AUTHOR:
*Renee Rubio*

PRIMARY REVIEWERS:
*Bryan Frank*

## 1. PURPOSE

This protocol describes the hybridization of a Cy labeled cDNA probe (mix of Cy3 and Cy5) onto coated slide spotted with PCR amplified cDNA.

## 2. SCOPE

This procedural format is currently utilized by Human Colon Cancer and Mouse microarray projects under the supervision of John Quackenbush within the Mammalian Genomics Dept.

## 3. MATERIALS

3.1 20X Saline-Sodium Citrate (SSC) (Sigma; Cat # S-6639)
3.2 10% Sodium Dodecyl Sulfate (SDS)(Life Technologies; Cat # 15553-035)
3.3 Bovine Serum Albumin (BSA) (Sigma; Cat # A-9418)
3.4 Formamide, redistilled (Life Technologies; Cat # 15515-081)
3.5 Isopropanol (Fisher Scientific; Cat # A451-1)
3.6 Coplin jar (VWR; Cat # 25457-200)
3.7 Human COT1-DNA (Life Technologies; Cat # 15279-011)
3.8 Mouse COT1-DNA (Life Technologies; Cat # 18440-016)
3.9 Poly(A)-DNA (Pharmacia; Cat # 27-7836-01)
3.10 Microscope Cover Glass (Fisher Scientific; Cat # 12-545J)
3.11 Corning UltraGAPS™ Coated Slides (bar-coded) (Corning; Cat # 40015)
3.12 Hybridization chamber (Corning Costar; Cat #2551)
3.13 1 L .22 µm CA (cellulose acetate) Filter System (Corning; Cat #430517)
3.14 Pressurized air duster (Fellowes; Cat # 99790) or clean in-house pressurized air source
3.15 Telechem ArrayIt™ Microarray High Speed Centrifuge (Telechem, Cat #MHC110V)

## 4. PROCEDURE

4.1 UV Cross-linking & Prehybridizaton

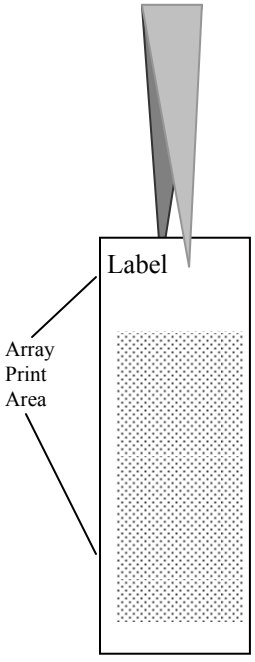
4.1.1 Aminosilane coated slides (Corning UltraGAPS™ Coated Slides) spotted with cDNA in 50% DMSO are UV cross-linked at ~120 mJ.

4.1.2 Prepare prehybridization buffer (5X SSC, 0.1% SDS, 1% BSA) and sterilize by filtration using a CA filter. Preheat at 42°C for ~30 minutes before use.

4.1.3 Place the printed slide(s) to be used for the hybridization in a Coplin jar containing preheated prehybridization buffer and incubate at 42°C for 45 minutes.

4.1.4 Washing Slides

- Fill two Coplin jars with MilliQ water and another with isopropanol.
- With forceps carefully grasp slide by the labeled end and vertically dip slide into the first Coplin jar (water) so that the slide is completely submerged. Dip slide four or five times.
- Dip the slide in the second Coplin jar of water four or five times, but only submerging the slide enough to wash the printed array itself.
- Finally, dip slide into the third Coplin jar (isopropanol) four or five times submerging the slide completely.




**NOTE:** Replace each water wash after every five slides.

4.1.5 Drying Slides

- Carefully insert the slide into a mini centrifuge (Telechem ArrayIt™ Products) with a slide attachment and spin for 10-20 seconds.
- Note the general appearance of the slide. Streaking or mottling on the slide surface indicates further washing is necessary.
- Repeat the water/water/isopropanol wash cycle as necessary to clean the slide. Centrifuge dry between each cycle.

4.1.6 Use slides immediately following prehybridization to ensure optimal hybridization efficiency.

4.2 Hybridization

4.2.1 Prepare 1X hybridization buffer (50% formamide, 5X SSC, and 0.1% SDS).

**NOTE:** The hybridization buffer must be preheated to 42°C prior to use.

4.2.2 Prepare Poly(A)-DNA by dissolving stock Poly(A)-DNA in a neutral buffer (i.e. 10 mM Tris, pH 7) to a final concentration of 20μg/μL.

**NOTE:** There are ~37ug per unit of Poly d(A). Multiply the number of units per bottle by 37 and then divide by 20 to get the amount of buffer to add to achieve the desired concentration. Using a spectrophotometer to validate the concentration is optional.

