CytoChip Summary Protocol

Experienced User Card

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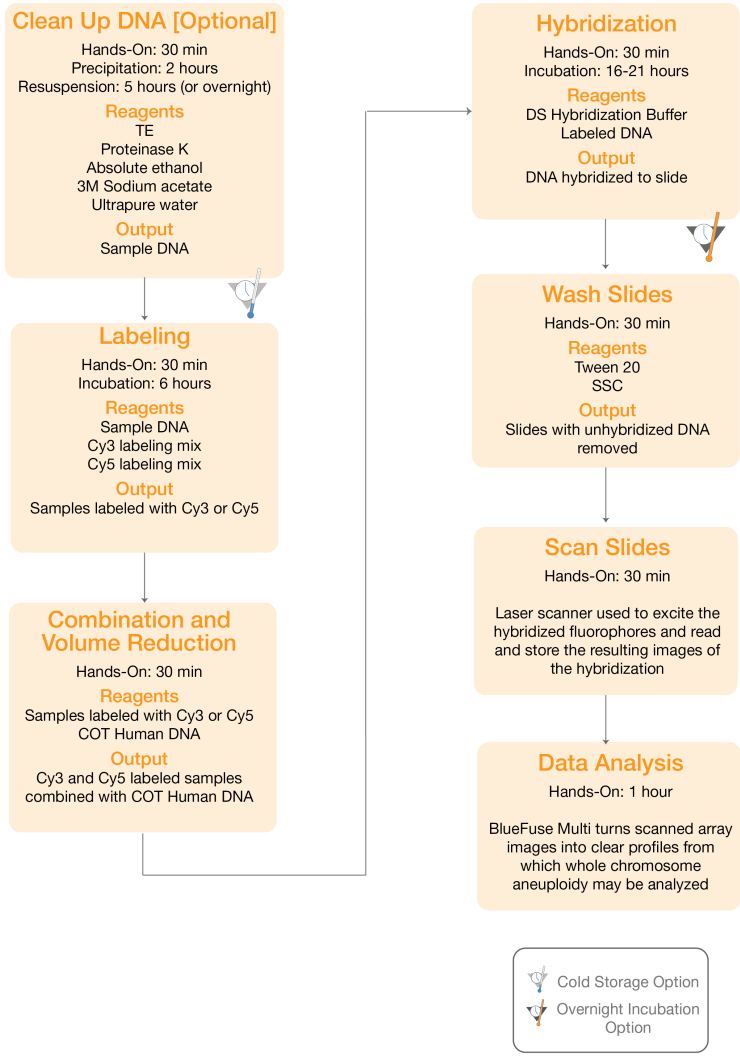

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Sample Preparation

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
3M sodium acetate (pH 5.2)	5 µl	Sigma S7899
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
Proteinase K (20 mg/ml)	1 µl	Sigma P4850

Clean Up DNA

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

[_] 2 Add 1 µl of Proteinase K and incubate for at least 5 hours at 56°C. Spin down.

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

[_] 5 Precipitate DNA for 2 hours at -25°C to -15°C.

[_] 6 Centrifuge at full speed (≥ 13,000 × g) for 15 minutes and decant the supernatant.

[_] 7 Add 500 µl of 70% ethanol and invert the tube three times to wash pellet.

[_] 8 Centrifuge at full speed (≥ 13,000 × g) for 5 minutes and decant the supernatant.

[_] 9 Pulse the tube in a centrifuge and remove the remaining ethanol with a P10 tip. Cap tube.

[_] 10 Remove the cap and allow the pellet to air dry for 5 minutes at room temperature.

[_] 11 Add ultrapure water to give a final concentration of approximately 100 ng/µl.

[_] 12 Resuspend for 5 hours, or overnight, at room temperature.

[_] 13 Quantify DNA to confirm that OD readings are in line with recommendations.

[_] 14 Proceed immediately to *CytoChip Labeling* or store DNA at 2°C to 8°C until required.

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CytoChip Labeling

Labeling Materials

The materials listed are for 32 reactions, sufficient for 16 hybridizations.

