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Microarray hybridization – RNA probes

The following is a protocol for the hybridization of dye-labeled RNA to microarrays. This protocol works well for 70mers (Operon) printed on poly-lysine or on Corning GAP slides. The amount of hybridization solution is sufficient for a 22 mm X 40 mm Lifterslip.

I: Slide Hybridization

1: Dry down purified probes in a speed-vac. Resuspend pellet in 5 µl 10 mM EDTA. Make sure that no precipitate is left in the sample.

2: Pre-heat the bottom part of a hybridization chamber to ~55°C. Clean a LifterSlip in 95% ethanol and dry it with a Kimwipe. Remove any dust from the Lifterslip as well as from the array with compressed air. Place the Lifterslip on the array.

3: Heat up sample at 80°C for 1 min.

4: Add 50 µl of Ambion SlideHyb Buffer #1 (pre-warmed for 15-30 min at 68°C; before use make sure that all precipitate has dissolved) and mix sample thoroughly by pipetting. Incubate for 2 min at 80°C.

5: Spin tube for 30 sec to collect the content at the bottom. Incubate at 80°C for a few sec. Place the hybridization chamber with the slide on top of a styrofoam block. Apply probe (55 µl) carefully without capturing air bubbles underneath the LifterSlip.

6: Distribute a total of 60 µl of 3XSSC in small drops left and right from the LifterSlip.

7: Close the hybridization chamber and incubate it in a water bath for 16-24 h at 48°C. Cover the water bath with a lid or with aluminum foil, to avoid any light exposure (high temperatures accelerate destruction of the fluorophores).

8: Put a glass dish and 1 liter of water in a 60°C incubator.

II: Slide Washes

1: Prepare 1 liter of the first wash buffer: 1XSSC, 0.2% SDS, 1 mM DTT (make up with pre-warmed water). Put a slide rack in the bottom of the pre-warmed glass dish and add the wash buffer. When the temperature of the wash buffer is ~48°C, dip a slide into the wash buffer, turn it up-side-down and shake it gently until the cover slip comes off. Insert slide into the slide tray without taking it out of the wash solution. Cover the dish with aluminum foil and gently shake it for 10 min.

2: Transfer the slide rack to a small glass dish with 0.5 liter of 0.1XSSC, 0.2% SDS, 1 mM DTT (room temp). Cover the slide chamber with aluminum foil and gently shake it for 10 min.

3: Transfer the slide rack to a small glass dish with 0.5 liter of 0.1XSSC, 1 mM DTT (room temp). Shake dish gently for 1 min.

4: Take the slides out of the last wash buffer and centrifuge them for 1 min at ~150 *g*. Scan the slides right away since the fluorescent signals (Cy5, in particular) may start to fade rapidly.

Alternatively, to avoid dye-fading, coat the slides immediately with DyeSaver. Since this reagent can cause significant background fluorescence particularly in the green channel, the coating should be thin and uniform. For this, put a horizontal shaker in a fume hood (Caution: the reagent is very toxic and flammable – keep the vial with DyeSaver closed whenever possible). Cover the platform of the shaker with paper towels. Tape the towels to the platform if necessary (they should not move much when the shaker is spinning at high rpms). Position a slide in the middle of the platform, array side up. The actual coating has to be done very quickly since the reagent will dry up very rapidly. Distribute 350 µl of DyeSaver over the entire length of the slide. Turn on shaker to high rpms for a few seconds until the entire array is covered with DyeSaver. Quickly lift slide up to a vertical position and gently tap the end of the slide onto a paper towel to get rid of as much excess DyeSaver as possible. Let the slide dry for a few minutes in an upright position.

MATERIALS AND REAGENTS

Ambion: SlideHyb Buffer #1 (Cat. No. 8861)

Erie Scientific: LifterSlip (e.g. Cat. No. 22x40I-2-4710)

Genishere: DyeSaver (Cat. No. Q100300)

20xSSC: 3 M NaCl (175.3g)
0.3 M Na-citrate (88.2g)
add H_2O to 1 l and adjust pH to 7 with NaOH

10% (w/v) SDS