direction assay workflow.

Before starting the assay protocol, review the required equipment, consumables, and reagents. See Chapter 4, "Equipment, consumables, and reagents required".

Laboratory setup and contamination prevention

The OncoScan™ CNV Plus Assay requires samples to be amplified using PCR. Proper laboratory setup and precautions are necessary to prevent previously amplified PCR product from being a source of contamination.

- Set up the laboratory areas for a single-direction workflow from Pre-PCR to Post-PCR.
- The Pre-PCR Room should be a low copy DNA template area, and should be free of PCR product (amplicons). No amplified product may be taken into the Pre-PCR Room.
- Do not open the seal of the 1st PCR reaction plate in the Pre-PCR Room.
- The Post-PCR Room has airborne contamination with the PCR amplified MIP-annealed template. It is recommended to not enter the Post-PCR Room while performing the Pre-PCR steps of the assay. After entering the Post-PCR Room, do not re-enter the Pre-PCR Room.
- Keep dedicated equipment (e.g., thermal cyclers, microfuges, pipettes and tips, ice buckets, etc.) in each room or area used for this assay.

- 2
- To avoid contamination, do not move equipment between the Pre-PCR Room and the Post-PCR Room.
- Use proper gowning procedures.
- Change gloves frequently throughout the assay and when instructed.
- Store reagents in the appropriate conditions as detailed on the packaging and in this user guide.
- Place separate copies of the assay procedure in the Pre-PCR Room and Post-PCR Room.
- Use nuclease-free pipette tips with aerosol barriers for all pipetting steps.

Equipment and calibration

Laboratory instrumentation plays an important role in the successful execution of this assay. It is critical to use equipment that conforms to the guidelines and specifications detailed in this user guide. To help maintain consistency across assay runs, all equipment must be well maintained and routinely calibrated per manufacturer recommendations.

- Only use calibrated thermal cyclers that meet the specifications outlined in this user guide. It
 is recommended to service the thermal cyclers at least once per year to help ensure they are
 operating within the manufacturer's specifications.
- Ensure that the single- and multichannel pipettes are calibrated and accurate.
- The GeneChip™ System 3000 should be serviced at least once a year to help ensure that the
 components are operating within specifications. The system includes the GeneChip™ Scanner
 3000 with the GeneChip™ AutoLoader, the GeneChip™ Fluidics Station 450, and the GeneChip™
 Hybridization Oven 645.
- Oven temperature is critical to the performance of the assay. Only use the GeneChip™ Hybridization Oven 645.

Reagent handling and storage

Proper storage and handling of reagents is essential to assay performance.

- Store all reagents at the recommended temperatures and conditions. Do not use reagents that have been improperly stored. Storage methods can profoundly impact activity.
- Keep enzymes at -25°C to -15°C until needed. Do not store enzymes at -80°C.
- Do not store enzymes in a frost-free freezer.
- Only store the reagents used for Anneal, Gap Fill, and 1st PCR in the Pre-PCR Room.

Sample quantification

The success of the OncoScan™ CNV Plus Assay is dependent on accurate quantitation of the input genomic DNA. It is mandatory to determine the sample concentration using a dsDNA specific quantification method. The Quant-iT™ PicoGreen™ Assay and the Qubit™ dsDNA Quantification Assay are 2 methods that have been verified for use in the OncoScan™ CNV Plus Assay. Other dsDNA quantitation kits that are commercially available may deliver different results.

IMPORTANT! Sample concentration determined by UV absorbance or NanoDrop™ Spectrophotometer *must not be used* in this assay.

The equipment and reagents required to quantitate the samples are not detailed in this site preparation guide.

GeneChip™ System 3000 installation

The Applied Biosystems™ GeneChip™ System 3000 and GeneChip™ Hybridization Oven 645 consists of the GeneChip™ Scanner 3000 7G System with Workstation and AutoLoader, barcode reader, computer, monitor, keyboard and the GeneChip™ Hybridization Oven 645.

Have your Field Service Engineer install the equipment in the Post-PCR Room on a bench that is free of possible vibration.



Laboratory setup

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Before you begin

Read Chapter 2, "Laboratory requirements and recommendations".

Equipment required

Equipment required in the Pre-PCR Room

- Ice bucket
- Cooling blocks (Quantity = 4)
- Vortexer
- Microfuge
- · Pipettes on stand
- Thermal cycler
- Plate centrifuge
- Refrigerator
- Freezer, -25°C to -15°C
- dsDNA specific quantitation device (required but not shown)
- Laminar flow cabinet or PCR cabinet (recommended)

Equipment required in the Post-PCR Room

- Ice bucket
- Cooling blocks (Quantity=3)
- Vortexer
- Microfuge
- · Pipettes on stand
- · Thermal cycler
- Plate centrifuge

- Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System
- Refrigerator
- Freezer, -25°C to -15°C
- GeneChip™ System 3000
 - Computer, monitor, and keyboard
 - GeneChip™ Scanner 3000 7G
 - GeneChip™ Fluidics Station 450 (Quantity = 2 or more)
 - GeneChip™ Hybridization Oven 645

Lab configurations

Two setup options are provided; one for 2 separate rooms and 1 for a single room with Pre- and Post-PCR separation. Both options require a single-direction workflow to be established and followed.