Table 3 Starting materials for labeling

Starting Materials	Amount
sample DNA (unsheared, undigested, genomic DNA, 100 ng/µl)	400 ng per hybridization

Table 4 Materials required for labeling

Materials Required	Amount	Part Number
Commercial control DNA (100 ng/µl)	400 ng per hybridization	Promega G1471/G1521
PCR tube (0.2 ml, thin walled, flip cap) or 96-well plate and adhesive seals	32 1	Thermo Scientific AB-0620 Thermo Scientific AB-0600, AB0558
Fluorescent Labeling System	1	Illumina PR-30-413103-00 or in pack
AutoSeq G50 columns and tubes	1	Illumina PR-40-413511-00 or in pack
COT Human DNA	400 µl	Illumina PR-40-413510-00 or in pack
3M sodium acetate (pH 5.2)	200 µl	Sigma S7899
Absolute ethanol	5 ml	
70% ethanol	5 ml	
Microcentrifuge tube (1.5 ml, flip cap)	34	Sarstedt 72.690.001

Labeling

Estimated Time

Hands-on time: 30 minutes

Incubation time: 6 hours

Steps

NOTE
Perform all steps on ice unless otherwise indicated.

[_] 1 Thaw the components from the Fluorescent Labeling System, vortex briefly and centrifuge to collect contents, retain on ice.

[_] 2 Prepare the labeling master mixes by adding the components in the quantities and order listed in Table 5 and briefly vortex to mix.

Table 5 Labeling master mix components and quantities

Component	Cap color	Cy3 labeling mix 1 rxn	Cy3 labeling master mix 16 rxns*	Cy5 labeling mix - 1 rxn	Cy5 labeling master mix 16 rxns*
Reaction buffer		10 µl	168 µl	10 µl	168 µl
Nuclease free water	Clear	19 µl	319.2 µl	19 µl	319.2 µl
Primer solution		10 µl	168 µl	10 µl	168 µl
dCTP-labeling mix		5 µl	84 µl	5 µl	84 µl
Cy3 dCTP		1 µl	16.8 µl		
Cy5 dCTP				1 µl	16.8 µl
Total		**45 µl**	**756 µl**	**45 µl**	**756 µl**

*Labeling master mix includes 5% excess.

[_] 3 Combine 4 µl (400 ng) of DNA with 45 µl of labeling mix in each PCR tube or in a 96-well plate.

[_] 4 Cap/seal and denature in a pre-warmed lidded thermal cycler for 5 minutes at 94°C. Then transfer *immediately* to ice or to a pre-cooled thermal cycler at 4°C for 5 minutes.

[_] 5 Add 1 µl of Klenow enzyme to each reaction, mix by flicking the tube (no vortex) and pulse to spin down contents.

[_] 6 Cap and incubate in a pre-warmed lidded thermal cycler for 6 hours at 37°C.

[_] 7 Add 5 µl EDTA solution to each reaction, mix, and centrifuge for 30 seconds.

[_] 8 Use AutoSeq G50 columns to clean up labeling reactions into the numbered microcentrifuge tubes.

[_] 9 Gently vortex the cleaned up samples and record DNA yield and dye incorporation.

[_] 10 Pulse each numbered microcentrifuge tube.

Combination

There are two alternative protocols for combination and volume reduction of the labeled material: see *Combination and Ethanol Precipitation* on page 4 and *Combination and Centrifugal Evaporation* on page 5.

Combination and Ethanol Precipitation

[_] 1 Centrifuge the PCR tubes from *CytoChip Labeling*. Combine sample and control DNA into numbered microcentrifuge tubes by adding the Cy5 labeling product to the Cy3 labeling product for each hybridization area.

[_] 2 Add 25 µl COT Human DNA and 12.5 µl 3 M sodium acetate to each tube and vortex.

[_] 3 Add to each tube 344 µl of absolute ethanol and invert twice to mix.

[_] 4 Precipitate in dark for 2 hours at -25°C to -15°C, or on dry ice.

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[_] 5 Pellet labeled DNAs at full speed (centrifuge at ≥ 13,000 × g) for 10 minutes, discard the supernatant.

[_] 6 Add 500 µl of 70% ethanol, invert to mix, and centrifuge at full speed for 5 minutes.

[_] 7 Decant the supernatant. Keeping tube inverted, gently tap out remaining droplets onto folded tissue.

[_] 8 Pulse tube in centrifuge and remove remaining ethanol with a P10 tip.

[_] 9 Allow the pellet to air dry for 5 minutes at room temperature.

[_] 10 Proceed to *CytoChip Hybridization*.

Combination and Centrifugal Evaporation

[_] 1 Pre-warm a centrifugal evaporator, rotor, and PCR plate racks (if using) to 75°C (or higher) for 30 minutes, or until warm.

