

# **cDNA Microarray Hybridization (v3.0.0)**

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This protocol details the automated hybridization and wash of labeled probes to cDNA expression arrays using the GeneTAC HybStation.

## **MATERIALS**

- Bovine albumin fraction V (Invitrogen, Cat # 11018-017)
- 20x SSC (Research Genetics, Cat # 750020), pH 8.0
- 10% SDS (Research Genetics, Cat # 750008), pH 8.0
- SlideHyb Buffer #3 (Ambion)
- Polyadenylic acid (Sigma, Cat # P-9403)
- 0.22  $\mu\text{m}$  CA 50 ml tube top filter (Corning, Cat # 430320)
- 0.22  $\mu\text{m}$  PES 500 ml bottle top filter (Corning, Cat # 431118)
- Human Cot-1 DNA (Invitrogen, Cat # 15279-011)
- Mouse Cot-1 DNA (Invitrogen, Cat # 18440-016)
- [GeneTAC HybStation](http://www.genomicsolutions.com/genomics/hybrid.html) (<http://www.genomicsolutions.com/genomics/hybrid.html>)

Note: All SSC, SDS, and H<sub>2</sub>O stocks are adjusted to pH 8.0 with NaOH.

## **Poly A**

Prepare Poly(A)-DNA by dissolving stock Poly(A)-DNA in a neutral buffer (i.e. 10 mM Tris, pH 7.0) to a final concentration of 20  $\mu\text{g}/\mu\text{l}$ . Aliquot and store at -20°C.

## **Cot-1 precipitation**

1. Add 1/10 volume of 3M NaAcetate (pH5.5) plus 2 volumes of 100% ethanol to the 500  $\mu\text{l}$  Cot-1. Vortex well.
2. Precipitate at -70°C for 30 min or -20°C O/N.
3. Centrifuge at full speed in an eppendorf microcentrifuge for 10 min.
4. Remove supernatant.
5. Wash pellet with 70% ethanol and spin at 8000 x g at 4°C for 5min.
6. Remove all of ethanol and dry pellet.
7. Resuspend pellet in TE (pH 8.0) to give 20 $\mu\text{g}/\mu\text{l}$ . If you have precipitated all 500  $\mu\text{g}$  of Cot-1, then resuspend pellet in 25  $\mu\text{l}$  TE.
8. Confirm concentration on spectrophotometer via A260 reading.

## **Procedure**

A) Preblocking of slides

NOTE: This should be done about 30-45 minutes before the labeled probe is ready to be hybridized. Preblocking is not necessary for poly-l-lysine prepped slides, only the Corning GAPS2 slides.

1. Prepare preblock solution in 50 ml tube (total volume 50 ml):

- 0.5 g BSA
- 12.5ml 20X SSC, pH 8.0
- 10% SDS, pH 8.0
- Add ddH<sub>2</sub>O to 50ml

2. Mix well and filter through a .22 µm 50 ml tube top filter.

3. Place slide(s) into HybStation module, preheated to 47°C. Add 110 µl of filtered preblock solution and incubate at 47°C for 30 minutes.

4. Wash slides 5 times with Milli-Q H<sub>2</sub>O.

5. Add 110 µl of Ambion SlideHyb Buffer #3 and incubate at 55°C.

B) Application of cy dye-labeled probe to slide

1. Heat Ambion SlideHyb Buffer #3 to 65°C in a heat block.

2. Resuspend labeled probe (prepared in “Indirect Direct Labeling” protocols) in 110 µl of SlideHyb Buffer #3, 2 µl Cot-1 and 2 µl PolyA.

3. Denature at 95°C for 5 minutes.

4. Place on ice for 1 minute, then spin at 13,000 rpm for 1 minute.

5. Drain prehyb buffer from slide.

6. Immediately, load probe onto slide. Careful to avoid bubbles.

7. Hybridize 16-20 hours at 55°C.

C) Slide washing

**FOR CORNING GAPSII SLIDES:** [all wash solution solutions are pH 8.0]

1. Warm the first wash solution (2x SSC, 0.1% SDS) in a wash chamber in a 42°C water bath before placing in HybStation.

2. Wash slides as follows:

2XSSC, 0.1%SDS	55°C	20sec flow, 30sec hold, 2 cycles
0.1XSSC, 0.1%SDS	45°C	20sec flow, 30sec hold, 2 cycles
0.1XSSC	45°C	20sec flow, 30sec hold, 2 cycles

3. Remove slides from HybStation.

4. Plunge the slide carrier 3x into a wash chamber of 95% EtOH.

5. Dry slides by spinning for 5 minutes at 500 rpm.

6. Incubate slides for 2 minutes at 80°C in oven.

D) Scanning of slides

Keep slides in the dark until ready to scan. Slides are scanned on an Affymetrix 428 array scanner. The cy5 channel is always scanned before

cy3 for each slide. The gain setting should be kept constant for every slide in a single experiment, and multiple gain setting scans may be required. After scanning, slides are stored in a slide box in the dark in case additional scans are needed at a later date.