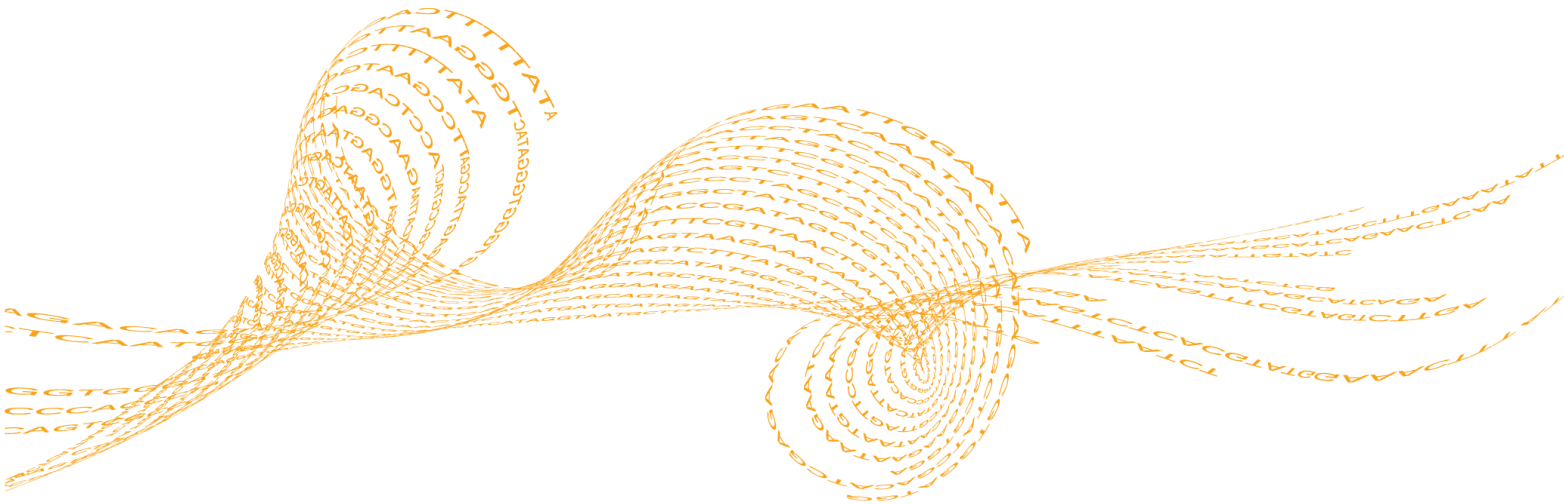
CytoChip Oligo Microarray Reference Guide



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Revision History

Part #	Revision	Date	Description of Change
15056493	C	June 2015	Updated hyperlinks and Technical Assistance page.
15056493	B	October 2014	Corrected wash buffer for wash 2 to Wash Buffer 2.
15056493	A	July 2014	Initial release

Introduction

CytoChip Oligo microarrays use array comparative genomic hybridization (array CGH) approaches to investigate genomic copy number imbalance. Array CGH enables independently labeled sample and control DNA to be competitively hybridized to genomic probes, of known sequence, immobilized on a glass microarray. Software analysis of the scanned microarray is used to estimate the amount of sample and control DNA bound to each probe location. Analysis is also used to estimate the copy number of the genomic sequence represented by that probe. This manual covers all stages of the array CGH approach with CytoChip Oligo microarrays.

Experimental Design and Array Format

Single Hybridization – Multiple Samples per Slide

CytoChip Oligo format arrays use large numbers of features and software smoothing to enable robust results to be reported. The results are based on a single hybridization of a Cy3 labeled sample against a Cy5 labeled control.

The CytoChip Oligo arrays are multiformat so that a single slide supports multiple samples. The CytoChip Oligo 2x105K array supports the analysis of 2 DNA samples on each physical slide (8 DNA samples on 4 slides, 16 per pack of 8). CytoChip ISCA 4x44K, CytoChip ISCA 4x180K, CytoChip Oligo SNP 4x180K, and CytoChip Focus 4x180K support 4 DNA samples per slide (16 per pack of 4). CytoChip ISCA 8x60K and CytoChip Focus 8x60K arrays support 8 DNA samples per slide (32 per pack of 4). Each format provides a different resolution of coverage of the genome.

Array Design

The **CytoChip Oligo** 2x105K and **CytoChip ISCA** arrays have a constitutional disease-focused design. The ICCG (International Collaboration for Clinical Genomics), committed to establishing a uniform array format for use in clinical cytogenetics laboratories worldwide, formulated the ISCA (International Standard Cytogenetic Array) design. For more information on the ISCA design, refer to: Baldwin et al. (2008), Genetics in Medicine, Jun;10(6):415-29 ihttp://www.ncbi.nlm.nih.gov/pubmed/18496225. The array design includes attention to cytogenetically important regions such as telomeres, centromeres, and pseudoautosomal regions. It also includes specific microdeletion/microduplication syndromes and important Mendelian disease loci associated with developmental delay and autism.

The **CytoChip Focus** array consists of a constitutional design for the investigation of challenging samples, such as those with low amounts of poor quality DNA extracted from CVS and amniocytes. This design is aimed to include genes associated with early developmental disorders and specifically exclude late-onset diseases. The backbone is replicated to provide more robust results while minimizing the reporting of Variants Of Unknown Significance (VOUS). The CytoChip Focus 4x180K array can be used with only 200 ng of starting material,

Combined Array CGH and SNP Arrays

CytoChip Oligo SNP 4x180K array is a SNP array with a constitutional design. CytoChip Oligo SNP consists of approximately 150,000 probes of the ISCA v2 4x180K targeted design. In addition, 27,000 SNP-specific probes are present, distributed as evenly as possible across the genome to provide approximately 10 Mb LOH resolution.

CytoChip Spike-in Controls

To improve the tracking of samples, especially on the more highly multiplexed formats, Illumina provides CytoChip Spike-in Controls. Probe targets for spike-in controls have been included on certain array designs and can be easily incorporated into the CytoChip laboratory protocol.

Table 1 CytoChip Spike-in Controls available

Description	Quantity	Catalog No.
CytoChip Spike-in Controls	8 x 80 µl	PR-40-415301-00

CytoChip arrays are available in packs that provide all the reagents required to perform an assay, including: CytoChip arrays, Fluorescent Labeling System [dUTP] (32 rxns) (PR-30-413401-00), or SureLabel32SNP [dUTP] Fluorescent Labeling System (32 rxns) (PR-30-413437-00), and COT Human DNA (PR-40-413503-00).

SureLabel32SNP [dUTP] Fluorescent Labeling System is provided specifically for use with the CytoChip Oligo SNP array and includes: reagents for the digestion step, characterized reference DNA (male and female) with a known SNP genotype, columns for clean-up post labeling, and reagents for the fluorescent labeling procedure. The genotype files required for SNP analysis with BlueFuse Multi are available for download from www.cambridgebluegnome.com/account-login.

Table 2 CytoChip arrays available in packs

Name	Description (AMADID/lot no.)	No. of samples	Spike-in Compatible	Catalog No.
CytoChip Oligo 2x105K Pack	142 disease regions and a 30 Kb backbone (21856)	16	No	PR-10-408001-PK
CytoChip ISCA v1.0 4x44K Pack	232 regions, 70 Kb backbone (23097)	16	No	PR-10-408003-PK
CytoChip ISCA v1.0 4x180K Pack	500 regions, 18 Kb backbone (24585)	16	No	PR-10-408006-PK
CytoChip ISCA v2.0 8x60K Pack	498 regions, 51 Kb backbone (26370)	32	Yes	PR-10-408006-PK
CytoChip ISCA v2.0 2x105K Pack	498 disease regions and a 30 Kb backbone (28740)	16	Yes	PR-10-408011-PK
CytoChip ISCA v2.0 4x180K Pack	500 disease regions and a 21 Kb backbone (30078)	16	Yes	PR-10-408013-PK
CytoChip ISCA v2.0 4x44K Pack	232 regions, 70 Kb backbone (28739)	16	Yes	PR-10-408010-PK
CytoChip Focus 8x60K Pack	162 severe early disorders, 97 Kb duplicated backbone (40942)	32	Yes	PR-10-408024-PK
CytoChip Focus 4x180K Pack	179 severe early disorders, 44 Kb triplicated backbone (45830)	16	Yes	PR-10-408026-PK
CytoChip Oligo SNP 4x180K Pack	500 regions, 20 Kb backbone, 10 Mb LOH resolution (33485)	16	Yes	PR-11-448006-PK