[_] 2 Centrifuge the PCR tubes from the labeling step to collect the contents.

[_] 3 Combine labeled sample and control DNA into numbered microcentrifuge tubes or PCR plate wells. Add the Cy5 labeling product to the Cy3 labeling product for each hybridization area.

[_] 4 Add 25 µl COT Human DNA to each tube or PCR plate well containing combined Cy3/Cy5 labeling products.

[_] 5 Transfer the tubes or plate to the pre-warmed centrifugal evaporator. Evaporate under centrifuge at 75°C (or high) until around 3 µl remains in each tube/well.

[_] 6 Proceed to *CytoChip Hybridization*.

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CytoChip Hybridization

Materials

The materials listed are for 32 reactions, sufficient for 16 hybridizations.

Table 6 Materials required for hybridization

Materials required	Amount	Part number
DS Hybridization Buffer (15% dextran sulphate)	336 µl	Part of Illumina PR-30-413103-00 inc. in pack
CytoChip Focus microarrays	8	2x Illumina PR-22-409501-00, inc. in pack
22x22 mm glass cover slips	16	Fisher Scientific FB58633, inc. in ClearPack Lite
ClearHyb Hybridization System	1	Illumina PR-70-432101-00 (230 V)/ PR-70-432102-00 (115 V)

Preparation

[_] 1 Place Hybridization Buffer in a heat block at 75°C to thaw and pre-warm.

[_] 2 Switch on the ClearHyb Hybridization System to pre-warm the chambers at 47°C for at least 30 minutes.

Hybridization

Estimated Time

Hands-on time: 30 minutes

Incubation time: 16 to 21 hours

Steps

[_] 1 Resuspend each pellet of combined labeled samples/references/COT in 21 µl of pre-warmed DS Hybridization Buffer at 75°C ensuring that pellet is dissolved. Pulse centrifuge to collect contents.

CAUTION
Add Hybridization Buffer in a fume cabinet.

[_] 2 Denature at 75°C for a further 10 minutes, centrifuge for 20 seconds to collect the contents.

[_] 3 Apply 18 µl of labeled DNA solution to each coverslip. Lower the slide barcode-down for each hybridization area in a fume cabinet.

[_] 4 Use the hybridization template to position coverslips and confirm which labeled DNAs are loaded on to each hybridization area.

[_] 5 Disassemble the pre-heated hybridization chambers and insert the slides into the racks at the open end. Take care to keep the loaded CytoChip slides flat and handle the slides by the edges (see Figure 1).

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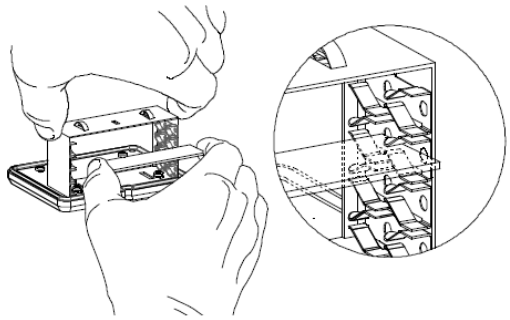
Figure 1

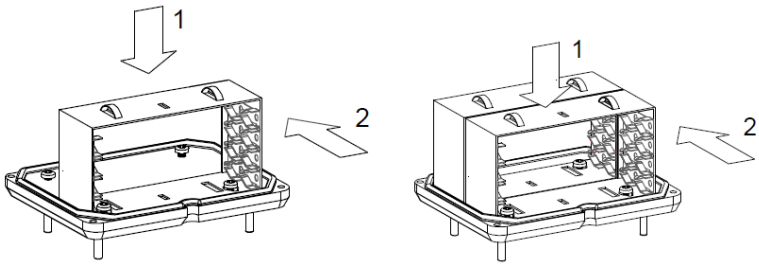


Hold slide by the ends and insert into rack.

Slides are held in place by spring clips.

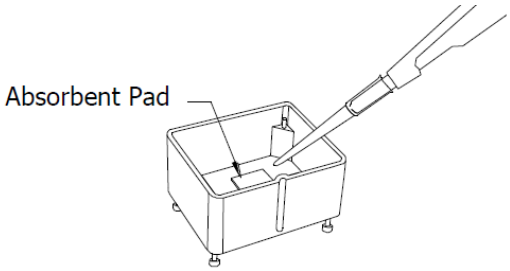
[_] 6 Clip the loaded racks into the base of the chamber by placing the racks on the base with the key holes over the keys and lock into place (see Figure 2).

