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aCGH Hybridization & wash

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

aCGH Hybridization

1 Turn on hybridization oven 65 °C
Turn on heat block 95 °C
Turn on heat block 37 °C

2 Mix Cy3 and Cy5 samples

3 Mix the following components in 1.5 centrifuge tubes

* Cy3/Cy5 gDNA mixture	39 µl
* Cot-1 DNA (1.3 µg/µl)	5 µl
* Agilent 10x blocking agent	11 µl
* Agilent 2x Hybridization buffer	55 µl
Total	110 µl

4 Mix sample by pipetting, by careful not to create any bubbles.

5 Spin down with maximum speed for a few seconds.

6 Heat for 00:03:00 at 95 °C and incubate immediately thereafter for 00:30:00 at 37 °C .

7 Leave samples in heat block, spin down for 00:01:00 at maximum speed.

8 Load gasket slide in hybridization chamber and dispense 100 µl hyb mix in a drag and drop manner (use a 200 µl pipette, be careful not to create any bubbles)

9 Place microarray slide – active side down – and assemble chamber.

10 Check for immobile air bubbles by rotating the assembled hybridization chamber. If necessary, slam side of the chamber on table to loosen the air bubbles.

11 Place assembled chamber in the hybridization oven (65 °C at 20 rpm) and make sure to balance with empty chambers.

12 Hybridize for 24:00:00 , 65 °C at 20 rpm.

aCGH wash

13 Turn on Agilent Slide Scanner

14 Wash buffer dishes with distilled water and blow dry with pressurized air.

15 Add buffers to dishes and have tin foil covering ready.-
Wash a maximum of 5 Agilent slides at the same time.

Dish	Method	Buffer	Temp (ºC)	Time (min)	
#1	Disassembly	Buffer 1	RT	-	
#2	1st wash step	Buffer 1	RT	5	(stirrer)
#3	2nd wash step	Buffer 2	37	1	(stirrer & hot plate)
#4	Dry step	Acetonitrile	RT	1	

16 Separate gasket slide from microarray slide in dish #1 (buffer 1)

17 Place slides in holding rack and was in dish #2 for 00:05:00 , rotation of stirrer is switched ON!

18 Cover dish with tin foil.

19 Start the "*Scan* Control *Software*"
For Agilent 180k, select the “AgilentG3_CGH” Option

20 Move holding rack to dish #3 (buffer 2) and wash for 00:01:00, rotation of stirrer and heating is switched ON!

21 Move holding rack to dish #4 (acetonitrile) leave for 00:01:00.

22 Remove slide gently with tweezers, thus preventing the formation of droplets.

23 Place slide in holder and load into the carousel of the scanner
Agilent Slide Scanner scans through the back of the slide (this side must contain the numeric barcode)

24 Click on “Set Values” to confirm all settings

25 Click Scan Slot m-n on the Scan Control main window
The letter m represents the Start slot where your first slide is located, and the letter n represents the End slot where your last slide is located.

26 Start the scan

27 Open “feature extraction” and check TIFF file