User Supplied Reagents

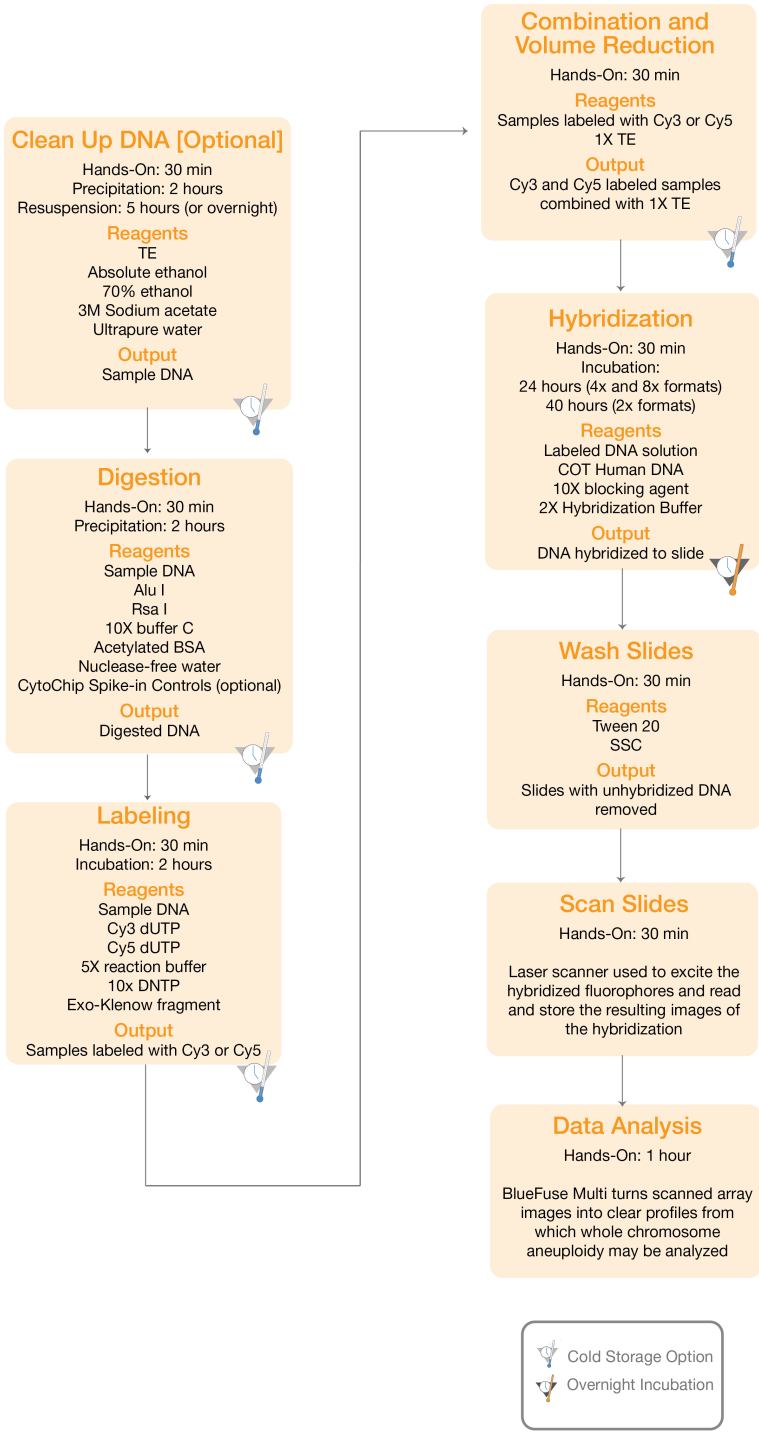
Table 3 Reagents required, not provided

Reagent	Part Number
Amicon Ultracel-30 membrane (columns included in SNP Packs)	Millipore UFC503024 (24), UFC503096 (96)
Commercial control DNA (100 ng/µl)	Promega G1521(female)/G1471 (male)
Aqueous glycogen solution	Sigma-Aldrich G1767
Oligo aCGH Wash Buffer 1 and 2	Illumina PR-70-413506-00
[Optional] CytoChip Spike-in Controls	Illumina PR-40-415301-00
[Optional] ClearPack Lite	Illumina PR-70-431001-00

CytoChip Oligo Workflow

CytoChip Oligo protocols have been developed to fit into a standard laboratory workflow as described in Figure 1. Protocols for CytoChip microarrays are performed within 2 days.

Figure 1 CytoChip Oligo Workflow Diagram



Sample Preparation

Ethanol precipitation is recommended to remove salts and other contaminants that might lead to labeling problems. See *DNA Quality* on page 25 for further discussion on when clean-up is required.

Materials

Table 4 Starting materials for sample preparation

Starting materials	Amount
DNA (unsheared, undigested, genomic DNA)	> 2 µg

Table 5 Materials required for sample preparation

Materials Required	Amount	Part Number
TE (10mM Tris, 1mM EDTA, pH7.0-8.0)	50 µl	Made up stock
Absolute ethanol	125 µl	
70% ethanol	500 µl	
Ultrapure water (Milli-Q, 18.2 MΩ.cm)	20 µl	
Microcentrifuge tube (1.5 ml, flip cap)		Sarstedt 72.690.001
Aqueous glycogen solution (1 µg/µl)	2 µl	Prepared from Sigma G1767

Clean Up DNA

NOTE
This step is optional.

1 Resuspend DNA in TE to a total volume of 50 µl.

2 Add 1/10th volume of 3M sodium acetate, vortex, then add 2.5 volume of absolute ethanol. Invert twice to mix.

3 If starting with less than 2 µg of DNA, add 2 µl of aqueous glycogen solution (1 µg/µl) as a coprecipitant.

4 Precipitate DNA for 2 hours at -25°C to -15°C.

NOTE
Alternatively, precipitation can be achieved in 30 minutes at -80°C.

5 Centrifuge at full speed (≥ 13,000 × g) for 15 minutes and decant the supernatant.

6 Add 500 µl of 70% ethanol and invert the tube three times to wash pellet.

7 Centrifuge at full speed (≥ 13,000 × g) for 5 minutes and decant the supernatant.

8 Pulse the tube in a centrifuge and remove the remaining ethanol with a P10 tip. Cap tube.

9 Remove the cap and allow the pellet to air dry for 5 minutes at room temperature.

NOTE
Make sure that there is no residual ethanol visible around the pellet before proceeding. Ethanol will inhibit subsequent labeling reactions.

10 Add 1x TE (pH 8.0) to give a final concentration of approximately 100 ng/µl.

11 Resuspend for 5 hours, or overnight, at room temperature.

12 Quantify DNA to confirm that OD readings are in line with recommendations (see *DNA Quality*).

13 Proceed immediately to *CytoChip Oligo Labeling* or store DNA at 2°C to 8°C until required.

CytoChip Oligo Labeling

Sample and reference DNAs are labeled with Cy3 and Cy5 fluorophores, respectively, using random primers. Labeling mixes are combined, concentrated, and reconstituted to the appropriate volume for hybridization.

To optimize dye incorporation using the fluorescent labeling system, include a restriction digestion step before labeling. A restriction digestion step is highly recommended. However, high-quality data can be achieved without digestion in certain circumstances. By including a digestion step and running an agarose gel of the products, a good impression of the DNA quality can be obtained that is independent of spectrophotometric readings. If sample DNA is digested, it is also necessary to digest the reference DNA to keep the DNA characteristics matched.

It is essential to perform restriction digestion of genomic DNA (sample and reference) hybridized to the CytoChip Oligo SNP platform for the LOH/UPD calling to be effective. Restriction digestion reagents are provided in the SureLabel32SNP [dUTP] Fluorescent Labeling System.

CytoChip Oligo Spike-in Controls, if used, are incorporated into the CytoChip protocol at the restriction digestion step **or** directly at the labeling step.

2x (CytoChip Oligo 2x105K) and 4x (CytoChip ISCA 4x44K and 4x180K, CytoChip Oligo SNP 4x180K, and CytoChip Focus 4x180K) formats use the same quantities of starting material and labeling reagents. The 8x (CytoChip ISCA 8x60K, CytoChip Focus 8x60K) formats require half the quantity of labeled material; the 8x format is highlighted where necessary.