Two separate rooms, single-direction workflow

The 2 separate room configuration option greatly reduces the risk of sample contamination due to previously-amplified PCR products. These rooms are called the:

- Pre-PCR Room
- Post-PCR Room

These 2 rooms are set up to perform specific assay steps. See Table 2.

Table 2 Assay workflow when 2 separate rooms are used.

Room	Assay steps	Template (genomic DNA)	PCR product
Pre-PCR Room	 Genomic DNA preparation dsDNA quantitation and normalization Anneal 1st PCR 		®
Post-PCR Room	 First QC Gel 2nd PCR HaellI Digest Second QC Gel Hybridization Washing and staining Scanning 	8	

Pre-PCR Room configuration

The Pre-PCR 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. (Figure 2.) After entering the Post-PCR Room, do not re-enter the Pre-PCR Room.

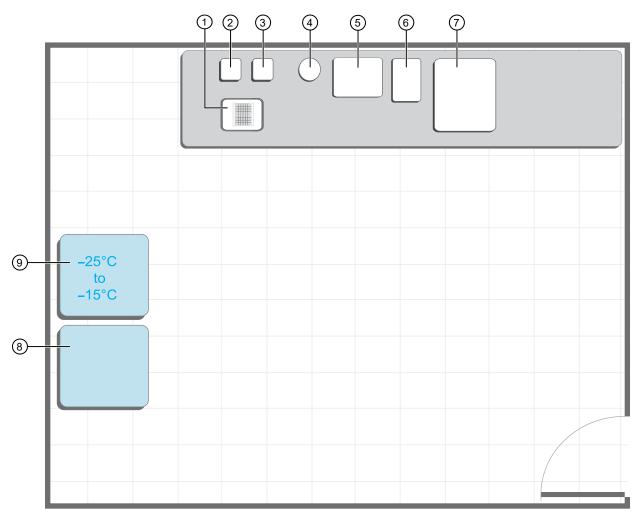


Figure 2 Pre-PCR Room configuration.

- 1 Ice bucket with cooling blocks (Quantity = 4)
- (2) Vortexer
- 3 Microfuge
- 4 Pipettes on stand
- 5 dsDNA specific quantitation device

- 6 Thermal cycler
- (7) Plate centrifuge
- 8 Refrigerator
- (9) Freezer, -25°C to -15°C

Post-PCR Room configuration

The Post-PCR Room is a high-copy DNA template lab. It contains PCR product (amplicons) created during the PCR amplification.

Setup and major pieces of dedicated equipment required are shown. (Figure 3.) After entering the Post-PCR Room, do not re-enter the Pre-PCR Room.

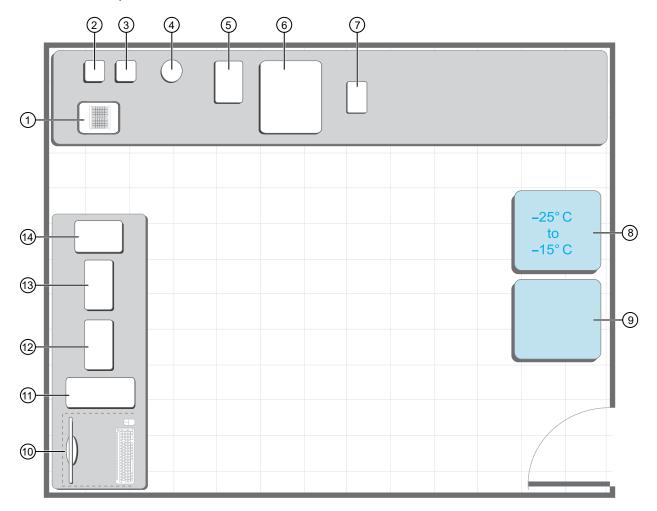


Figure 3 Post-PCR Room configuration.

- 1 Ice bucket with cooling blocks (Quantity = 3)
- (2) Vortexer
- 3 Microfuge
- 4 Pipettes on stand
- (5) Thermal cycler
- 6 Plate centrifuge
- (7) Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System
- 8 Freezer, -25°C to -15°C
- 9 Refrigerator
- 10 Computer, monitor, keyboard
- 1 GeneChip™ Scanner 3000 7G
- (12) GeneChip™ Fluidics Station 450
- (13) GeneChip™ Fluidics Station 450
- (14) GeneChip™ Hybridization Oven 645

One room, single-direction workflow

A 1-room configuration can be used, but care is required to prevent sample contamination from previously-amplified PCR products. The use of a laminar flow cabinet or PCR cabinet and maintaining a strict single direction workflow will reduce the chance of contamination.

