

aRNA Labeling and Hybridization Protocol

1. Prepare the reaction for each aRNA sample.

	aRNA	Rhx Primer (8 μ g/ μ l)	ddH ₂ O	Total
Cy5	X μ l (3 μ g)	1 μ l	16-(X+1)	16 μ l
Cy3	Y μ l (3 μ g)	1 μ l	16-(Y+1)	16 μ l

2. Heat to 70°C for 10 min and cool on ice.
3. Prepare reaction mixture for each Cy dye.

5X First-Strand Buffer	6 μ l
50X dNTPs	0.7 μ l
Cye Dyes	3 μ l
0.1 M DTT	3 μ l
Superscript II	1.7 μ l
Total Volume	14.4 μ l

4. Add 14.4 μ l of reaction mixture to the 16 μ l reaction tube, mix well, and incubate at 42°C for 1 hour.
5. Add another 1 μ l of SSII to each sample and incubate at 42°C for 1 hour.
6. Add 15 μ l of 0.1 N NaOH (with 2mM EDTA) and incubate at 65°C for 8 min.
7. Neutralize by adding 15 μ l of 0.1 HCl.
8. Add 450 μ l of TE to a Microcon YM-30 Microcon. Add 15 μ l of COT1 (1 mg/ml) DNA.
9. Add Cy5 and Cy3 labeled probe to the same Microcon.
10. Wash 1: Spin column for 7 min at 14,000 x g.
Wash 2. Remove flow through and add 500 μ l TE Spin for 7 min.
Wash 3. Repeat Wash 2 and concentrate probe to less than or equal to 30-35 μ l.
11. Invert the Microcon into a clean 1.5 ml tube and spin for 2 min. at 14,000 RPM to recover the probe.
12. Transfer 26.1 μ l of probe to new PCR tube:

Sample	26.1 μ l
Poly (A) DNA	2 μ l (10 μ g/ μ l)
Yeast tRNA	1 μ l (10 μ g/ μ l)
20X SSC	5.3 μ l
10% SDS	0.6 μ l
Total hybridization volume	35 μ l
13. Denature probe by heating for 2 min at 92°C and 42°C for 20-30 min.
14. Spin briefly and place 35 μ l to on array.
15. Place a coverslip (24 X 60 mm size) on to array and add 20-30 μ l of 3X SSC to each corner of the hybridization chamber.
16. Hybridize at 65°C for 14-18 hours.
17. Wash slide once with 2X SSC/ 0.05% SDS, for 2 min.
18. Transfer slide to 1X SSC for 2 min.
19. Wash slide in 50°C 0.2X SSC for 2 min, twice.
20. Spin slide in 600RPM for 5min.