Restriction Digestion of gDNA

Table 6 Starting materials for restriction digestion of gDNA

Starting Materials	Amount (2x and 4x formats)	Amount CytoChip Focus 4x180K	Amount (8x formats)
Sample/Reference DNA	1.0–1.5 µg in 20.2 µl per hybridization	0.2–1.5 µg in 20.2 µl per hybridization	0.4–0.5 µg in 10.1 µl per hybridization
Sample DNA when using CytoChip Oligo Spike-ins	1.0–1.5 µg in 19.2 µl per hybridization	0.2–1.5 µg in 19.2 µl per hybridization	0.4–0.5 µg in 9.1 µl per hybridization

Table 7 Reagents required for restriction digestion of gDNA

Reagents Required	Company and Part Number
PCR tube (0.2 ml, thin walled, flip cap) or 96-well plate and adhesive seals	Thermo Scientific AB-0620 Thermo Scientific AB-0600, AB-0558
Commercial control DNA (100 ng/µl)	Promega G1521(female)/G1471(male) or SNP reference*
Alu I (10 U/µl)	Promega R6281 or provided in SureLabel32SNP [dUTP]
Rsa I (10 U/µl)	Promega R6371 or provided in SureLabel32SNP [dUTP]

Reagents Required	Company and Part Number
10X buffer C OR 10X RE buffer	Supplied with Rsa I Supplied with SureLabel 32SNP [dUTP]
Acetylated BSA (10 µg/µl)	Supplied with Rsa I or with SureLabel 32SNP [dUTP]
Nuclease-free water	Supplied in labeling kit Illumina PR-30-413401 or PR-30-413437-00
CytoChip Spike-in Controls (optional)**	Illumina PR-40-415301-00

*For SNP arrays, use a fully genotyped human reference DNA (provided with the SureLabel32SNP [dUTP] Fluorescent Labeling System).
**CytoChip Oligo Spike-in Controls can be added at the digestion OR labeling step.

Restriction Digestion of gDNA Procedures

1. Thaw 10X buffer C or RE buffer and acetylated BSA. To collect contents, briefly vortex and centrifuge. Retain reagents on ice while in use and return promptly to -25°C to -15°C.
2. For each reaction:
   a. Add the amount of genomic DNA to the appropriate nuclease-free tube or well in the PCR plate.
   b. Add enough nuclease-free water to bring the final volume to 20.2 µl (2x and 4x formats) or 10.1 µl (8x format).
   c. If using spike-ins controls, add enough nuclease-free water to bring the sample volume to 19.2 µl (2x and 4x formats) or 9.1 µl (8x format).
3. If using spike-in controls:
   a. Remove a spike-in strip from the freezer and thaw on ice. Make sure that caps are tightly closed.
   b. To collect contents, briefly vortex the strip and then centrifuge shortly. Orient the strip with the cap hinge away from you.
   c. Accurately transfer 1 µl of Spike-in from each well to each tube or each well of the 96-well plate containing the sample genomic DNA.
   d. Record the spike-in that has been added to each sample.

CAUTION
Add the sample tracking spike-ins to the sample DNA, do not add spike-ins to the wells containing reference DNA.

4 Prepare the Digestion Master Mix on ice in the order listed in Table 8. Briefly vortex and centrifuge the Digestion Master Mix.

Table 8 Digestion Master Mix

Component	Amount (2x and 4x formats) 1 rxn	Amount (2x and 4x formats) 16 rxns*	Amount (8x formats) 1 rxn	Amount (8x formats) 16 rxns*
Nuclease-free water	2.0 µl	33.6 µl	1.0 µl	16.8 µl
10X buffer C or RE buffer	2.6 µl	43.7 µl	1.3 µl	21.8 µl
acetylated BSA	0.2 µl	3.4 µl	0.1 µl	1.7 µl
Alu I (10U/µl)	0.5 µl	8.4 µl	0.25 µl	4.2 µl
Rsa I (10U/µl)	0.5 µl	8.4 µl	0.25 µl	4.2 µl
Final volume	**5.8 µl**	**97.5 µl**	**2.9 µl**	**48.7 µl**

*master mix includes 5% excess

5 Add 5.8 µl (2x or 4x formats) or 2.9 µl (for 8x formats) of the Digestion Master Mix to each reaction tube containing the genomic DNA. Make a total volume of 26 µl (2x or 4x formats) or 13 µl (for 8x formats). Pipette to mix.

6 Transfer the samples to a thermal cycler and run the program in Table 9.

Table 9 Thermal cycler program for restriction digestion of gDNA

Step	Temperature	Time
1	37°C	2 hours
2	65°C	10 minutes
3	4°C	hold

7 Take 2 µl of the digested genomic DNA and run on a 0.8% agarose gel to assess the completeness of the digestion. Make sure that most of the digested products are less than 500 bp in length (see *Agarose Gel Visualization of Digested gDNA* on page 25).

a There will be 24 µl (2x or 4x formats) or 11 µl (8x formats) of digested genomic DNA remaining.

b Add back the 2 µl as nuclease-free water to bring the volumes back to 26 µl and 13 µl in preparation for labeling.

NOTE
Complete digestion is essential for UPD/LOH calling to be effective with SNP microarrays.

8 Proceed directly to *Labeling (2x and 4x Formats)* on page 13 or *Labeling (8x formats)* on page 15. Digested gDNA can be stored for up to a month at -25°C to -15°C.

Labeling Materials

The materials listed are for 16 reactions, sufficient for 8 hybridizations.

Table 10 Starting materials for labeling

Starting Materials	Amount (2x and 4x formats)	Amount CytoChip Focus 4x180K	Amount (8x formats)
Sample/reference DNA	1.0–1.5 µg in 26 µl per hybridization	0.2–1.5 µg in 26 µl per hybridization	0.4–0.5 µg in 13 µl per hybridization
Sample DNA when using CytoChip Oligo Spike-ins	1.0–1.5 µg in 25 µl per hybridization	0.2–1.5 µg in 25 µl per hybridization	0.4–0.5 µg in 12 µl per hybridization

Table 11 Materials required for labeling

Materials Required	Amount (2x and 4x formats)	Amount (8x formats)	Part Number
Commercial control DNA* (100 ng/µl)	0.2–1.5 µg per hybridization	0.4–0.5 µg per hybridization	Promega G1471/G1521
PCR tube (0.2 ml, thin walled, flip cap) or 96-well plate and adhesive seals	16 PCR tubes or 1 96-well plate	16 PCR tubes or 1 96-well plate	Thermo Scientific AB-0620 Thermo Scientific AB-0600, AB-0558
Fluorescent Labeling System [dUTP]	Half a kit	Quarter of a kit	Illumina PR-30-413401/ PR-30-413437-00 or in pack
Amicon Ultracel-30 membrane (columns included in SNP Packs)	16	16	Millipore UFC503024 (24), UFC503096 (96)
TE (pH 8.0)	16 ml	16 ml	
Microcentrifuge tube (1.5 ml, flip cap)	50	50	Sarstedt 72.690.001
CytoChip Spike-in Controls (optional)**	1 µl per sample	1 µl per sample	Illumina PR-40-41530-00

*For the control, match the DNA quantity used in the sample - CytoChip Focus 4x180K can be as low as 200 ng.

**CytoChip Oligo Spike-in Controls can be added at the digestion OR labeling step.

For more information on required materials, see *Labeling Notes* on page 25.

Labeling (2x and 4x Formats)

NOTE
Perform all steps on ice unless otherwise indicated.

1 Thaw components from Fluorescent Labeling System, and then vortex briefly. To collect contents, centrifuge the components. Retain on ice.

2 If using spike-in controls, remove a spike-in strip from the freezer and thaw on ice. Make sure that caps are tightly closed.

a Briefly vortex the strip followed by a short centrifugation. Orient the strip with the cap hinge away from you.

b Accurately transfer 1 µl of spike-in from each well to each tube or each well of the 96-well plate containing the sample genomic DNA.

c Record the spike-in that was added to each sample.

CAUTION

Add the sample tracking spike-ins to the sample DNA, do not add spike-ins to the wells containing reference DNA.

3 Add 5 µl of random primers to each reaction tube containing 26 µl of gDNA to make a total volume of 31 µl. Pipette up and down gently to mix. Centrifuge to collect the contents.

