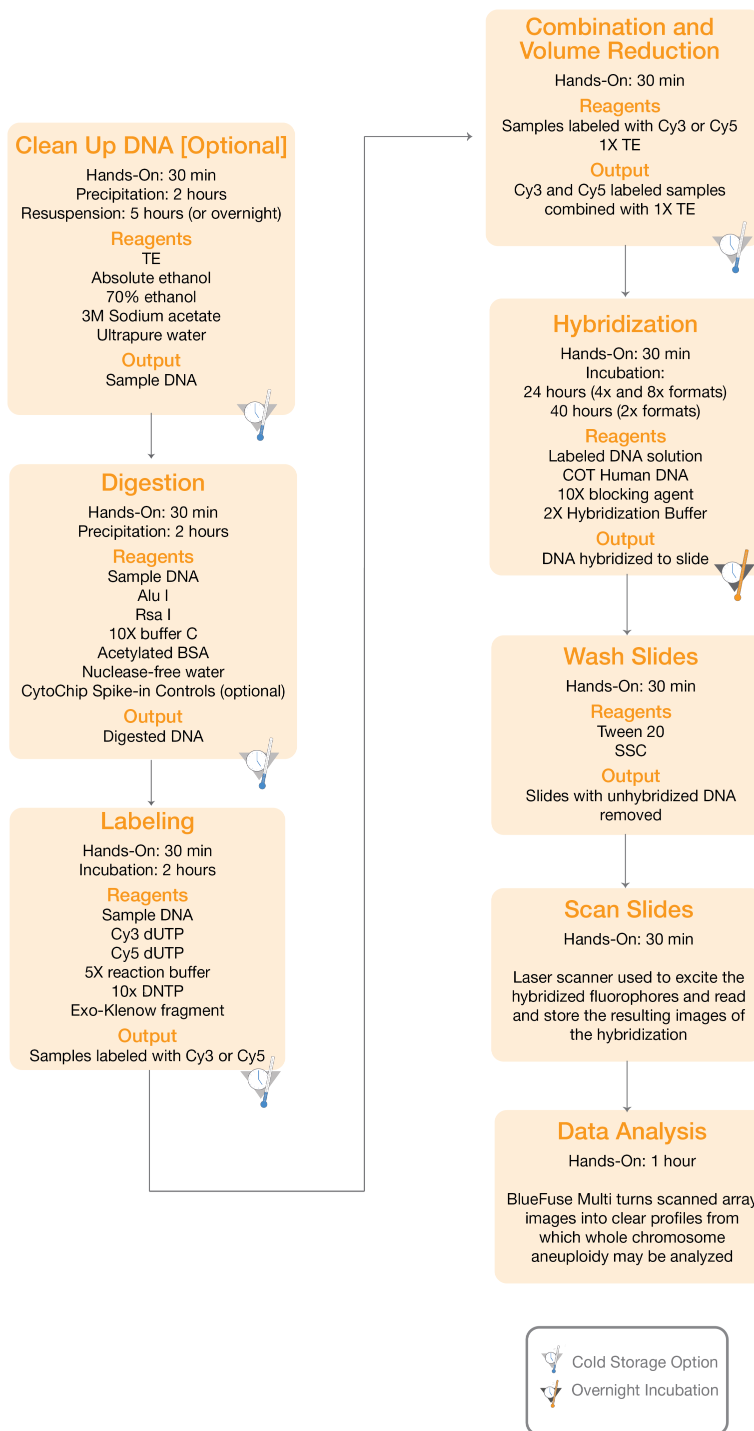
# CytoChip Oligo Summary Protocol

## Experienced User Card

FOR RESEARCH USE ONLY



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# Sample Preparation

## Materials

Table 1 Starting materials for sample preparation

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

Table 2 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 co-precipitant.

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

[_] 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.

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

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# CytoChip Oligo Labeling

## Restriction Digestion of gDNA

Table 3 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 4 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] |
| 10X buffer C<br>OR 10X RE buffer | Supplied with Rsa I<br>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

### Estimated Time

Hands-on time: 30 minutes

Incubation time: 2 hours

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## Steps

[_] 1 Thaw 10X buffer C or RE buffer and acetylated BSA. Briefly vortex and centrifuge to collect contents. 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 Briefly vortex the strip followed by a short centrifuge to gather the contents. 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 5. Briefly vortex the Digestion Master Mix and centrifuge to collect the contents.

Table 5 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.

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[_] 6 Transfer the samples to a thermal cycler and run the program in Table 6.