To greatly reduce the chance of contamination to the stock gDNA samples, it is suggested to perform gDNA extractions in a completely separate room or area.

The one room is split into 2 areas called the:

- Pre-PCR Area
- Post-PCR Area

These 2 areas are set up to accommodate the assay steps. See Table 3.

IMPORTANT! We strongly recommend the use of a laminar flow cabinet or PCR cabinet when the entire assay is to be performed in 1 room.

Note: Entering and exiting through the Pre-PCR area only is permissible as long as the Post-PCR area has not been entered.

Table 3 Assay workflow when 2 separate areas are used.

Area	Assay steps	Template (genomic DNA)	PCR product
Pre-PCR Area	 Genomic DNA preparation dsDNA quantitation and normalization Anneal 1st PCR 		×
Post-PCR Area	 First QC Gel 2nd PCR HaellI Digest Second QC Gel Hybridization Washing and staining Scanning 	⊗	

One-room configuration

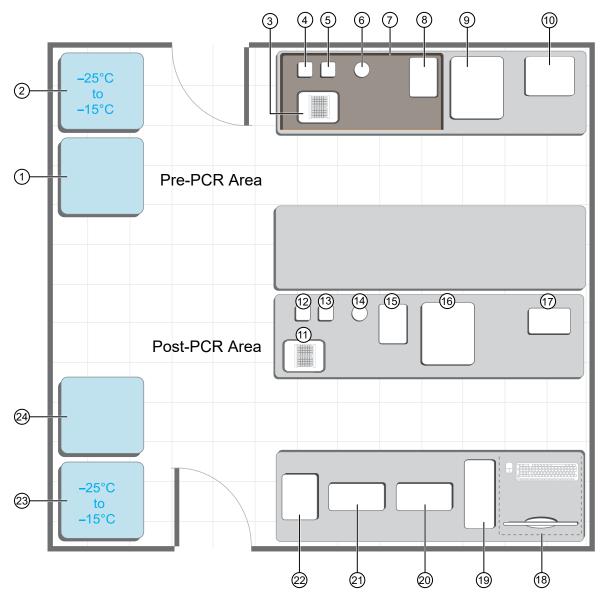


Figure 4 One-room, single direction configuration.

- (1) Refrigerator
- ② Freezer, -25°C to -15°C
- (3) Ice bucket with cooling blocks (Quantity = 4)
- 4 Vortexer
- (5) Microfuge
- (6) Pipettes on stand
- (7) Laminar flow cabinet or PCR cabinet (recommended)
- 8 Thermal cycler
- 9 Plate centrifuge
- 10 dsDNA specific quantitation device
- (1) Ice bucket with cooling blocks (Quantity = 3)

- (12) Vortexer
- 13 Microfuge
- 14 Pipettes on stand
- 15 Thermal cycler
- 16 Plate centrifuge
- (17) Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System
- 18 Computer, monitor, keyboard
- 19 GeneChip™ Scanner 3000 7G
- ② GeneChip™ Fluidics Station 450
- ②1 GeneChip™ Fluidics Station 450

- ② GeneChip™ Hybridization Oven 645
- 23 Freezer, -25°C to -15°C

2 Refrigerator



Equipment, consumables, and reagents required

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Equipment required but not provided	24
Labware and consumables required	28

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.

Thermo Fisher Scientific materials required

Equipment and software required

The OncoScan™ CNV Plus Array can be processed on the GeneChip™ System 3000 and the GeneChip™ System 3000Dx v2.

Table 4 GeneChip™ System 3000 equipment and software required.

Item	Source	
Equipment		
GeneChip™ System 3000		
GeneChip™ Scanner 3000 7G System with the Workstation and AutoLoader	• 00-0218	
GeneChip™ Fluidics Station 450 (2 or more units required)	• 00-0079	
GeneChip™ Hybridization Oven 645		
Tubing, silicone peristaltic for the GeneChip™ Fluidics Station 450 (Quantity = 1 tube)		
Software		
Applied Biosystems™ GeneChip™ Data Collection Software (GCDC) (Version 1.0 or higher)	611403	
or		
Applied Biosystems™ GeneChip™ Command Console™ (GCC) (Version 6.1.3 or higher)		
Applied Biosystems™ Chromosome Analysis Suite software (Version 4.1 or higher)	901394	

Table 5 GeneChip™ System 3000Dx v2 equipment and software required.

Item	Source
Equipment	
GeneChip™ System 3000Dx v2 Instrument System	00-0334
 GeneChip™ Scanner 3000Dx v2 with GeneChip™ AutoLoader Dx and GeneChip™ Fluidics Station 450Dx v2 	
GeneChip™ Hybridization Oven 645	00-0331
GeneChip™ Fluidics Station 450Dx v2 (Optional)	00-0335
Tubing, silicone peristaltic for the GeneChip™ Fluidics Station 450 (Quantity = 1 tube)	400110
Software	
Applied Biosystems™ GeneChip™ Data Collection Software Dx (GCDC Dx) (Version 1.0 or higher)	611403
or	or
Affymetrix™ Molecular Diagnostics Software (AMDS) (Version 1.1 or higher)	610449
Applied Biosystems™ Chromosome Analysis Suite software (Version 4.1 or higher)	901394

Thermo Fisher Scientific reagents and array required

Table 6 OncoScan™ CNV Plus reagents and array kits.