4 Transfer samples to a prewarmed lidded thermal cycler at 95°C for 5 minutes (if restriction digestion used) or for 10 minutes (if no restriction digestion is used). Then transfer *immediately* to ice or to a precooled thermal cycler for 5 minutes at 4°C.

NOTE

Rapid cooling of the denatured labeling mixes is critical for high efficiency labeling. Poor cooling can be associated with increased dye bias in GC-rich regions, for example 1 p and chromosomes 19 and 22.

5 Centrifuge the samples for 1 minute at 6,000 × g to collect the contents at the bottom of each tube. Centrifuge PCR plates at 170 × g.

6 Prepare the labeling master mixes by adding the components in the quantities and order listed in Table 12.

Table 12 Labeling master mix components and quantities (2x and 4x formats)

Component	Cap color	Cy3 labeling mix 1 rxn	Cy3 labeling master mix 8 rxns*	Cy5 labeling mix 1 rxn	Cy5 labeling master mix 8 rxns*
5x Reaction buffer		10 µl	84 µl	10 µl	84 µl
10x dNTP		5 µl	42 µl	5 µl	42 µl
Cy3 dUTP		3 µl	25.2 µl		
Cy5 dUTP				3 µl	25.2 µl
Exo-Klenow fragment		1 µl	8.4 µl	1 µl	8.4 µl
Total		**19 µl**	**159.6 µl**	**19 µl**	**159.6 µl**

*Labeling master mix includes 5% excess.

7 Add 19 µl of the labeling master mix to each reaction tube to make a total volume of 50 µl. Cy3 mix is added to the sample gDNA and Cy5 is added to the reference. Gently pipette up and down to mix. Centrifuge to collect the contents.

8 Transfer the samples to a thermal cycler and run the program in Table 13.

Table 13 Thermal cycler program for labeling (2x and 4x formats)

Step	Temperature	Time
1	37°C	2 hours
2	65°C	10 minutes
3	4°C	hold

9 Proceed directly to *Combination* on page 16. Alternatively, reactions can be stored up to a month at -25°C to -15°C in the dark.

Labeling (8x formats)

NOTE
Perform all steps on ice unless otherwise indicated.

1 Thaw components from Fluorescent Labeling System, vortex briefly. Centrifuge to collect the contents. Retain on ice.

2 If using spike-in controls, remove a spike-in strip from the freezer and thaw on ice. Make sure that caps are tightly closed.
   a Briefly vortex the strip, then briefly centrifuge. Orient the strip with the cap hinge away from you.
   b Accurately transfer 1 µl of spike-in from each well to each tube or each well of the 96-well plate containing the sample genomic DNA.
   c Record the spike-in that was added to each sample.

CAUTION
Add the sample tracking spike-ins to the sample DNA, do not add spike-ins to the wells containing reference DNA.

3 Add 2.5 µl of random primers to each reaction tube containing 13 µl of gDNA to make a total volume of 15.5 µl. Pipette up and down gently to mix. Centrifuge to collect the contents.

4 Transfer samples to a prewarmed lidded thermal cycler at 95°C for 5 minutes (if restriction digestion used) or for 10 minutes (if no restriction digestion is used). Then transfer immediately to ice or to a precooled thermal cycler for 5 minutes at 4°C.

NOTE
Rapid cooling of the denatured labeling mixes is critical for high efficiency labeling. Poor cooling can be associated with increased dye bias in GC-rich regions, for example 1 p and chromosomes 19 and 22.

5 Centrifuge the samples for 1 minute at 6,000 × g to collect the contents at the bottom of each tube. Centrifuge PCR plates at 170 × g.

6 Prepare the labeling master mixes by adding the components in the quantities and order listed in Table 14.

Table 14 Labeling master mix components and quantities (8x formats)

Component	Cap color	Cy3 labeling mix 1 rxn	Cy3 labeling master mix 8 rxns*	Cy5 labeling mix 1 rxn	Cy5 labeling master mix 8 rxns*
5x Reaction buffer		5 µl	42 µl	5 µl	42 µl
10x dNTP		2.5 µl	21 µl	2.5 µl	21 µl
Cy3 dUTP		1.5 µl	12.6 µl		
Cy5 dUTP				1.5 µl	12.6 µl
Exo-Klenow fragment		0.5 µl	4.2 µl	0.5 µl	4.2 µl
Total		**9.5 µl**	**79.8 µl**	**9.5 µl**	**79.8 µl**

*Labeling master mix includes 5% excess.

7 Add 9.5 µl of the labeling master mix to each reaction tube to make a total volume of 25 µl. Cy3 mix is added to the sample gDNA and Cy5 mix is added to the reference. Gently pipette up and down to mix. Centrifuge to collect the contents.

8 Transfer the samples to a thermal cycler and run the program in Table 15.

Table 15 Thermal cycler program for labeling (8x formats)

Step	Temperature	Time
1	37°C	2 hours
2	65°C	10 minutes
3	4°C	hold

9 Proceed directly to *Combination*. Alternatively, reactions can be stored up to a month at -25°C to -15°C in the dark.

Combination

The labeled genomic DNA is cleaned up using Amicon Ultracel-30 membrane filters (AU-30), purchased separately or provided with SureLabel32SNP [dUTP], followed by vacuum concentration if necessary. Labeled sample and reference genomic DNA is combined for each hybridization area. If many samples are being processed simultaneously, use a 96-well plate format for more convenient processing. See www.chem.agilent.com (part G4410-90010) for the recommended use of AutoScreen-96A Well plates (GE Healthcare p/n 25- 9005- 98).

1 Centrifuge the labeled genomic DNA samples for 1 minute at 6,000 × g to collect the contents at the bottom of each tube, or at 170 × g for PCR plates. Transfer each sample or reference to a separate 1.5 ml microcentrifuge tube.

2 Add 430 µl of 1x TE (pH 8.0) to each reaction tube.

3 For each sample or reference, place an AU-30 filter into a collection tube (supplied with Amicon filters) and load each labeled gDNA into the filter. Centrifuge at 14,000 × g at room temperature for 10 minutes, discard flow through.

4 Return the filter to the collection tube, add 480 µl of 1x TE (pH 8.0) to each filter, centrifuge at 14,000 × g at room temperature for 10 minutes, discard flow through.

5 Invert the filter into a fresh collection tube (supplied), centrifuge at 1,000 × g at room temperature for 1 minute to collect the purified sample.

6 Measure the volume collected with a pipette. There will be approximately 21 µl, add 1x TE, or use a vacuum concentrator to bring the sample to the volume required:

Table 16 Sample volume required for combination

	2x105K	4x Format	8x60K
Collected Volume	21 µl	21 µl	21 µl
1x TE	20 µl	-	-
Volume required	**41 µ**	**21 µl**	**9.5 µl**

7 For the 8x60K formats, concentration of the sample is required. Place sample tubes in a vacuum concentrator (prewarmed to 75°C or higher) with tops open and evaporate to dryness (~20–40 minutes). Then reconstitute with 9.5 µl of 1x TE. If no vacuum concentrator is available, ethanol precipitation can be used (see *Ethanol Precipitation of Labeling* on page 26).

NOTE

With a miVAc Duo (Genevac) fitted with a swing rotor for microplates (Genevac #DRS-00000-200), evaporation is completed in 40–45 minutes at 75°C.

8 Take 1.5 µl of each sample and use a NanoDrop Spectrophotometer to determine DNA concentration, dye incorporation, and specific activity for each sample (see *Recording DNA Yield and Dye Incorporation* on page 27).

9 Combine the Cy3 labeled sample and the Cy5 labeled reference DNA for each hybridization area.

10 Proceed to *CytoChip Oligo Hybridization*. Alternatively, reactions can be stored up to a month at -25°C to -15°C in the dark.

CytoChip Oligo Hybridization

This section describes combination of the labeled DNA with blocking agents and Hybridization Buffer, followed by hybridization using Agilent hybridization chambers.

Before proceeding with *Hybridization*, prepare the 10X blocking agent. Add 900 µl of nuclease-free water to the vial containing the lyophilized 10X blocking agent. Leave the vial at room temperature for 1 hour. Vortex before use or store the vial at -25°C to -15°C.

For more comprehensive guidance on loading samples and assembly/disassembly of Agilent Microarray Hybridization Chambers, refer to the Agilent Microarray Hybridization Chamber User Guide (G2534-90001).

NOTE
The "Agilent"-labeled barcode is on the active side of the slide.