Table 6 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. Most of the digested products should be less than 500 bp in length.

- [_] 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 6 or *Labeling (8x formats)* on page 7. 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 7 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 8 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<br>Thermo Scientific AB-0600, AB-0558 |

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| Materials Required | Amount (2x and 4x formats) | Amount (8x formats) | Part Number |
|---|---|---|---|
| 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.

## Labeling (2x and 4x Formats)

### Estimated Time

Hands-on time: 30 minutes

Incubation time: 2 hours

### Steps

NOTE
Perform all steps on ice unless otherwise indicated.

[_] 1 Thaw components from Fluorescent Labeling System, vortex briefly, and centrifuge to collect 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 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. If necessary, centrifuge to collect the contents at the bottom of the tube.

[_] 4 Transfer samples to a pre-warmed 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 pre-cooled thermal cycler for 5 minutes at 4°C.

[_] 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 9.

Table 9 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. If necessary, centrifuge to collect the contents at the bottom of the tube.

[_] 8 Transfer the samples to a thermal cycler and run the program in Table 10.

Table 10 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 9. Alternatively, reactions can be stored up to a month at -25°C to -15°C in the dark.

## Labeling (8x formats)

### Estimated Time

Hands-on time: 30 minutes

Incubation time: 2 hours

Date/Time: ______________________________ Operator: ______________________________



## Steps

NOTE
Perform all steps on ice unless otherwise indicated.

[_] 1 Thaw components from Fluorescent Labeling System, vortex briefly, and centrifuge to collect 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. If necessary, centrifuge to collect the contents at the bottom of the tube.

[_] 4 Transfer samples to a pre-warmed 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 pre-cooled thermal cycler for 5 minutes at 4°C.

[_] 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 11.

Table 11 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. If necessary, centrifuge to collect the contents at the bottom of the tube.

[_] 8 Transfer the samples to a thermal cycler and run the program in Table 12.

Table 12 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

[_] 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 13 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 (pre-warmed 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.

[_] 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.

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[_] 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.

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# CytoChip Oligo Hybridization

## Materials

Table 14 Starting materials for hybridization

| Starting Material | 8 hybs on four 2x format slides | 8 hybs on two 4x format slides | 8 hybs on one 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 Pre-warm a hotblock to 95°C.

[_] 2 Pre-warm the Agilent Hybridization Oven to 65°C.

[_] 3 Pre-warm a heat block or waterbath to 37°C.

Date/Time: ______________________________ Operator: ______________________________



# Hybridization

## Estimated Time

Hands-on time: 30 minutes

Incubation time: 24 hours (4x and 8x formats) or 40 hours (2x formats)

## Steps

[_] 1 To each tube containing labeled DNA, add COT, blocking agent, and Hybridization Buffer according to Table 15. Mix and pulse centrifuge to collect the contents at the bottom of the tube.

Table 15 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 pre-warmed hotblock. Transfer immediately to a pre-warmed 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.

  [_] 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.

[_] 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.

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

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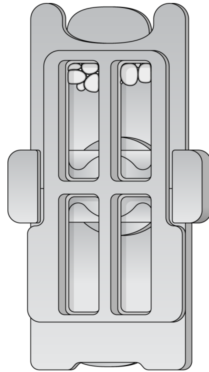
[_] 7 Holding the assembled chamber vertically (see Figure 1), rotate clockwise three times to wet the slides and assess the mobility of the bubbles. If necessary, tap the chamber on a hard surface to move stationary bubbles.

Figure 1 Assembled hybridization chamber



[_] 8 Place the assembled slide chamber in the rotator rack in a pre-warmed 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*.

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# CytoChip Oligo Washing

## Materials and Equipment

### Prepare the following buffers:

Table 16 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 2, Illumina PR-70-413506-00 |

### Prepare the following equipment:

Table 17 CytoChip Oligo wash equipment

| Item | Number | Comments |
|---|---|---|
| 400 ml lidded square glass staining dishes | 2 | For disassembly and wash 1. |
| ClearHyb Wash System | 1 | Waterbath insert should be pre-filled CytoChip Oligo aCGH Wash Buffer 2 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.

## Wash Procedures

### Estimated Time

Hands-on time: 10 minutes

Incubation time: 1 minute

### Steps

[_] 1 Disassemble hybridization chambers on the bench.

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[_] 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, touching only 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 pre-warmed CytoChip Oligo aCGH Wash Buffer 2 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 should take 10–12 seconds to remove the slide rack. Store the slides in the original packaging.