Hybridizing sample for 60k

Prepare the 10× Blocking Agent:

1. Add 1,350 µL of DNase/RNase-free distilled water to the vial containing lyophilized 10× aCGH Blocking Agent (included in the Oligo aCGH/ChIP-on-chip Hybridization Kit).

2. Leave at room temperature for more than 6 hours and mix on a vortex mixer to reconstitute sample before use or storage.

Hybridizing sample

1. Check if the incubator is at 95°C

2. Re-hydrate pellets with 11.9µL of ddH2O (put on the wall of the tube).

3. Spin down, keep in dark.

4. Prepare Hybridization Solution Master Mix

60k	Each	1 slide	2 slides	3 slides	4 slides
2*HI-RPM Hybridization Buffer	27.5	247.5	495	742.5	962.5
10*Acgh Blocking Agent	5.5	49.5	99	148.5	192.5
Formamide	5.5	49.5	99	148.5	192.5
Cot-1 DNA	2.4	21.6	43.2	64.8	84
Universal standard	2.2	19.8	39.6	59.4	77
total buffer volume	43.1	387.9	775.8	1163.7	1508.5

* Add US in dark room

5. Add 43.1 µL of Hybridization Solution Master Mix into the tube

6. Mix well (~15 seconds) and spin down

7. Denature labeled DNA at 95°C for 3 minutes

8. Immediately transfer tubes to a 37°C incubator. Incubate at 37°C for 30 minutes

9. Spin 1 minute at 6000 × g, keep in 37°C incubator

10. Hybridization

- Check if hybridization oven is setting at 67°C and 20 rpm.

- Load a gasket slide into the Agilent SureHyb chamber base with label "Agilent" facing up

- Take 50 µL of the mixture and load about 48 µL onto a gasket well

Caution: Do not touch the gasket slides when loading the hybridization sample mixture.

- Put a microarray slide with label "Agilent" down onto the gasket slide, the numeric barcode side is facing up.

- Put the SureHyb chamber cover onto the sandwiched slides.

- Slide the clamp assembly onto both pieces.

- Hand-tighten the clamp firmly onto the chamber.

- Vertically rotate the assembled chamber, make sure that bubbles can smoothly move.

-Tap the assembly on a hard surface if necessary to move stationary bubbles.

- Place assembled slide chamber in the hybridization oven.

Caution: Be sure to balance the loaded hybridization chambers on the rack.

11. Hybridize the sample for 22-24 hours at 67°C.

12. Pre-warm 200mL **Wash Buffer 2** at 43°C overnight.

Washing Arrays

	Dish	Wash buffer	Temperature	Stirrer	Time
Disassembly	#1	Wash Buffer 1	RT		
1st wash	#2	Wash Buffer 1	RT	200 rmp	5 min
2nd wash	#3	Wash Buffer 2	37 °C	140 rmp	1 min

1. Minimize exposure of the slide to air.
2. Touch only the barcode portion of the microarray slide or its edges.
3. Spin dry the slide.

Scanning

1. Insert each slide with label "Agilent" side up and barcode end first into the slide holder.
2. Scan using the Multi-TIFF settings.