Materials

Table 17 Starting materials for hybridization

Starting Material	8 hybs on 4 2x format slides	8 hybs on 2 4x format slides	8 hybs on 1 8x format slide	Part number, or included in Packs
COT Human DNA	200 µl	40 µl	16 µl	PR-40-413503-00
2X Hi-RPM buffer	1040 µl	440 µl	180 µl	Supplied with Labeling Kit
10X blocking agent (reconstituted)	208 µl	88 µl	36 µl	Supplied with Labeling Kit
CytoChip Oligo 2x105K slides and gasket slides	4			PR-21-408001-00
CytoChip ISCA 4x44K slides and gasket slides		2		PR-21-408003-00/ PR-21-408010-00
CytoChip ISCA 4x180K slides and gasket slides		2		PR-21-408006-00/ PR-21-408013-00
CytoChip ISCA 8x60K slides and gasket slides			1	PR-21-408005-00
CytoChip Focus 8x60K slides and gasket slides			1	PR-21-408024-00
CytoChip Focus 4x180K slides and gasket slides		2		PR-21-408026-00
CytoChip Oligo SNP 4x180K slides and gasket slides		2		PR-23-438006-00
Agilent Microarray Hybridization Chambers	4	2	1	Agilent G2534A

CAUTION
Microarray slides and gasket slides are made of glass. Handle with care. Inspect the slides for any damage or imperfections before removing from the packaging to make sure that no damage has occurred in transit.

Preparation

1 Prewarm a hotblock to 95°C.

2 Prewarm the Agilent Hybridization Oven to 65°C.

3 Prewarm a heat block or waterbath to 37°C.

Hybridization

1 To each tube containing labeled DNA, add COT, blocking agent, and Hybridization Buffer according to Table 18. To collect the contents, mix and pulse centrifuge each tube.

Table 18 CytoChip hybridization reagents

Reagent	Volume per 2x105K hybridization	Volume per 4x format hybridization	Volume per 8x60K hybridization
Labeled DNA solution (test and reference combined)	79 µl	39 µl	16 µl
COT Human DNA	25 µl	5 µl	2 µl
10X blocking agent	26 µl	11 µl	4.5 µl
2X Hybridization Buffer (Hi-RPM)	130 µl	55 µl	22.5 µl
Total	**260 µl**	**110 µl**	**45 µl**

2 Incubate for 3 minutes at 95°C in a prewarmed hotblock. Transfer immediately to a prewarmed hotblock or waterbath at 37°C, and incubate for a further 30 minutes. Cool to room temperature and pulse centrifuge.

3 Load a clean gasket slide of the correct format (2x, 4x, or 8x) into an Agilent Microarray Hybridization Chamber base with the gasket label facing upwards over the rectangular alignment area.

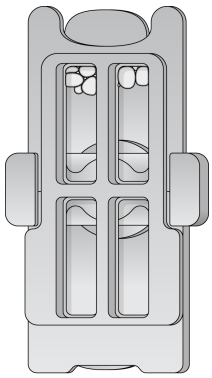
4 Using a "drag and dispense" method, slowly dispense 245 µl (2x105K), 100 µl (4x formats), or 40 µl (8x60K) of labeled hybridization mixture onto a gasket well. For more information, see *Loading Hybridization Solution* on page 28.
   a To prevent leakage, avoid contacting the o-ring of the gasket slide with the hybridization mixture.
   b Load hybridization mixture into all gasket wells before applying the CytoChip Oligo slide. See *Multiformat Subarray Layout* on page 29 for the naming of subarrays on the different CytoChip Oligo formats.

5 Place a CytoChip Oligo slide array-side down onto the gasket slide, with the numeric barcode facing upwards and the Agilent-labeled barcode downwards. The Agilent-labeled side is the active side of the array. Make sure that the slide and gasket are aligned (see *Assembly of Agilent Hybridization Chamber* on page 29).

6 Place the hybridization chamber cover onto the chamber base. Slide the clamp assembly onto the chamber and hand-tighten.

7 Holding the assembled chamber vertically (see Figure 2), rotate clockwise 3 times to wet the slides and assess the mobility of the bubbles (see *Quality of Bubbles*). If necessary, tap the chamber on a hard surface to move stationary bubbles.

Figure 2 Assembled hybridization chamber



8 Place the assembled slide chamber in the rotator rack in a prewarmed Hybridization Oven at 65°C. Rotate at 20 rpm and hybridize at 65°C for 24 hours (4x formats or 8x60K), or 40 hours (2x105K).

9 Proceed with *CytoChip Oligo Washing*.

CytoChip Oligo Washing

Hybridized CytoChip Oligo slides are washed to remove unhybridized labeled DNA. This protocol uses a ClearHyb Wash System. If a ClearHyb Wash System is not available, see *CytoChip Oligo Oven-Based Wash* on page 31. For general guidance on wash conditions, see *Additional Wash Notes* on page 32. Cover the room temperature wash in foil to keep dark.

Materials and Equipment

Prepare the following buffers:

Table 19 CytoChip Oligo wash buffers

Wash	Volume	Temp	Times	Agitation	Buffer
Disassembly	400 ml	RT		None	CytoChip Oligo aCGH Wash Buffer 1, Illumina PR-70-413506-00
1	400 ml	RT	5 min	2.5 cm stir bar	CytoChip Oligo aCGH Wash Buffer 1, Illumina PR-70-413506-00
2	500 ml	37 °C	1 min	None	CytoChip Oligo aCGH Wash Buffer 1, Illumina PR-70-413506-00

Prepare the following equipment:

Table 20 CytoChip Oligo wash equipment

Item	Number	Comments
400 ml lidded square glass staining dishes	2	Use for disassembly and wash 1.
ClearHyb Wash System	1	Pre-fill the waterbath with CytoChip Oligo aCGH Wash Buffer 1 preheated to 37 °C for 30 minutes in preparation for wash 2.
25 position stainless steel staining rack	1	Place the rack in wash 1
Magnetic stirrer	1	
2.5 cm stir bar	1	

NOTE
Glass containers are recommended for washing. For room temperature washes, use foil covers over glass jars to protect slides from light.

NOTE
Calibrate the ClearHyb unit following the manufacturer instructions. When the ClearHyb is calibrated, recheck the temperature using a calibrated thermometer before each wash.

Wash Procedures

1 Disassemble hybridization chambers on the bench (see *Disassembly of Agilent Hybridization Chambers* on page 32).

a Submerge the gasket and microarray slide in the CytoChip Oligo aCGH Wash Buffer 1 in a square glass staining dish.
b Separate the gasket slide from the microarray.
c Transfer the CytoChip Oligo microarrays to the slide rack in wash 1. Only touch the edge or barcode of the slide.

2 When the rack is fully loaded (wash no more than 12 slides at a time), replace glass lid, switch on stirrer and adjust for good, but not vigorous, stirring. Cover with foil and stir for 5 minutes at room temperature.

3 Transfer the slide rack to the ClearHyb containing prewarmed CytoChip Oligo aCGH Wash Buffer 1 at 37°C, and incubate for 1 minute.

4 *Optional*: In a high-ozone environment, use the Ozone-Barrier slide cover kit supplied by Agilent (G2505-60550). The Ozone-Barrier slide cover protects Cy dyes from ozone degradation.

5 Slowly lift the slide rack out of the ClearHyb, allowing the liquid to draw droplets off the microarray surface. It takes 10–12 seconds to remove the slide rack. Store the slides in the original packaging.

6 Proceed with *Scanning*.

Scanning

A laser scanner is used to excite the hybridized fluorophores and record the resulting images of the hybridization. A two channel scanner is required to image the Cy3 and Cy5 signals produced by the independently labeled sample and control DNA. The resulting images are stored in TIFF format file, which the BlueFuse Multi analysis software reads.

Any 2 channel laser scanner equipped with the following lasers is able to scan a CytoChip Oligo slide.

- Green laser, 532 nm wavelength – used to excite and read the Cy3 signal.
- Red laser, 635 nm wavelength – used to excite and read the Cy5 signal.

White light, CCD based, scanners can also be used. However, such devices are likely to produce poorer results, which are more difficult to interpret and report.

Although there some laser scanners available from commercial suppliers, they all share a basic workflow.

NOTE
2x105K and 4x44K formats require 5 µm scanning resolution.
4x180K and 8x60K formats require 3 µm scanning resolution.
Illumina does not support 2 µm scanning.