Item	Source
OncoScan™ CNV Plus Reagent Kit for Research	902294
OncoScan™ CNV Plus Array (12 pack)	902292
OncoScan™ CNV Plus Assay for Research, consists of: • OncoScan™ CNV Plus Reagent Kit for Research • OncoScan™ CNV Plus Array (48 arrays)	902293
OncoScan™ CNV Plus Assay Training Kit, consists of: • OncoScan™ CNV Plus Reagent Kit for Research • OncoScan™ CNV Plus Array (36 arrays) • Training controls	902305

OncoScan™ CNV Plus Reagent Kit for Research

Table 7 OncoScan™ CNV Plus Reagent Kit for Research (Cat. No. 902294).

OncoScan™ CNV Plus Somatic Mutation Probe Mix 1.0 902247 -25°C to -15°C OncoScan™ CNV Plus Copy Number Probe Mix 1.0 & Controls (Part No. 902268) ● Positive Control (12 ng/μL) 902249 ● Negative Control (12 ng/μL) 902248 ● Negative Control 902248 ● Double Probe Mix 1.0 902248 ● Buffer A 902246 OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269) ● Buffer A 902246 ● Gap Fill Enzyme Mix 902252 ● SAP, Recombinant (1 U/μL) 902251 ● dNTP Mix (G/C) 902254 ● Los Mix 902255 ● Cleavage Buffer 902257 ● Cleavage Enzyme (2 U/μL) 902258 ● PCR Mix 902259 □ Taq Polymerase (5 U/μL) 902259 □ Taq Polymerase (5 U/μL) 902259 □ Taq Polymerase (5 U/μL) 902260 ● Buffer B 902261 ● Haelll Enzyme (10 U/μL) 902262 ● Exo I Enzyme (20 U/μL) 902263 ● Hybridization Mix 902264	Component and cap color	Part No.	Storage
OncoScan™ CNV Plus Copy Number Probe Mix 1.0 & Controls (Part No. 902268) ● Positive Control (12 ng/μL) 902249 ● Negative Control 902250 ● Copy Number Probe Mix 1.0 902248 ● Buffer A 902246 OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269) ● Buffer A 902246 ● Gap Fill Enzyme Mix 902252 ● SAP, Recombinant (1 U/μL) 902251 ● dNTP Mix (A/T) 902254 ● dNTP Mix (G/C) 902255 Nuclease-Free Water 902256 ● Cleavage Buffer 902257 ● Cleavage Enzyme (2 U/μL) 902258 □ PCR Mix 902259 □ Taq Polymerase (5 U/μL) 902260 ● PCR Mix 902259 □ Taq Polymerase (5 U/μL) 902260 ● Buffer B 902261 ● Haelll Enzyme (10 U/μL) 902262 ● Exo I Enzyme (20 U/μL) 902263 ○ Nuclease-Free Water 902253	OncoScan™ CNV Plus Somatic Mutation Probe Mix 1.0 (Part No. 902272)		
Positive Control (12 ng/μL) 902249 Negative Control 902250 Copy Number Probe Mix 1.0 902248 Buffer A 902246 OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269) Buffer A 902246 Gap Fill Enzyme Mix 902252 SAP, Recombinant (1 U/μL) 902251 dNTP Mix (A/T) 902254 dNTP Mix (G/C) 902253 Nuclease-Free Water 902253 Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 Haelll Enzyme (10 U/μL) 902262 Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Somatic Mutation Probe Mix 1.0	902247	–25°C to −15°C
Negative Control 902250 Copy Number Probe Mix 1.0 902248 Buffer A 902246 OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269) Buffer A 902246 Gap Fill Enzyme Mix 902252 SAP, Recombinant (1 U/μL) 902251 dNTP Mix (A/T) 902254 dNTP Mix (G/C) 902255 Nuclease-Free Water 902253 Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 Haelll Enzyme (10 U/μL) 902262 Algebra Free Water 902253	OncoScan™ CNV Plus Copy Number Probe Mix 1.0 &	Controls (Part No. 90	2268)
Copy Number Probe Mix 1.0 902248 Buffer A 902246 OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269) Buffer A 902246 Gap Fill Enzyme Mix 902252 SAP, Recombinant (1 U/μL) 902251 ♠ dNTP Mix (A/T) 902254 ♠ dNTP Mix (G/C) 902255 Nuclease-Free Water 902253 ♠ Cleavage Buffer 902257 ♠ Cleavage Enzyme (2 U/μL) 902258 ♠ PCR Mix 902259 ♠ Taq Polymerase (5 U/μL) 902260 ♠ PCR Mix 902259 ♠ PCR Mix 902259 ♠ Taq Polymerase (5 U/μL) 902260 ♠ Buffer B 902261 ♠ Haelll Enzyme (10 U/μL) 902262 ♠ Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Positive Control (12 ng/µL)	902249	
	Negative Control	902250	25°C to 15°C
OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Part No. 902269) Buffer A 902246 Gap Fill Enzyme Mix 902252 SAP, Recombinant (1 U/μL) 902251 dNTP Mix (A/T) 902254 dNTP Mix (G/C) 902255 Nuclease-Free Water 902253 Exo Mix 902256 Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 Haelll Enzyme (10 U/μL) 902262 Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Copy Number Probe Mix 1.0	902248	-25 C t0 -15 C