Figure 2



[_] 7 Pipette 0.25 ml of distilled water onto a clean and dry absorbent pad (supplied with the ClearHyb Hybridization Kit) secured inside the lid and allow to soak in (see Figure 3).

Figure 3



[_] 8 Place the lid over the base and tighten the screws using the tool provided, starting with one corner, then the one diagonally opposed to keep the pressure even (see Figure 4). To avoid stripping the threads, take great care not to tighten the screws too tightly. Fingertip pressure is enough as the O ring provides a good seal.

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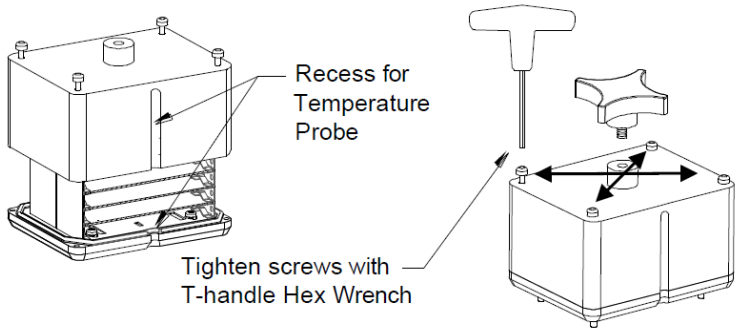
Figure 4



[_] 9 Load the hybridization unit into the ClearHyb Heating base unit for 16 to 21 hours at 47°C.

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CytoChip Washing

Materials and Equipment

Prepare the following buffers:

Table 7 CytoChip wash buffers

Buffer	Wash	Recipe
1000 ml 2x SSC/0.05% Tween 20	Remove cover slips and Wash 1	100 ml 20x SSC [pH 7.0], 899.5 ml H_2O, 0.5 ml Tween 20
500 ml 1x SSC	Wash 2	25 ml 20x SSC [pH 7.0], 475 ml H_2O
1000 ml 0.1x SSC	Wash 3 and 4	5 ml 20x SSC [pH 7.0], 995 ml H_2O

Prepare the following equipment:

Table 8 CytoChip wash equipment

Item	Number	Comments
Coplin jar/Hellendahl jar	1	Containing 2x SSC/0.05% Tween 20 at room temperature for removal of cover slips
400 ml lidded square glass staining dishes	3	Washes 1 (2x SSC/0.05% Tween 20), 2 (1x SSC) and 4 (0.1x SSC) at room temperature, inc. in ClearPack Lite
ClearHyb Wash System, ensure the ClearHyb unit is calibrated.	1	Wash 3 - waterbath insert should be pre-filled with 0.1x SSC and **preheated to 60°C for 30 minutes**
24 position stainless steel staining rack	2	Required as a balance in the centrifuge, inc. in ClearPack Lite
Magnetic stirrer	1	Inc. in ClearPack Lite
2.5 cm stir bar	3	Inc. in ClearPack Lite

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: 30 minutes

Steps

[_] 1 Prepare wash 1. Add 400 ml of 2xSSC/0.05% Tween 20 to a staining dish at room temperature. Add a 2.5 cm stir bar and the 24 position stainless steel rack.

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[_] 2 When hybridization incubation time is complete, disassemble the hybridization units and remove the slides from racks by pushing from behind, taking care to handle only the slides by the edges.

[_] 3 Remove the coverslips from each slide by manually agitating in 2xSSC/0.05% Tween 20 in a Coplin jar at room temperature. Transfer immediately to the stainless steel rack sitting in dish prepared in step 1. Repeat for all slides.

[_] 4 When rack is fully loaded, replace lid, turn on stirrer, start timer, cover with foil and complete wash 1 and subsequent washes summarized in Table 9.

Table 9 Wash Summary

Wash	Volume	Temp	Times	Agitation	Buffer
0	100 ml	RT	-	-	2x SSC/0.05% Tween 20 (to remove coverslips in Coplin jar)
1	400 ml	RT	10 min	2.5 cm stir bar	2x SSC/0.05% Tween 20
2	400 ml	RT	10 min	2.5 cm stir bar	1x SSC
3	500 ml	60 °C	5 min	None	0.1x SSC
4	400 ml	RT	1 min	2.5 cm stir bar	0.1x SSC

[_] 5 Dry slides by centrifugation at 170 × g for 3 minutes and store in original blue box.