Prescan

CytoChip oligos have 2 (2x105K), 4 (4x44K, 4x180K) or 8 (8x60K) hybridization areas. The prescan provides a low-resolution image of the entire slide, which is used to identify the hybridization areas. Most scanners also read and interpret the barcode of the slide during the prescan, which can be used when saving the file.

For some scanners, such as those supplied by Agilent, it can be more convenient to scan the whole slide at higher resolution. The image can then be split using Image Viewer software available for download at www.cambridgebluegnome.com/account-login. Alternatively, images can be split during batch import into BlueFuse Multi.

Scan Resolution

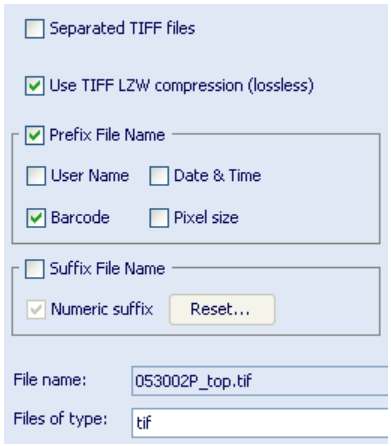
Typically scans are produced from 2 to 10 µm resolution. High-resolution scans of 5 µm (2x105K, 4x44K) and 3 µm (4x180K, 8x60K) are recommended for CytoChip Oligo arrays.

Saving of TIFF images

To simplify the workflow, it is recommended that the following settings are used when saving TIFF images (see Figure 3).

- Place the barcode within the first or second string of the file name. For example, **158028A**_S01_TOP.tif or US85103613_**158028A**_S01_TOP.tif, and NOT rimilab_2010-07-16_12h38m_**158028A**_CY3-34627_CY5-34759_TOP.tif.
- Store all the scans, potentially 2 prescans (Cy3 and Cy5) and 2 high-resolution scans, in a single, multiframe TIFF file.
- Use a consistent suffix, such as “top” and “bottom”, to identify the hybridization area that is included in the file.

Figure 3 Saving TIFF images



If these recommendations are followed, the BlueFuse Multi Software automatically loads the resulting images, saving time and minimizing a potential source of error.

Proceed with Data Analysis. Refer to the BlueFuse Multi Walkthrough guide.

Appendix

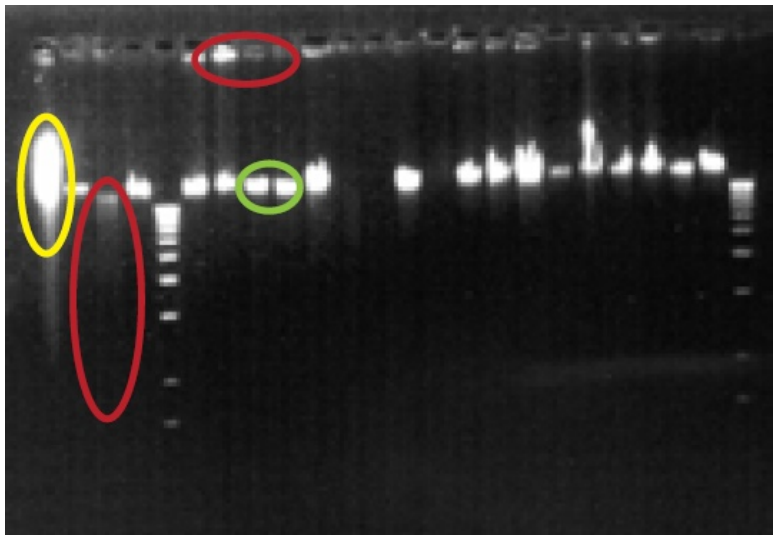
DNA Quality

Confirm that the unsheared, undigested, genomic DNA is:

High molecular weight	> 10–20 kbp
Protein/RNA free	OD 260/280 > 1.8
Solvent free	OD 260/230 > 2.0

As an additional quality check, run the DNA on a 0.8–1% agarose gel (seeFigure 4). DNA of high molecular weight appears as a series of tight bands towards the middle of gel (marked in green). Fragmented DNA, with a mixture of molecular weights appear as a vertical smear (marked in red on left). Impurities appear as small bands at the top (marked in red at top). Overloading of the gel can result in overly bright bands, which make it difficult to interpret the gel correctly (marked in yellow).

Figure 4 DNA that is run on 1% agarose gel



DNA that fails to meet these quality criteria can be prepared using this protocol, which describes ethanol precipitation, to reduce salt contamination.

Store NanoDrop readings, gel images, and extraction protocols in the sample record of the BlueFuse Multi analysis software.

Labeling Notes

Agarose Gel Visualization of Digested gDNA

Figure 5 Examples of good gDNA digestion and poor gDNA digestion

Fully digested gDNA is visible on an agarose gel with most fragments sized less than 500 bp, as shown on the left gel in Figure 5. Poorly digested samples, shown on the right gel in Figure 5, have a continuous smear of DNA, with no significant size concentration of fragments. Full digestion is imperative for effective LOH calling when using SNP arrays. The digested samples from the gel on the right did not perform well on CytoChip Oligo SNP arrays.

gDNA Input Amount and Volume per Microarray

At least 1.0 µg of genomic DNA is required in labeling reactions for 2x105K or 4x formats (0.4 µg minimum is required for 8x60K format). Equal amounts of genomic DNA are required for both the experimental and reference channels.

Table 21 gDNA input amount and volume per microarray

Microarray format	gDNA input requirement (µg)	Volume of gDNA with restriction digestion (µl)	Volume of gDNA without restriction digestion (µl)
2x105K or 4x formats	1.0 to 1.5	20.2	26
8x60K	0.4 to 0.5	10.1	13

Choice of Reference DNA

If the sex of the sample is known, selection of reference DNA is based on the sex of the sample. In general, sex-mismatched designs are used when array CGH approaches are first implemented because the offset of the X chromosome provides useful information on assay performance. Sex-matched designs are more commonly used when consistent results are obtained. If the sex of the sample is unknown, use a dyeswap design with one hybridization undertaken with male control DNA and the other undertaken with female control DNA.

For standard CytoChip Oligo arrays, commercial pooled DNA is recommended. However, there is evidence that results are improved when sample and control DNA are extracted using the same technique.

SNP platforms require a single well-characterized reference with a known SNP genotype. Do not pool reference DNA. Illumina supplies SureLabel32SNP [dUTP] with SNP arrays that includes SNP reference DNA.

Component Quantities for Single Reactions

The Fluorescent Labeling System is supplied in a 32 reaction format optimized to produce master mixes for 16 reactions for each Cy dye. Take care when making master mixes for smaller numbers of reactions, particularly with Cy dyes, so that there are sufficient reagents to complete the total of 16 reactions. For example, a volume of 0.8 µl/hour is lost to evaporation from an open tube.

Ethanol Precipitation of Labeling

For the 8x60K array format, it is necessary to reduce the volume of the labeled samples after cleaning up with the AU-30 filters. A vacuum concentrator is recommended However, there is an alternative method using ethanol precipitation. First check the DNA yield and specific activity using a NanoDrop spectrophotometer (and record in BlueFuse Multi if necessary). Then combine the labeled sample and reference together with the COT human DNA before performing the ethanol precipitation. This step improves the DNA recovery.

NOTE

If the volumes recovered from the Amicon columns are greater than 22 µl, it is also necessary to concentrate the samples for 4x format. Therefore volumes are also included here for 4x formats in **bold**.

Table 22 Acceptable values for DNA yield and dye incorporation after labeling and clean-up. Values are based on the volumes recovered from the clean-up step from 500 ng of starting material.

Volume	Concentration (ng/µl)	Cy3 incorporation (pmol/µl)	Specific activity Cy3 (pmol/µg)	Cy5 incorporation (pmol/µl)	Specific activity Cy5 (pmol/µg)
21 µl	117 to 167	3–6.8	>11.3	2.2–5.9	>9
25 µl	98 to 140	2.6–5.7	>9.5	1.9–5.0	>7.6

NOTE
Without restriction digestion, the specific activity values will be about 2 pmol/µg lower than values indicated in this table.

1 Combine ~21 µl of labeled test, ~21 µl of labeled reference with 2 µl (**5 µl**) of COT human DNA.

2 Add 5.5 µl of sodium acetate, mix thoroughly and add 150 µl of 100% ethanol. Invert to mix.

3 Precipitate for 2 hours at -25°C to -15°C (recommended). Alternatively, precipitation can be achieved in 30 minutes at -80°C.