Buffer A 902246 Gap Fill Enzyme Mix 902252 SAP, Recombinant (1 U/μL) 902251 ♠ dNTP Mix (A/T) 902254 ♠ dNTP Mix (G/C) 902255 Nuclease-Free Water 902253 ♠ Exo Mix 902256 ♠ Cleavage Buffer 902257 ♠ Cleavage Enzyme (2 U/μL) 902258 ▶ PCR Mix 902259 ▼ Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) ▶ PCR Mix 902259 ▼ Taq Polymerase (5 U/μL) 902260 ♠ Buffer B 902261 ♠ HaellI Enzyme (10 U/μL) 902262 ♠ Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Buffer A	902246	
Gap Fill Enzyme Mix 902252 SAP, Recombinant (1 U/μL) 902251 ♠ dNTP Mix (A/T) 902254 ♠ dNTP Mix (G/C) 902255 Nuclease-Free Water 902253 ♠ Exo Mix 902256 ♠ Cleavage Buffer 902257 ♠ Cleavage Enzyme (2 U/μL) 902258 ♠ PCR Mix 902259 ♠ Taq Polymerase (5 U/μL) 902260 ♠ PCR Mix 902259 ♠ Taq Polymerase (5 U/μL) 902260 ♠ Buffer B 902261 ♠ Haelll Enzyme (10 U/μL) 902262 ♠ Exo I Enzyme (20 U/μL) 902263 ♠ Nuclease-Free Water 902253	OncoScan™ CNV Plus Gap Fill and 1st Stage PCR (Pa	rt No. 902269)	
SAP, Recombinant (1 U/μL) dNTP Mix (A/T) dNTP Mix (G/C) Nuclease-Free Water Exo Mix Cleavage Buffer Cleavage Enzyme (2 U/μL) PCR Mix PCR Mix 902259 Taq Polymerase (5 U/μL) PCR Mix 902259 PCR Mix 902259 PCR Mix 902250 DncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 Haelll Enzyme (10 U/μL) 902263 Nuclease-Free Water 902253	Buffer A	902246	
■ dNTP Mix (A/T) 902254 ■ dNTP Mix (G/C) 902255 Nuclease-Free Water 902253 ■ Exo Mix 902256 ■ Cleavage Buffer 902257 ● Cleavage Enzyme (2 U/µL) 902258 ▶ PCR Mix 902259 ▼ Taq Polymerase (5 U/µL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) ▶ PCR Mix 902259 ▼ Taq Polymerase (5 U/µL) 902260 ■ Buffer B 902261 ■ HaellI Enzyme (10 U/µL) 902262 ■ Exo I Enzyme (20 U/µL) 902263 Nuclease-Free Water 902253	Gap Fill Enzyme Mix	902252	
MNTP Mix (G/C) 902255 Nuclease-Free Water 902253 Exo Mix 902256 Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	SAP, Recombinant (1 U/µL)	902251	
Nuclease-Free Water 902253 -25°C to -15°C Exo Mix 902256 Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	dNTP Mix (A/T)	902254	
Exo Mix 902256 Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	odntp Mix (G/C)	902255	
Cleavage Buffer 902257 Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Nuclease-Free Water	902253	–25°C to −15°C
Cleavage Enzyme (2 U/μL) 902258 PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaelII Enzyme (10 U/μL) 902262 −25°C to −15°C Exo I Enzyme (20 U/μL) 902253 Nuclease-Free Water 902253	Exo Mix	902256	
PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaelII Enzyme (10 U/μL) 902262 -25°C to -15°C Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Cleavage Buffer	902257	
Taq Polymerase (5 U/μL) 902260 OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 -25°C to -15°C Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Cleavage Enzyme (2 U/μL)	902258	
OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Processing (Part No. 902270) PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaelII Enzyme (10 U/μL) 902262 -25°C to -15°C Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	PCR Mix	902259	
PCR Mix 902259 Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 -25°C to -15°C Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	Taq Polymerase (5 U/μL)	902260	
Taq Polymerase (5 U/μL) 902260 Buffer B 902261 HaellI Enzyme (10 U/μL) 902262 -25°C to -15°C Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	OncoScan™ CNV Plus 2nd Stage PCR & Post PCR Pro	ocessing (Part No. 902	2270)
Buffer B 902261 HaelII Enzyme (10 U/μL) 902262 -25°C to -15°C Exo I Enzyme (20 U/μL) 902263 Nuclease-Free Water 902253	PCR Mix	902259	
Haelll Enzyme (10 U/μL) Exo I Enzyme (20 U/μL) Nuclease-Free Water 902262 -25°C to -15°C 902263 902253	Taq Polymerase (5 U/μL)	902260	
Exo I Enzyme (20 U/µL) 902263 Nuclease-Free Water 902253	Buffer B	902261	
Nuclease-Free Water 902253	Haelll Enzyme (10 U/μL)	902262	–25°C to −15°C
	Exo I Enzyme (20 U/μL)	902263	
Hybridization Mix 902264	Nuclease-Free Water	902253	
	Hybridization Mix	902264	

