

Protocol for Microarray Hybridization of cDNA and Oligo Microarray Slides

Notes:

- Adapted from HGMP Protocol (http://www.hgmp.mrc.ac.uk/Research/Microarray/HGMP-RC_Microarrays/protocols.jsp)
- Make sure solution and materials are dust free.
- Start at step 14 with pre-fixed slides.
- For drying slides we centrifuge in open metal staining racks in a Heraeus MegaFuge 2.0 using plate adapters. These steps can also be done in a 50 ml tube in a Camlab plastic slide holder.
- Steps 1-25 can be done during the early stages of labeling in the 1 hour incubations. Place the chamber lid on loosely and place in safe area (usually in drawer).

Solutions Required:

- Pre-Fixing Buffer:
 - 10g BSA
 - 150 ml 20x SSC Solution
 - Fill to 1000 ml with DEPC treated H₂O
- Pre-Hybridization Buffer:
 - 10g BSA
 - 100 µl 10% SDS solution
 - 150 ml 20x SSC solution
 - Fill to 1000 ml with DEPC treated H₂O.
- Solution 1:
 - 25 ml 20x SSC solution
 - 10 ml 10% SDS solution
 - Fill to 1000 ml with Milli-Q H₂O
- Solution 2:
 - 25 ml 20x SSC solution
 - 1000 µl 10% SDS solution
 - Fill to 1000 ml with Milli-Q water
- Solution 3:
 - 3 ml 20x SSC solution
 - Fill to 1000 ml with Milli-Q water.

Experiment:

1. Use samples prepared according to Microarray labeling short.doc
2. Turn on water bath to 55°C and one onto 42°C.
3. Pre-warm 45 ml of the pre-fixing buffer at 55°C for 20 mins.
4. Place the slide into a slide holder (Camlab) and place into the pre-fixing buffer. Turn the tube up-side down several times to remove air bubbles.
5. Incubate at 55°C for 1 hour.
6. Fill a 50 ml tube with Milli-Q highly filtered water.

7. Wash the slide in the water by lifting the slide holder up and down (2 times) in the tube containing the water.
8. Remove the slide from the holder, and place the slide into a rack.
9. Centrifuge the slide at 1000 rpm for 5 minutes to dry.
10. Place a Pyrex dish (or 2L beaker) into the microwave and bring water to near boiling, and then place the dish onto a hot plate, and bring to boil.
11. Turn off heat and immerse rack into basin containing boiling water, and leave for 2 minutes.
12. Remove rack, and place the rack into a glass chamber containing 95% Ethanol. Leave for 1 minute.
13. Centrifuge in rack at 1000 rpm for 5 minutes.
14. Fill a 50 ml tube with 45 ml of pre-hybridization buffer and place at 55°C to pre-warm for at least 20 minutes.
15. Add slide to a slide holder and place it into the tube containing pre-hybridization solution tube and incubate at 55°C for 25 minutes.
16. Fill two 50 ml tubes with 40 ml Milli-Q water and one with 100% Ethanol.
17. Remove slide and place into a tube containing Milli-Q water.
18. Raise and lower the slide holder 5 times to wash.
19. Repeat step 18 with second tube of water.
20. Place slide into tube containing 100% ethanol, and raise and lower the slide holder 5 times.
21. Remove and place in rack and centrifuge for 5 minutes at 1000 rpm.
22. Clean the hyb chamber (Array-IT) and remove dust by using compressed air.
23. Place the slide array side up in the hyb chamber.
24. Away from the array, clean a Lifter Slip coverslip (Erie Scientific) with compressed air to remove dust.
25. Place the coverslip onto the slide covering the array area making sure that the white strips are down creating a slight gap between the array and the coverslip.
26. Pipette 38 μ l of sample prepared using Microarray Labeling. Doc at the front of the coverslip (half at each end, capillary action will take sample under).
27. Make sure no air bubbles are present and the whole array is covered.
28. Add 20 μ l of 3x SSC into each end of a chamber once cleaned with Mill-Q water.
29. Close the hybridization chamber clamp lid down tightly (use screw driver if necessary).
30. Place whole apparatus in a water bath or oven at 42°C.
31. Leave ON (at least 10 hours).
32. Fill one 50 ml falcon tube with solution 1.
33. Add the slide to the tube, and shake off the cover slip by lifting the slide up and down.
34. Fill one glass box was filled with Solution 1, one with solution 2 and one with solution 3.
35. The rack was placed in the box containing solution 1.
36. Place the slide into the rack in the chamber filled with solution 1.
37. Cover the box with tin foil.
38. The whole box should then be placed on the shaker for 4 minutes mixing at a gentle speed.
39. Remove the rack and place in the box containing solution 2, cover the box with tin foil. Shake again for 4 minutes.
40. Remove the rack and place in the box containing solution 3, cover the box with tin foil. Shake again for 4 minutes.
41. Remove the rack, and centrifuge for 5 minutes at 1000 rpm (large Heraeus Megafuge).
42. Once dry place in dark box and take to scanner.