MATERIALS

1. Microarray slide
2. RNase free water (Ambion #9920)
3. 20X SSC (Ambion, #9763G)
4. Bovine Serum Albumin (Invitrogen, # 15561-020)
5. 20% SDS (Fisher Biotech, #BP1311-200)
6. 2X GeneTAC Hybridization Buffer (Genomic Solutions, #16003050)
7. Ultrapure formamide (Sigma, #F-9037)
8. Medium stringency (Genomic Solutions, #16004001)
9. High stringency (Genomic Solutions, #16004501)
10. Post wash buffer (Genomic Solutions, #16003501)
11. Prepared Cy3/Cy5 labeled cDNA mix
12. Manifold O-ring (Genomic Solutions, #HYB10155)

EQUIPMENT

1. Genomic Solutions GeneTAC Hybridization Station
2. Rotating platform
3. Pipetman adjustable micropipettors (various)
4. Microfuge slide soaking tank(s) and rack(s)
5. Eppendorf 5810R centrifuge with slide holder buckets

REAGENT PREPARATION

1. Hybridization Mix (120 µl total):
    a. 60 µl of 2X GeneTAC Hybridization Buffer
    b. 36 µl of Formamide (30% final concentration)
    c. 24 µl of Cy3/Cy5 cDNA mix in water

PROCEDURE

1. Prewashing
    a. Slides are placed in a rack and gently placed in a soaking tank containing 0.2% SDS at RT for 10 minutes with very gentle agitation on a rotating platform.
    b. Slides are next transferred to a new tank containing 70% ethanol, rinsed briefly and air-dried.
    c. They may now be stored at RT in the dark until ready to use.
    d. NOTE: In most cases, an excess of DNA has been placed onto each spot and this DNA will come off during the hybridization. This can cause a

reduced signal as well as a higher background. It is important to treat the slides very gently, in order to minimize any shearing which can lead to increased loss of DNA from the slide.

2. Set up the Genomic Solutions GeneTAC Hybridization Station according to the manufacturer's instructions.
   a. Install a new O-ring in the each station manifold for every slide to be hybridized.

3. Prehybridization Blocking (optional but strongly recommended):
   a. Load each slide (array) onto the Station according to manufacturer's instructions.
   b. Inject 120 µl 5X SSC buffer containing 0.1% SDS and 1% BSA (1% by volume from stock 50 mg/ml solution of BSA)
   c. Incubate the spotted slide at 40°C for 45 min on the Hybstation, and then wash 5 cycles with filtered water at 40 °C, flow 30 sec, hold 1 min.
   d. Dry slide by spinning at 1,000 rpm for 10 min.
   e. Use slide immediately for hybridization experiment.

4. Hybridization
   a. Reload slide onto hybridization station
   b. Preheat probe at 70°C for 2 min.
   c. Preheat the Station at 75°C for 2 min, and then introduce 120 µl probe at 75°C.
   d. Hybridization program:
      1. Hybridize at 45°C for 3hours, then at 42 °C for 14 hours or longer using standard setting for probe agitation.
         1. Total hybridization time should be from 17 hours to 24 hours.
      2. Washing program:
         1. 2 cycles med stringency at 42°C, flow 30 sec, hold 1min
         2. 2 cycles high stringency at 42°C, flow 30 sec, hold 1min
         3. 2 cycles post wash at 30°C, flow 30 sec, and hold 1 min
   e. Dry slide by spinning at 1000 rpm for 10 min. in Eppendorf 5810R centrifuge.
   f. Clean Hybridization Station according to manufacturer's instructions.