

**Fluorescent Probe (aRNA) labeling and
Hybridization to cDNA MicroArrays.**
(V5.4 jun 2003)

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Fluorescent Probe (aRNA) labeling for microarray hybridization

- mix

aRNA (amplified antisense RNA)	2 μ g
random hexamer pd(N) ₆	3 μ g
H ₂ O	till 21 μ l

- Incubate 10 min @ 70°C

- Add:

5x Superscript 1 st strand buffer	8 μ l
0.1 M DTT	4 μ l
0.5 mM dA/C/G TP, 0.2 mM dTTP	2 μ l
RNAsin	1 μ l
Cy3 or Cy5 dUTP	2 μ l
Superscript II	2 μ l

- Incubate 1hr @ 42°C, add 1 μ l Superscript II

- Incubate 1hr @ 42°C then place on ice

- Purify the probes using a Qiagen mini-elute PCR clean up column
 1. Add 5 volumes buffer PB to labeled probe
 2. Transfer to Qiagen column
 3. Spin 1 minute 14k rpm, discard flow through
 4. Add 750 μ l buffer PE (alternatively use 80% EtOH)
 5. Spin 1 minute 14k rpm, discard flow through
 6. Repeat steps 4 and 5 one more time
 7. Spin additional minute to dry membrane
 8. Apply collection tube to column
 9. Add 10 μ l Elution buffer (buffer EB, or Tris pH 8)
 10. Spin 1 minute max speed (14k rpm)

Alternatively purify probes using a Qiagen PCR clean up column

Step 1- 8 as above

9. Add 50 μ l Elution buffer (buffer EB, or Tris pH 8)

10. Spin 1 minute max speed (14k rpm)

Then perform an EtOH precipitation to reduce volume

Add 3 μ g COT-1 DNA as carrier

Add 5 μ l SodiumAcetate (3 M, pH 5.6)

Add 150 μ l EtOH (100%)

Mix by vortexing

Store in -20°C for 15 minutes

Spin 20' MAX speed

Wash pellet with 150 μ l EtOH (70%)

Resuspend in 5 μ l RNase free H₂O

Random Hexamers	20 µg	Pomega	Cat# C1181
SuperscriptII rev transcriptase	10000 units	Gibco	Cat# 18064-014
Cy3 dUTP	25 nmol	Amersham	Cat# PA55022
Cy5 dUTP	25 nmol	Amersham	Cat# PA53022
RNAasin	1000 units	Gibco	Cat#15518-012
dNTPs	25 µM ea	Amersham	Cat# 27-2035-01
MinElute PCR Purification Kit	50	Qiagen	Cat# 28004
Qiaquick PCR purification Kit	50	Qiagen	Cat# 28104

Hybridization of cDNA microarrays

Pre-hybridization of cDNA microarrays (hybridized in Ambion buffers or other buffers that contain formamide)

- Prehybridize for at least 2 hrs at 42°C in a plastic 5-slide mailer using 5xSSC, 0.1% SDS, 1% BSA.

20 ml: 5 ml 20x BSA
0.2 ml 10% SDS
12.8 ml H₂O



- Wash slides 2 x 5 minutes in sterile water, wash on a rotating platform, or mix the washing buffer carefully every min.
- Wash slides 2 minutes in isopropanol (alternatively: wash the slides 3 min in 80% EtOH, followed by 3 min 100% EtOH).
- Air-dry or spin-dry the slides. They are ready for use in microarray hybridizations.

Preparing Probe/Hybridization mixture:

Microarrays (18k) are hybridized in a final volume of 40-80 µl depending on the size of the cover slips. This protocol is further written for a hybridization volume of 45 µl

- Heat probe 5 min @ 100°C (dry heat block)
- Add to 35 µl preheated (42°C) slidehyb buffer 1 (Ambion)
- Apply probe/hyb mix to microarray.

Hybridization Setup

- Clean the hybridization cassette with alcohol and remove any dust using compressed air (preheat hybridization cassette to 42°C).
- Add 5 µl of water to the far ends of the chamber
- Insert microarray in chamber, array side up
- Clean cover slip (lifterslip) and place it on the array
- Add 45 µl probe/hyb mix to the array; the probe will diffuse under the cover slip.
- Close the hybridization chamber and hybridize the array overnight at 42°C in an incubator.

Hybridization washes

- Following the hybridization reaction remove the slide from the cassette.
- Quickly transfer the slide to a 50 ml tube containing preheated (42°C) wash solution 2x SSC, 0.1%SDS. The cover slide will slide down the array slowly.
- Transfer the array to a glass slide rack with an open bottom and immerse in a staining dish containing 1xSSC, wash 2 times 5 min at 42°C
Perform the washes on a rotating platform, or mix the washing buffer carefully every min.
- Wash 1 x 5 min in 0.2xSSC (buffer preheated to 42°C).
- Wash app. 30 sec in 0.05xSSC (RT)
- Spin-dry the array 3 min @ 600 rpm (or air dry)
- Scan the array immediately (if possible, otherwise store slide dark and dust free).

NOTES:

Prior to pre-hybridization, filter all solutions. Salt particles in the SSC might cause bad spots on the array and 10% BSA is an ideal growth medium for bacteria.

Make sure you wash the slides thoroughly in water after pre-hybridization. Any BSA that is still on the slide causes a lot of Cy3 background in the hybridization, which cannot be washed away.

When the hybridization solution does not cover the slide completely, you may push the cover slip gently using a pipette tip to make sure the solution will spread evenly over the array.

Slidehyb buffer 1	5x2 ml	Ambion	Cat# 8861
Hybridization cassettes	ea	Ambion	Cat# 10040
Lifterslips		Erie Scientific	Cat#25x60I-2-4789