4 Centrifuge at full speed (≥ 13,000 × g) for 15 minutes and decant supernatant

5 Add 300 µl of 70% ethanol and invert the tube three times to wash the pellet.

6 Centrifuge at full speed (≥ 13,000 × g) for 5 minutes and decant supernatant.

7 Pulse tube in centrifuge and remove remaining ethanol with a P10 tip. Cap tube.

8 Remove cap and allow the pellet to air dry for 5 minutes at room temperature. Make sure that there is no residual ethanol visible around the pellet before proceeding as ethanol will inhibit subsequent labeling reactions.

9 Resuspend in 18 µl (**44 µl**) of 1x TE (pH 8.0).

10 Return to *Hybridization* on page 19. At step 1, there is no need to add COT.

Recording DNA Yield and Dye Incorporation

Use the NanoDrop 2000 UV-VIS Spectrophotometer or equivalent to measure the yield, degree of dye incorporation of labeling reactions.

1 From the main menu, select MicroArray Measurement, then from the Sample Type menu, select DNA-50.

2 Use 1.5 µl of TE to blank the instrument.

3 Use 1.5 µl of purified labeled genomic DNA for quantification. Measure the absorbance at A260nm (DNA), A550nm (cyanine 3), and A650nm (cyanine 5).

4 Record the dye incorporation (pmol dye/µl) for Cy3 or Cy5 for each sample or reference.

5 Record the gDNA concentration (ng/µl) for each sample.

6 Calculate the specific activity of your sample:

$$\text{Specific activity (pmol/µg)} = \frac{\text{dye incorporation pmol per µl dye}}{\text{DNA concentration µg per µl genomic DNA}}$$

7 Acceptable values, after labeling and clean-up:

Table 23 Acceptable values if vacuum concentrator is used

CytoChip Oligo array	Concentration (ng/µl)	Cy3 incorporation (pmol/µl)	Specific activity Cy3 (pmol/µg)	Cy5 incorporation (pmol/µl)	Specific activity Cy5 (pmol/µg)
8x60K	260 to 370	7–15	>25	5–13	>20
4x44K, 4x180K	240 to 330	6–13	>25	5–12	>20
2x105K	120 to 170	3–7	>25	2–6	>15

NOTE
Without restriction digestion, the specific activity values are about 5 pmol/µg lower than values indicated in Table 23.

8 Record DNA yield and dye incorporation for each reaction or save NanoDrop file for later import into the BlueFuse Multi analysis software.

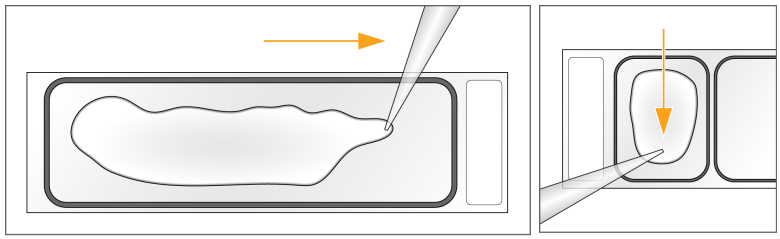
NOTE
If a NanoDrop instrument is not available, use other spectrophotometers (eg, Epoch BioTek Gen 5). Measure the Cy3 incorporation by taking the absorbance at 553 nm and, after removing the value of the blank, divide by 0.015 to give pmol/µl. Measure the Cy5 incorporation by taking the absorbance at 649 nm and, after removing the value of the blank, divide by 0.025 to give pmol/µl.

Hybridization Notes

Loading Hybridization Solution

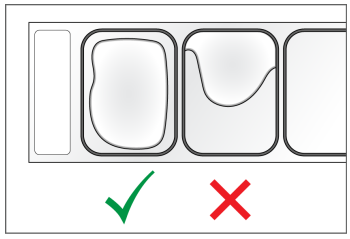
Load the hybridization solution onto the appropriate gasket slide for the array format. With the gasket slide already in the hybridization chamber, slowly dispense your sample while moving your pipette to the opposite end of the well without touching the slide. Use a "drag and dispense" pipetting method (see Figure 6).

Figure 6 Drag and dispense method



To avoid leakage of the hybridization solution, avoid touching the gasket with the pipette. Dispense the solution in the center of the gasket (see Figure 7).

Figure 7 Correct placement of hybridization solution in gasket slides



NOTE

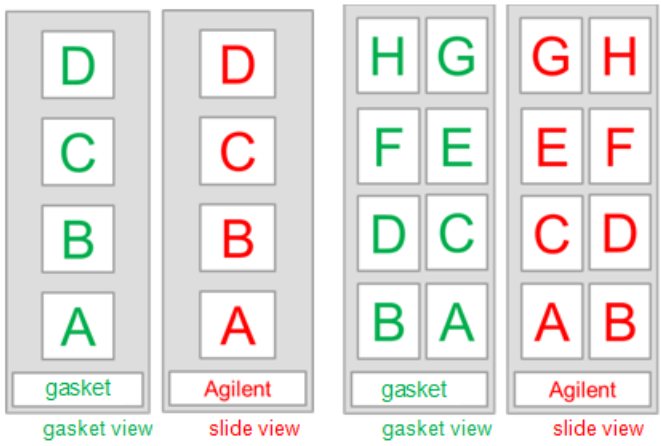
These images are for demonstration purposes only. Load gasket slides in the hybridization chamber before loading the hybridization solution.

Multiformat Subarray Layout

The 2 subarrays of the CytoChip Oligo 2x105K microarray are named top and bottom by the image viewer software/BlueFuse Multi batch import process.

Similarly, the 4 subarrays of the 4x array formats are labeled A-D, and the 8x60K format subarrays are labeled A-H. Gasket view is how the gasket slide looks as you apply the samples and slide view is how the slide looks on screen after scanning (see Figure 8).

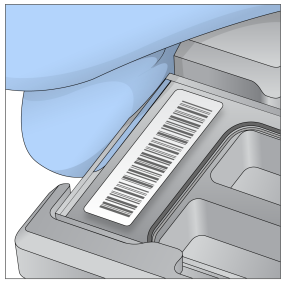
Figure 8 Gasket View and Slide View



Assembly of Agilent Hybridization Chamber

1 When all gaskets are loaded with the correct hybridization solution, retrieve the CytoChip Oligo slide. With the numeric barcode facing up, carefully lower on top of the gasket slide.

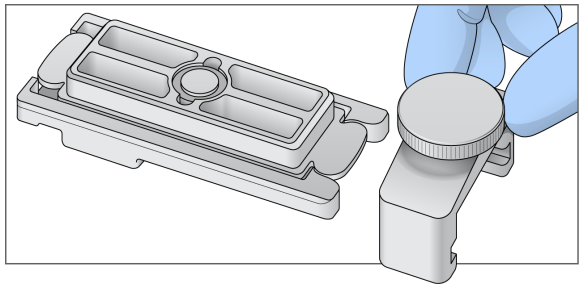
Figure 9 CytoChip Oligo placed in hybridization chamber gasket slide



2 Place the chamber cover, correct side facing up, onto the chamber base containing both slides.

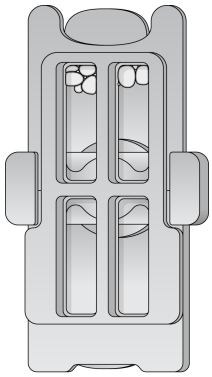
3 Slip the clamp assembly onto the chamber base and cover until it stops firmly in place. Tighten the thumbscrew on the completed chamber assembly.

Figure 10 Hybridization chamber and clamp assembly



4 Rotate the final assembled chamber 2 to 3 times to wet the gaskets and to make sure that the hybridization solution will coat the entire surface of the microarray during the incubation process. Assess the mobility of the bubbles.

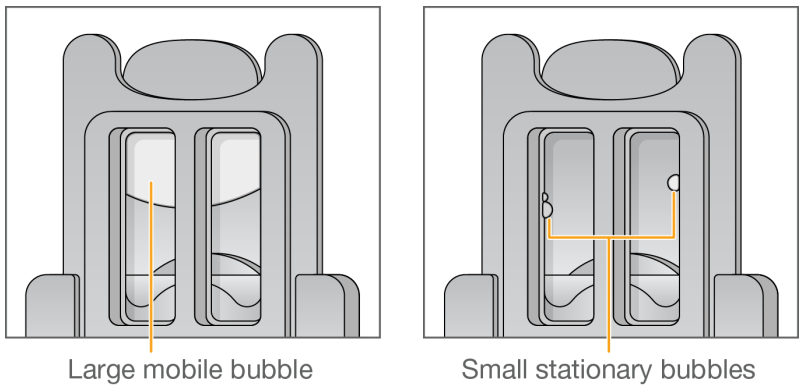
Figure 11 Assembled hybridization chamber



Quality of Bubbles

A single, large mixing bubble that freely moves around the chamber when rotated is best. Multiple bubbles are acceptable as long as they move when you rotate the chamber. If necessary, remove stationary bubbles by gently tapping each corner of the chamber assembly on a firm surface.