Table 7 OncoScan CNV Plus Reagent Kit for Research (Cat. No. 902294). (continued)

Component and cap color	Part No.	Storage
OncoScan™ CNV Plus Stain Reagents (Part No. 90227	1)	
Stain 1	902265	
Stain 2	902266	2-8°C
Array Holding Buffer	901733	
Individual bottles		
Wash A	901680	15 2000
Wash B	901681	15–30°C

OncoScan™ CNV Plus Array

Component	Cat. No.	Storage
OncoScan™ CNV Plus Array (12 pack)	902292	2-8°C

Recommended dsDNA quantitation kits and equipment

The success of the OncoScan™ CNV Plus Assay requires accurate quantification of the input gDNA using a dsDNA specific quantification method. Two methods that have been verified for use are the Quant-iT™ PicoGreen™ Assay and the Qubit™ dsDNA Quantification Assay. Sample concentration determined by UV absorbance or NanoDrop™ spectrophotometer *must not* be used in this assay.

Table 8 PicoGreen™ reagents and equipment required.

Item	Source
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P7589
Required: Fluorometer	VL0000D0
Recommended: Varioskan™ LUX Multimode Microplate Reader	

Table 9 Qubit™ reagents and equipment required.

Item	Source
Qubit™ dsDNA HS Assay Kit	Q32851
Qubit™ fluorometer	Q33327 or Q33238
Qubit™ assay tubes specific to the Qubit™ instrument	
Qubit [™] 4 Fluorometer: Qubit [™] Assay Tubes	• Q32856
Qubit™ Flex Fluorometer: Qubit™ Flex Assay Tube Strips	• Q33252

Other reagents

Item	Source
TE Buffer, 1X Solution pH 8.0, Low EDTA	J75793.AE
Nuclease-Free Water (from different source)	10977015
Applied Biosystems™ 25-bp DNA Ladder	931343
Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer	10482028
Invitrogen™ E-Gel™ 48 Agarose Gels with SYBR™ Safe DNA Gel Stain, 4%	G820804
Bleach, Sodium Hypochlorite solution without additives	MLS

Equipment required but not provided

Pre-PCR Room

Table 10 Pre-PCR Room—required equipment not provided.

Item	Source
If assay is to be performed in 1 room:	
Laminar flow cabinet, 6 foot, or	MLS
PCR cabinet	
Required markers:	
1 blue permanent marker, extra-fine tip	MLS
1 red permanent marker, extra-fine tip	
Required: Rectangular ice bucket, large, 9 L (16 × 13 in; 41 × 33 cm)	Fisher Scientific™,
Recommended: Corning™ Rectangular Ice Pan, Maxi 9L	07-210-093
Required: Cooling block with the capacity to hold 96-well plates with a maximum volume	
capacity of 330 μL.	Fisher Scientific™,
Recommended: Quantity = 4, Electron Microscopy Sciences CoolSafe™ Cooling Chamber for 0.2 mL tubes	50-334-22
Required: Vortexer, 600-3,200 rpm, cup head or 3' head.	Fisher Scientific™,
Recommended: Fisherbrand™ Analog Vortex Mixer	02-215-414
Required: Microfuge (for tubes and strip tubes)	Fisher Scientific™,
Recommended: Fisherbrand™ Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey	12-006-901

Table 10 Pre-PCR Room—required equipment not provided. (continued)

