SGED_SOP_6.2.4:Hybridization with Indirectly Labeled Aminoallyl Probes

Effective Date: 2/3/04

1. **Materials**
   - 20X Saline-Sodium Citrate (SSC) (Sigma; Cat # S-6639)
   - 10% Sodium Dodecyl Sulfate (SDS)(Life Technologies; Cat # 15553-035)
   - Bovine Serum Albumin (BSA) (Sigma; Cat # A-9418)
   - Formamide, redistilled (Life Technologies; Cat # 15515-081)
   - Isopropanol (Fisher Scientific; Cat # A451-1)
   - Coplin jar (VWR; Cat # 25457-200)
   - Poly(A)-DNA (Invitrogen Custom Primer, 1U scale of synthesis, 20-mer)
   - Salmon Sperm DNA (10mg/ml) (Invitrogen Cat # 15632-011)
   - Microscope Lifter Slip (Erie Scientific Cat #25x60I-2-4789)
   - Corning UltraGAPS™ Coated Slides (bar-coded) (Corning; Cat # 40015)
   - Hybridization chamber (Corning Costar; Cat #2551)
   - 1 L .22 µm CA (cellulose acetate) Filter System (Corning; Cat #430517)
   - Pressurized air duster (Fellowes; Cat # 99790) or clean in-house pressurized air source
   - Staining dish (Wheaton, Cat# 900200)
2. **Methods**

a. UV Cross-linking & Prehybridizaton

1) Aminosilane coated slides (Corning UltraGAPS™ Coated Slides) spotted with cDNA in 50% DMSO are UV cross-linked at ~120 mJ (***NOTE:** The slides you receive have already been cross-linked***)

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.

3) Place the printed slide(s) to be used for the hybridization in a Coplin jar containing prehybridization buffer preheated to 42°C. Incubate at 42°C in water bath for 45 minutes.

b. Washing Slides

1) Wash the slides by shaking in glass slide staining dish filled with room temperature MilliQ water. Shake for 5 minutes.

2) Repeat step 1 with fresh MilliQ water for 5 minutes.

3) Shake the slides at room temperature in isopropanol for 2 minutes.

Note: Replace each water wash after every five slides.

c. Drying Slides

Dry with pressurized air (Dust Off XL VWR# 21899-094),

OR

Dry down in centrifuge by placing slides in slide rack on a swinging plate tray (500rpm for 5 minutes). If you see white streaks on the slide repeat water/water/isopropanol wash cycle.

1) To avoid blowing debris from the forceps onto the array, first blow from where the array ends toward the forceps. Then dry the array itself by blowing down the slide away from the forceps.

2) Finally dry the back of the slide.

3) Note the general appearance of the slide. Streaking or mottling on the slide surface indicates that further washing is necessary.

4) Repeat the water/water/isopropanol wash cycle as necessary to clean the slide. Blow-dry between each cycle.

5) Use slides immediately following pre-hybridization to ensure optimal hybridization efficiency.

d. Hybridization

1) Prepare 1X hybridization buffer (50% formamide, 5X SSC, and 0.1% SDS).
2) Prepare Poly (A)-DNA by dissolving stock Poly(A)-DNA in molecular biology grade water to a final concentration of 20 μg/μL.
3) Resuspend labeled probe (Cy3/Cy5 probe mixture) in 50-60 μL of 1X hybridization buffer.
Note: Expose Cy labeled probe to light as little as possible during the hybridization process.
5) To block nonspecific hybridization add:

Salmon Sperm DNA (10 mg/mL)…….0.75μL

Poly (A)-DNA (20μg/μL)…..……………..1μL

6) To denature, heat the probe mixture at 95°C for 3 minutes and snap cool on ice for 30 sec.
7) Centrifuge the probe mixture at maximum angular velocity for 1 minute. Keep at room temperature and use immediately.

e. Applying the Labeled Probe Mixture

1) Place a prehybridized microarray slide (array side up) between the guide teeth in the bottom half of a hybridization chamber.
2) Take a lifter slip and dip it in MilliQ water and let air dry.
3) Place lifter slip with raised edges side down (rough side) and smooth side of lifter slip facing up. Place the lifter slip so the top edge is about 2-3 mm from the top of the array (the top being the end of the array opposite of the barcode).
4) Add probe (~50-60ul) to bottom end of lifter slip (end closest to barcode), little by little. The probe will get sucked under the slip by capillary action. If the probe is not wicking up the slide, lightly tap with pipette tip. *Depending on the humidity of your lab, you may need to increase the volume of the probe. In cold, dry weather this may be the case.*
5) Work any large bubbles toward the edge by gently tapping the lifter slip surface; small bubbles will absolve themselves during hybridization.
6) Add 10-20 uL of water to each of the small wells at each end of the chamber and seal the chamber.
7) Wrap the chamber in foil (light-tight) and incubate in a 42°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 lifter slip to shift from the slide and adversely affect the hybridization.

f. Washing the slides

1) Prepare a low stringency wash buffer (~500mL) containing 2X SSC and 0.1% SDS and a high-stringency wash buffer (~500mL) containing 0.05X SSC and 0.1% SDS.
2) After the incubation remove foil and unseal hybridization chamber. Remove the slide from the chamber, taking care not to disturb the lifter slip.
3) To remove lifter slip submerge slide in a dish containing low stringency wash buffer for about 5 minutes (preheated to 42°C). With forceps shake the slide gently to loosen the lifter slip. With time the lifter slip 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) After the lifter slip is removed place slide in a staining dish containing low stringency wash buffer (preheated to 42°C) and agitate for 5 minutes.
5) Wash the slide in a staining dish with high stringency wash buffer by agitating for 10 minutes at room temperature.
6) Wash the slide in 0.05X SSC agitating for 1 minute at room temperature. Repeat 4 times with fresh 0.05X SSC solution each time.
7) Dry slides as described in section 2 c.
8) Place slides in a light tight slide box until they can be scanned, preferably as soon as possible.