Synthesis and Labeling of cDNA

1. Bring 0.5-5 µg RNA (Typically 3 µg) to 11 µl and add 2 µl (~2 mg/ml) random hexamers.
2. Heat 10 min at 65C (or 2 min at 98C), snap cool on ice.
3. Add 11µl

	10 rxns	20 rxns
5.0 µl 5X First-Strand buffer	(51)	(102)
2.5 µl 100 mM DTT	(25.5)	(51)
2.0 µl dNTP (5 mM A,G,C and 0.2 mM T)	(20.5)	(41)
1.5 µl Cy3 or Cy5 (Typically use Cy5 for sample that should change)	(15.3)	(30.6)

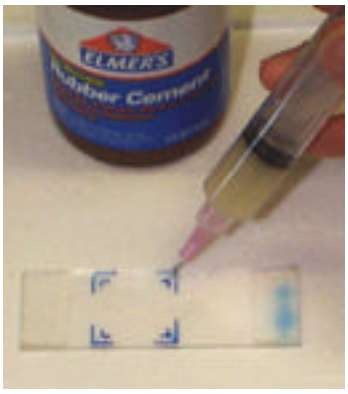
   Then add 1.2-1.8 µl Stratascript or 0.8-1.2 µl Superscript II RTase.

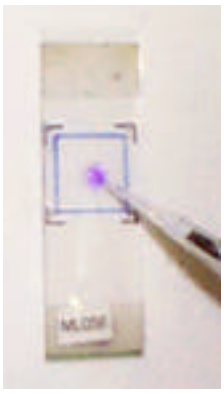
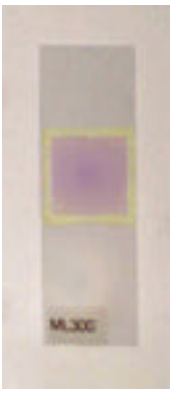
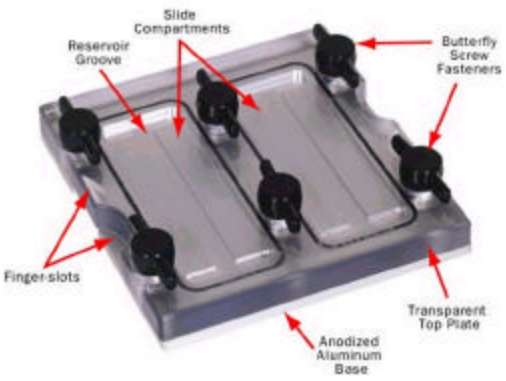
4. Incubate 10m at 25C followed by 90m at 42C in PCR machine. Can freeze or leave at 4C o/n.

Hybridization

1. Add both rxns to a microcon10 (Amicon) with 400 µl TE. Centrifuge at max speed until ~25 µl remaining (~20min). Add 100 µl more TE and spin until almost dry (~8min) (< 7 µl).
2. Recover cDNA by inverting microcon into new collection 1.5 ml tube and centrifuge at half max speed for 1 min.
3. Bring sample to 7 µl with TE and transfer to a 0.5 ml tube.
4. Add the following to sample.

   0.67 µl tRNA 10 mg/ml
   1.9 µl 20X SSC
   1.35 µl 2% SDS
5. Heat to 98C for 2m. Quick spin in centrifuge. Let it cool at R.T for a minute before applying to the array.
6. Apply hybridization solution and seal with rubber cement.
   a) Remember that the array is invisible after post-processing. So, make a template of your array using a clean glass slide, or by drawing on a piece of paper. Indicate the corners of the array and where the coverslip would lie as shown in the figure on the next page.
   b) Lay the array on top of your template and apply 1 microdot of rubber cement with a needle and syringe to each corner (just inside the marked coverslip corners).
   c) Apply 10 µl hybe solution to microarray. Place 22 mm coverslip with bent precision forceps right on top of the 4 dots of rubber cement. If there are bubbles under coverslip, try to free the bubbles by tapping on the top of coverslip with the forceps.
   d) COMPLETELY seal edges of coverslip with a bead of rubber cement (practice this). It is <u>not</u> critical to move quickly at this step. The rubber cement can be allowed to dry for a few minutes before sealing the chambers to avoid sticking to the top of the chamber.
   e) Hybe in standard hybe chambers with ~100 µl water for 6 hr to O/N in 65C water bath. (We use GeneMachine HybChambers, which fit 2 slides)



7. Take the array out of the hybe chamber and immediately submerge the slide into a flat container filled with the first washing solution, 1X SSC+0.05% SDS. Remove the coverslip with a clean razorblade by lifting it at the corner and pulling up. Transfer the array to a slide rack, submerged in 1X SSC+0.05% SDS and rinse for 1-2 min.
8. Rinse in 0.06X SSC to remove traces of SDS, which will produce autofluorescence if left on the slide. Rinse again for 2 min in fresh 0.06X SSC.
9. Centrifuge 1m at 500 rpm to dry, and scan.