Item	Source
Pipettors:	
• 12-channel, 2 μL to 20 μL	
• 12-channel, 20 μL to 200 μL	
• Single-channel, 2 μL to 20 μL	MLS
• Single-channel, 20 μL to 200 μL	
• Single-channel, 100 μL to 1,000 μL	
Note: Electronic multichannel pipettes are not recommended.	
Thermal cycler: capable of holding 200 μ L 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. The use of a compatible compression pad is recommended throughout the assay.	
Recommended: One of the following verified thermal cyclers.	
 Applied Biosystems[™] ProFlex[™] 96-well PCR System 	
 Applied Biosystems™ Veriti™ 96-Well Thermal Cycler, 0.2 mL,^[1] 	• 4484075
Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver	• 4375786
block)	• 4314878
 Applied Biosystems™ 2720 Thermal Cycler Bio-Rad™ T100 Thermal Cycler 	43596591861096
Bio-had 1100 Mermai Cycler	1001090
Required: Centrifuge, plate, multipurpose, 330 µL capacity with swinging buckets	
Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Ventilated) with swinging bucket rotor and unsealed buckets (Set of 2)	75007200 with 75005706 and 75005723
or	or
Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Refrigerated) with swinging bucket rotor and unsealed buckets (Set of 2)	75007203 with 75005706 and 75005723
Refrigerator, 2—8°C, 6 cu ft	MLS
Freezer, -25°C to -15°C; deep freeze; manual defrost; 17 cu ft	MLS
Required: Storage racks, tube	Fisher Scientific™,
Recommended: Thermo Scientific™ 4-Way Flipper™ Rack	21-402-27
Required: PCR tube storage rack, 96-well, with cover	Fisher Scientific™,
Recommended: Axygen™ PCR Tube Storage Rack	14-222-396

Table 10 Pre-PCR Room-required equipment not provided. (continued)

Item	Source
Required: Benchtop cooler, with the capacity to hold 8-12 tubes (1.5 mL)	Fisher Scientific™,
Recommended: Thermo Scientific™ Benchtop Cooler	15-350-61
Required: Adhesive film applicator (hard plastic)	Fisher Scientific™,
Recommended: MicroAmp™ Adhesive Film Applicator	4333183

^[1] The Veriti™ Fast 96-Well Thermal Cycler, Cat. No. 4375305 and the and the Eppendorf™ Mastercycler™ pro S, Cat. No. 950030020 are not compatible with this assay.

Post-PCR Room

Table 11 Post-PCR Room—required equipment not provided.

Item	Source
Required markers:	
1 blue permanent marker, extra-fine tip	MLS
1 red permanent marker, extra-fine tip	
Required: Rectangular ice bucket, large, 9 L (16 × 13 in; 41 × 33 cm)	Fisher Scientific™,
Recommended: Corning™ Rectangular Ice Pan, Maxi 9L	07-210-093
Required: Cooling block with the capacity to hold 96-well plates with a maximum volume capacity of 330 μ L.	Fisher Scientific™,
Recommended: Quantity 3, Electron Microscopy Sciences CoolSafe™ Cooling Chamber for 0.2 mL tubes	50-334-22
Required: Vortexer, 600-3,200 rpm, cup head or 3' head.	Fisher Scientific™,
Recommended: Fisherbrand™ Analog Vortex Mixer	02-215-414
Required: Microfuge (for tubes and strip tubes)	Fisher Scientific™,
Recommended: Fisherbrand™ Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey	12-006-901
Pipettors:	
• 12-channel, 2 μL to 20 μL	
• 12-channel, 20 μL to 200 μL	
• Single-channel, 2 μL to 20 μL	MLS
• Single-channel, 20 μL to 200 μL	
• Single-channel, 100 μL to 1,000 μL	
Note: Electronic multichannel pipettes are not recommended.	

Table 11 Post-PCR Room—required equipment not provided. (continued)

Item	Source
Thermal cycler: capable of holding 200 μ L 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. The use of a compatible compression pad is recommended throughout the assay.	
Recommended: One of the following verified thermal cyclers.	
Applied Biosystems™ ProFlex™ 96-well PCR System	
 Applied Biosystems[™] Veriti[™] 96-Well Thermal Cycler, 0.2 mL,^[1] 	• 4484075
 Applied Biosystems[™] GeneAmp[™] PCR System 9700 (with gold-plated or silver block) 	43757864314878
Applied Biosystems™ 2720 Thermal Cycler	• 4359659
Bio-Rad™ T100 Thermal Cycler	• 1861096
Required: Centrifuge, plate, multipurpose, 330 µL capacity with swinging buckets	
Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Ventilated) with swinging bucket rotor and unsealed buckets (Set of 2)	75007200 with 75005706 and 75005723
or	or
Recommended: Sorvall™ ST 8 Small Benchtop Centrifuge (Refrigerated) with swinging bucket rotor and unsealed buckets (Set of 2)	75007203 with 75005706 and 75005723
Required: Electrophoresis supplies	
Recommended for electrophoresis and image capture:	
E-Gel™ Power Snap Plus Electrophoresis Device	• G9110
Safe Imager™ Viewing Glasses	• \$37103 ^[2]
E-Gel™ agarose gel cassette	• _[2]
E-Gel™ Power Snap Plus Camera	• G9200
Additional options for electorphoresis and image capture:	
Invitrogen™ Mother E-Base™ Device	• EBM03 (Mother)
 Invitrogen™ Daughter E-Base™ Device 	EBD03 (Daughter)
Invitrogen™ iBright™ CL750 Instrument	• A44116
Refrigerator, 2–8°C, 6 cu ft	MLS
Freezer, -25°C to -15°C; deep freeze; manual defrost; 17 cu ft	MLS
Required: Storage racks, tube	Fisher Scientific™,
Recommended: Thermo Scientific™ 4-Way Flipper™ Rack	21-402-27
Required: PCR tube storage rack, 96-well, with cover	Fisher Scientific™,
Recommended: Axygen™ PCR Tube Storage Rack	14-222-396