Figure 12 Examples of bubbles in hybridization chamber



Washing Notes

CytoChip Oligo Oven-Based Wash

Prepare the following buffers

Table 24 CytoChip Oligo oven-based wash buffers

Wash	Volume	Temp	Times	Agitation	Buffer
Disassembly	400 ml	RT		None	CytoChip Oligo aCGH Wash Buffer 1, Illumina PR-70-413506-00
1	400 ml	RT	5 min	2.5 cm stir bar	CytoChip Oligo aCGH Wash Buffer 1, Illumina PR-70-413506-00
2	500 ml	37 °C	1 min	None	CytoChip Oligo aCGH Wash Buffer 2, Illumina PR-70-413506-00

Prepare the following equipment

Table 25 CytoChip Oligo oven-based wash equipment

Item	Number	Comments
400 ml lidded square glass staining dish	1	Prewarmed to 37 °C in an oven (2 hrs)*
400 ml lidded square glass staining dish	1	For disassembly
25 position stainless steel staining rack	1	
Magnetic stirrer	2	One prewarmed to 37 °C in an oven (2 hrs)*
2.5 cm stir bar	1	

*For optimal stringency it is important that CytoChip Oligo microarrays are washed in oligo aCGH Wash Buffer 2 at 37°C. Prewarm the buffer, staining dish, and magnetic stirrer in a calibrated oven for 2 hours.

1 Disassemble the hybridization chambers on the bench. Separate the gasket slide from the microarray in 400 ml square glass staining dish. Transfer the CytoChip Oligo microarrays to the slide rack in wash 1 touching only the edge or barcode of the slide (see *Disassembly of Agilent Hybridization Chambers* on page 32).

2 When rack is fully loaded, replace lid and protect from light with a foil cover. Switch on stirrer and adjust for good, but not vigorous, stirring. Stir for a further 5 minutes at room temperature. Meanwhile, prepare wash 2.

3 Prepare wash 2.
   a Working quickly to keep materials warm, empty the water from the prewarmed square glass dish.
   b Add a 2.5 cm stir bar and place on the magnetic stirrer in the oven at 37°C.
   c Pour 400 ml of prewarmed CytoChip Oligo aCGH Wash Buffer 2 into the dish and replace the lid.
   d Close the oven door to keep the materials at 37°C.

4 Transfer slide rack to wash 2 in oven for 1 minute.

5 *Optional:* In a high-ozone environment, use the Ozone-Barrier slide cover kit supplied by Agilent (G2505-60550). The Ozone-Barrier slide cover is a highly effective way of protecting Cy dyes from ozone degradation.

6 Slowly lift the slide rack out wash 2, allowing the liquid to draw droplets off the microarray surface. It takes 5–10 seconds to remove the slide rack. Store slides in original packaging.

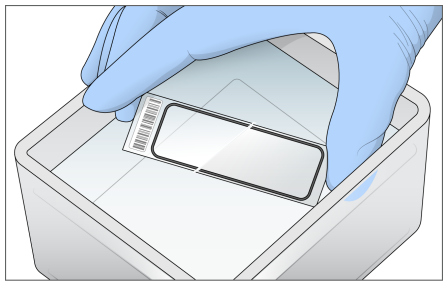
Additional Wash Notes

- Complete all room temperature washes in dishes covered with silver foil.
- Always keep the rack of CytoChips in buffer. Transfer the rack of CytoChips immediately after the completion of each wash.
- To reduce risk of contact between the stir bar and the array surface, place the CytoChips in the rack facing away from the stir bar.
- When using a stirred wash, the rack holds the CytoChips above the stir bar. A vortex is visible, but the CytoChips are always covered by liquid.

Disassembly of Agilent Hybridization Chambers

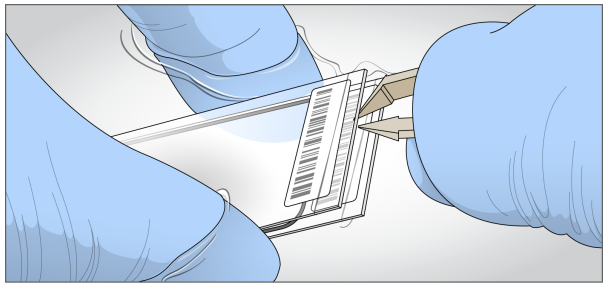
1 Remove 1 hybridization chamber from the incubator and place on a flat surface.
   a Loosen the thumbscrew and slide the clamp off the assembly.
   b Remove the chamber cover.
   c With gloved fingers, hold the slide/gasket pair by the short ends and remove from the chamber base.
   d Submerge and hold in the disassembly bath with the numeric barcode of the CytoChip Oligo facing upwards.

Figure 13 Slide and gasket submerged in the disassembly bath



2 Keeping the slide/gasket pair submerged, pry the sandwich open at the barcode end using plastic forceps.
   a Allow the gasket slide to drop to the bottom of the dish.
   b Minimizing exposure to air, quickly transfer the CytoChip Oligo microarray to the slide rack in the wash 1, touching the edges or barcode of the slide only.

Figure 14 Removal of CytoChip Oligo slide from the gasket slide



Equipment List

Table 26 Equipment required for the CytoChip Oligo protocol

Name	Description	Alternative Recommendations	Company and Part No.
Benchtop centrifuge	Variable speed benchtop centrifuge—1.5 ml tubes adjustable from 2000 × g upwards.		eg, Eppendorf 5430/5424
Lidded thermal cycler	To take microplates with temperature range 4°C to 95°C.		eg, Applied Biosystems:Veriti (4375786)
Swing out centrifuge	Large centrifuge with swing out bucket stainless to hold steel slide racks. Typically a microplate swing out bucket is appropriate.	If a swing out centrifuge is unavailable, it is possible to complete labeling in microplates, which simplifies workflow.	eg, ThermoScientific Heraeus Megafuge 40, Eppendorf 5804 with A-2-DWP rotor
Agarose gel electrophoresis equipment	To enable the visualization of the digestion products.		Numerous options
Spectrophotometer	UV-VIS spectrophotometer.		NanoDrop ND-2000
Evaporator centrifuge	miVAc Duo (Genevac)		eg, Genevac #DRS-00000-200
Hotblock	Hotblock to take 1.5 ml tubes with temperature range up to 95°C.	Lidded thermal cycler or 95°C water bath.	eg, Techne FDB02DD
Agilent Hybridization Oven	For hybridizing CytoChip Oligo arrays	SciGene Mai Tai® Hybridization System	Agilent (G2545A)
Hybridization oven rotator for Agilent Microarray Hybridization Chambers	Accessory for fitting Agilent Hybridization Chambers into Hybridization Oven	SciGene Mai Tai® Hybridization System	Agilent (G2530-60029)

Name	Description	Alternative Recommendations	Company and Part No.
Agilent Microarray Hybridization Chamber	Chambers for clamping gasket slides to CytoChip Oligo arrays	SciGene Mai Tai® Hybridization System	Agilent (G2534A)
400 ml square glass staining dishes and lids	To take stainless steel slide racks.		Part of PR-70-431001-00
Clearhyb Wash system	Precision water bath to maintain wash temperature		PR-70-432201-00 (230 V)/PR-70-432202-00 (115 V)
Coplin jar	Coverslip removal		eg, Solmedia SJ320
Magnetic stirrer	Magnetic stirrer with 2.5 cm stir bar.		eg, Bibby Stuart SB161-3 (3 position stirrer)
Laser scanner	Dual channel fluorescent laser scanner with 532 nm and 635 nm lasers.		Innopsys Innoscan 710 and 710AL with Mapix-CS software
Agilent Ozone-Barrier Slide Cover Kit	Covers the oligo array surface and protects against ozone degradation of signal		Agilent (G2505-60550)

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 27 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 28 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.