

Stripping Protocol for Agilent Oligonucleotide Microarrays Hybridized with cRNA

Reagents:

1. 0.5 N EDTA from Sigma Cat# E-7889 (available at TCF)
2. 10 N NaOH from LabChem Inc Cat# NOLC24500-1 (On Chemical Shelf)
3. Triton X-102 (10%) from Agilent (Same as the one from amplification kit)

Solution Preparation:

1. 2N NaOH: 10 ml of 10 N NaOH and 40 ml distilled water
2. 1N NaOH/0.25N EDTA (Stock): 25 ml of 2N NaOH and 25 ml of 0.5 N EDTA

Name	20XSSC(ml)	TX-102 (ul)	1N NaOH/0.25N EDTA(ml)	H2O(ml)
8mM NaOH	20	300-500	3.2	380
4mM NaOH	20	300-500	1.6	380
2XSSC	100	500	0	900
1XSSC	50	0	0	950

Note: If you have used Agilent's Stabilization and Drying Solution to treat your slide, you need to clean your slide by immersing into acetonitrile for about 1 minute in a ventilated hood. If a slide is 3 month or more from product receipt, you may not achieve the good result with this protocol because of possible degradation of array's coating surface.

1. Pre-warm 380 ml ddH₂O in a 500ml bottle (you need 2, one for 8mM NaOH, the other for 4mM NaOH) to 60 to 62°C in a water-bath. **Make sure temperature can't be higher than 63°C. If so, add some distilled water into water bath to cool down a bit.**
2. Make 8mM NaOH and 4mM NaOH according to the above table.
3. Warm the bottles for another 20 minutes.
4. Pour the solution into a stain dish that was pre-warm in the water-bath. Wait 60 minutes. **This will allow solution to get rid of soluble air, hence avoid bubbles between array surface and the solution. (You may see those air bubbles if you do it without waiting).**
5. Immerse the glass slides into the solution with 8 mM NaOH in the stain dish for 8 minutes.
6. Transferred the glass slide to another stain dish containing 4mM NaOH in the water-bath for 5 minutes. Leave this solution in water bath in case you need more treatment cycle as indicated in step 14.
7. 2xSSC Wash, 20 minutes at room temperature.
8. Repeat step 7.
9. 1xSSC Wash, 30 minutes at room temperature.
10. Repeat step 9.
11. Wash with 0.1x SSC for 10 minutes.
12. Take slides out slowly one by one and make sure no water droplets on glass surface. Wipe the four edges and barcode area. Left dry in the air. **Note: Do not spin**
13. Scan at Red PMT 550 and Green PMT 450 with Axon scanner. **Rule of Thumb (empirically): To determine whether a slide needs to be treated another cycle, set the image intensity at 91 and contrast at 99 when scanning. If no clear feature (spots) is observable, your slide is ok and doesn't need another cycle and jump to step 15. Otherwise, go to step 14 to next cycle.**
14. Go to stripping Cycle at **step 6 to 13** if necessary. **If you go back to step 6 too often, you may want empirically to increase time of treatment at step 2 up to 15 minutes to avoid "Cycle".**
15. Do as described at step 12.
16. Wash for 1 minute with acetonitrile.
17. Store under vacuum until used within 1 month.