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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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21851

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 µlTotal 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 (°C)	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