4.2.3 Prepare COT1-DNA (stock conc.1μg/μL) by ethanol precipitation:

- Add 2 to 3 volumes of ethanol and 0.1 volumes of 3 M Sodium Acetate (NaOAc) to the stock tube.
- Mix well and place on dry ice for 20-30 minutes or in –20$^{o}$C freezer overnight.
- Centrifuge for 20-30 minutes in a cold room microfuge at maximum angular velocity.
- Remove supernatant and allow excess ethanol to dry off.
- Dissolve precipitated COT1 in a neutral buffer (i.e. 10 mM Tris, pH 7) to the final concentration of 20μg/μL.

**NOTE**: Take the total number of μg per tube and divide by 20 to determine the amount of buffer to add to achieve the desired concentration. Using a spectrophotometer to validate the concentration is optional.

4.2.4 Resuspend labeled probe (Cy3/Cy5 probe mixture: *see SOP-M004* *http://intranet.jtc.jcvsf.org/sops/M004.pdf* ) in 24μL of preheated 1X hybridization buffer.

**NOTE:** Expose Cy labeled probe to light as little as possible during the hybridization process.

4.2.5 To block nonspecific hybridization add:

COT1-DNA (20μg/μL)…….1μL
Poly(A)-DNA (20μg/μL)…...1μL

**NOTE:** The COT1–DNA is organism specific: add mouse COT1 to labeled mouse probes and human COT1-DNA to labeled human probes.

4.2.6 To denature, heat the probe mixture at 95$^{o}$C for 3 minutes and snap cool on ice for 30 sec.

4.2.7 Centrifuge the probe mixture at maximum angular velocity for 1 minute. Keep at room temperature and use immediately.

4.2.8 To Apply Labeled Probe Mixture

- Place a prehybridized microarray slide (array side up) between the guide teeth in the bottom half of a hybridization chamber.
- Pipette the labeled probe mixture (~26μL) to the slide surface near one end of the array print area keeping bubbles to a minimum.
- Take a 22mm x 60mm microscope glass coverslip, dust it with compressed air, and grasp one end with forceps.
- Holding the coverslip over the array print area, lower the end nearest the pool of cDNA probe until solution wicks to the surface of the coverslip.
- Gradually lower the opposite end of the coverslip (held by the forceps) onto the slide. The solution may take a minute or two to wick across the entire length of the slide.
- After probe has wicked across the slide carefully adjust the coverslip's position with the tip of the forceps so that there is an even margin between the edge of the coverslip and the edge of the slide.
- Work any large bubbles toward the edge by gently tapping the coverslip surface; small bubbles will absolve themselves during hybridization.

4.2.9 To the small wells at each end of the chamber add 10μL of water (20μL total), cover, and seal the chamber.

4.2.10 Wrap the chamber in foil (light-tight) and incubate in a 42$^{o}$C water bath for 16-20 hours. To ensure chamber remains level and does not float to the surface place a small weight upon it.

**NOTE:** Do not flip the hybridization chamber upside down during hybridization; this may cause the coverslip to shift from the slide and adversely affect the hybridization.

4.2.11 Prepare a low stringency wash buffer (~500mL) containing 1X SSC and 0.2% SDS and a high-stringency wash buffer (~500mL) containing 0.1X SSC and 0.2% SDS.

**NOTE:** The low stringency wash must be preheated to 42°C prior to use. The other two washes should be at room temperature.

4.2.12 After the incubation remove foil and unseal hybridization chamber. Remove the slide from the chamber, taking care not to disturb the coverslip.

4.2.13 To remove coverslip submerge slide in a dish containing low stringency wash buffer (preheated to 42$^{o}$C). With forceps shake the slide gently to loosen the coverslip. With time the coverslip will slide free of the slide surface.

**NOTE:** Once the slide has been hybridized it should be exposed to light as little as possible. Therefore, all staining dishes should be covered with foil to make them light tight.

4.2.14 After the coverslip is removed place slide in a staining dish containing low stringency wash buffer (preheated to 42$^{o}$C) and agitate for 4 minutes.

4.2.15 Wash the slide in a staining dish with high stringency wash buffer by agitating for 4 minutes at room temperature.

4.2.16 Wash the slide in 0.1X SSC agitating for 4 minutes at room temperature. (alternatively one can wash twice in 0.1X SSC agitating for 2.5 minutes to minimize SDS carryover.)

**NOTE:** The slide can be dipped one to two times in a Coplin jar containing water; however, this step is optional.

4.2.17 Dry the slides by using a microcentrifuge adapted to microarray slides according to step 4.1.5.

4.2.18 Place slides in a light tight slide box until they can be scanned, preferably as soon as possible.