Protocol for Array Hybridization

1. Prepare probe as described at end of labeling protocol.

   Cy3 + Cy5 samples: 18.5 µl
   20X SSC 3.6 µl
   polyA (10mg / ml) 1 µl (optional)
   1M HEPES pH 7.0 0.5 µl
   Optional: Filter with Millipore unit. See RNA labeling protocol.

   Add 0.5 µl 10%SDS.

2. Set slide in hybridization chamber. Put 10µl 3XSSC at end of slide on top of the slide label. This is to ensure a constant humidity in the chamber during hybridizations, if it is on the label, then it will be less likely to spread across the glass and into your sample. If the 3XSSC is not applied, the array will dry out. To avoid unpleasant surprises, the concentration of salt in the re-hydration drop and the probe must be the same!

3. Clean a Lifterslip with EtOH and Kimwipes. Place slip on array using either fingers or forceps. Slip should be applied with dull white strips on the long axis of the chip touching the glass. This creates a platform which allows even distribution of the hybridization solution across the array. Use of the Lifterslip greatly improves data quality by preventing non-uniform hybridization.

4. Boil probe for 2 min. at 100°C. Let cool 5-10 min. at room temp. Boiling denatures the sample and makes it accessible for hybridization.

5. Slowly inject the probe under one corner of the coverslip until the array surface is covered. Continue to apply remaining probe at the other corners. To prevent bubbles make sure there is no debris on the array before laying down coverslip. The arrays may be dusted with compressed air briefly. Bubbles may also be caused by not allowing the solution to completely cover the array before injection at a different corner begins.

6. Tightly screw down chamber lid and carefully place chamber in a 63°C water bath. Take caution to keep array completely flat during transfer and hybridizations.

7. Allow hybridization to run for at least 6 hours. Human RNA samples should hyb for at least 12 hours. The optimal time for hybridization will depend on the complexity and concentration of your sample.