Table 11 Post-PCR Room—required equipment not provided. (continued)

Item	Source
Required: Benchtop cooler, with the capacity to hold 8–12 tubes (1.5 mL) Recommended: Thermo Scientific™ Benchtop Cooler	Fisher Scientific™, 15-350-61
Required: Adhesive film applicator (hard plastic) Recommended: MicroAmp™ Adhesive Film Applicator	4333183
Required: Microtube storage rack, capacity to hold 96 tubes (1.5 mL) Recommended: Thermo Scientific™ 96-Well Flipper™ Microtube Rack	21-402-18
Fluidics station recommended bottles: • Media Bottle, SQ, 500 mL • Media Bottle, SQ, 1,000 mL	400118400119

^[1] The Veriti™ Fast 96-Well Thermal Cycler, Cat. No. 4375305 and the and the Eppendorf™ Mastercycler™ pro S, Cat. No. 950030020 are not compatible with this assay.

Labware and consumables required

Table 12 Consumables required but not provided.

Labware	Source	Room	Image
 Required: Plates, 96-well unskirted PCR with a maximum volume of 330 μL Recommended: Amplifyt™ 96-Well PCR Plate, Semi-Skirted Alternative: Applied Biosystems™ MicroAmp™ Optical 96-Well Reaction Plate Alternative: Bio-Rad™ Multiplate™ 96-Well PCR Plates, high profile, unskirted, clear 	 Thomas Scientific™, 1148A74 N8010560 Bio-Rad™, MLP9601 	Both	80000000000000000000000000000000000000
Required: Adhesive films, clear, PCR-certified, 96-well plates Recommended: Applied Biosystems™ MicroAmp™ Clear Adhesive Film	4306311	Both	
Pipette tips with aerosol barriers, 20 μL, 200 μL, and 1,000 μL	MLS	Both	_
Tube strips, nuclease-free, 8-well or 12-well, 0.2 mL polypropylene	MLS	Both	00000000000
Optional: 8-tube or 12-tube strip caps	MLS	Both	_
Microcentrifuge tubes, 1.5 mL, non-stick, polypropylene, DNAse and RNAse-free Note: Required for all sample workflows.	MLS	Both	

 $^{^{[2]}}$ Included with E-Gel $^{\rm m}$ Power Snap Plus Electrophoresis Device.

Table 12 Consumables required but not provided. (continued)

Labware	Source	Room	Image
Microcentrifuge tubes, 2.0 mL, non-stick, polypropylene, DNAse and RNAse-free	MLS	Post	
Note: Only required if running the 25-sample workflow.			
Conical centrifuge tubes, nuclease-free, sterile, non-stick, 15 mL	MLS	Post	
Required: Reagent reservoir, 25 mL	8093	Post	
Recommended: Matrix™ Reagent Reservoirs			السسا
Microcentrifuge tubes, nuclease-free, non-stick, 1.5 mL natural polypropylene	MLS	Post	
Microcentrifuge tubes, nuclease-free, non-stick, 1.5 mL blue polypropylene	MLS	Post	
Microcentrifuge tubes, nuclease-free, non-stick, 1.5 mL amber, polypropylene	MLS	Post	
Adhesive label dot (Tough-Spots™ labels), 1/2-inch roll and 3/8-inch roll	MLS	Post	_



Documentation and support

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Related documentation

Document	Publication number	Description
OncoScan™ CNV Plus Assay User Guide	MAN0027850	This document provides instruction on running the OncoScan™ CNV Plus Assay workflow and array processing with the GeneChip™ Hybridization Oven 645, GeneChip™ Fluidics Station 450 and the GeneChip™ Scanner 3000 7G System.
OncoScan™ CNV Plus Assay – 7 Samples Quick Reference	MAN0029389	An abbreviated reference for the assay protocol steps of the OncoScan™ CNV Plus Assay workflow for 7, 9, 13, and 25 samples. These quick reference documents are intended for experienced
OncoScan™ CNV Plus Assay—9 Samples Quick Reference	MAN0029622	users.
OncoScan™ CNV Plus Assay—13 Samples Quick Reference	MAN0029623	
OncoScan™ CNV Plus Assay—25 Samples Quick Reference	MAN0027126	
GeneChip™ Data Collection Software (GCDC) User Guide	MAN0026726	This user guide provides instructions on using GeneChip™ Data Collection Software (GCDC) used to control GeneChip™ instrument systems. GeneChip™ Data Collection Software provides an intuitive set of tools for instrument control and data management used in the processing of Applied Biosystems™ cartridge arrays.
Chromosome Analysis Suite User Guide	MAN0027798	This user guide provides instructions on 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.

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

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