

Hybridization Protocol for the 32K Arrays

Random Prime

- 1) Set up 25µl random prime reactions for both test and reference DNA samples using a BioPrime Aray CGH Kit (*Invitrogen #45-0048*).

a) In a PCR 8-tube strip:

<u>Test</u>	<u>Reference</u>
10 µl Primers	10 µl Primers
X µl Test DNA(~600ng)	X µl Reference DNA(~600ng)
<u>Y µl Water</u>	<u>Y µl Water</u>
21 µl Total	21 µl Total

b) In a PCR machine, denature at 100°C and ramp temperature to 4°C then add:

2.5 µl dNTP Mix*	2.5 µl dNTP Mix*
1.0 µl 1mMdCTP-Cy3**	1 µl 1mMdCTP-Cy5**
<u>0.5 µl Klenow</u>	<u>0.5 µl Klenow</u>
25 µl Final Volume	25 µl Final Volume

c) Incubate at 37°C overnight.

*dNTP Mix

184.6 µl Water
2.0 µl 1M Tris pH 7.6
0.4 µl 500mM EDTA
8.0 µl each of 100mM dATP, dGTP, dTTP
<u>4.0 µl of 100mM dCTP</u>
215 µl Total

**Cy Dyes

Perkin Elmer #NEL576 (Cy3), #NEL577 (Cy5)
OR
Amersham #PA53021 (Cy3), #PA55021 (Cy5)

- 2) Remove unincorporated label using G-50 Sephadex column (*Amersham #27-5330-02*).

a) Spin the columns in 1ml tubes at 770ccf for 1 minute to remove excess buffer.

b) Transfer columns to new 1ml tubes

- c) Spin the columns at 770ccf for 2 minutes to isolate labeled probe.
- d) Combine the labeled probe pair in a 1ml tube.

Prepare hybridization mix

- 1) Add 100µg of Cot-1 to the probe pair and ethanol precipitate (2x volume cold ethanol + 10% volume 3M Sodium Acetate) and spin at 14000rpm for 45min.
- 2) Decant the ethanol solution and swab the tube dry. Dissolve the pellet into

8µl water
16µl 20% SDS
56µl MM#1
80µl

- 3) Denature the Hybe Mix at 73°C for 15 minutes and incubate at 37°C for 60 minutes.

Prepare Slide

- 1) Treat the slide with 2600 X100µjoules of UV in a Stratalinker.
- 2) Using 1mil polyimide film tape (*McMaster-Carr #7648A715*) cut 6 spacers (~2mm x ~4mm) and apply to the slide around the array, pressing to flatten the tape.
- 3) Soak a slide-sized piece of paper towel with ~400µl of blank Hybe Mix and place on the top of the slide chamber (*ArrayIt #AHCXD*). Assemble the slide chamber and warm to 37°C.
- 4) Apply the Hybridization Mixture to the array in a T shape with the elongated top of the T along the third row of both subarrays. Place the edge of the coverslip on the spacers at the top of the T and carefully lower the coverslip until it touches the Hybe Mix. Release the coverslip and quickly guide it into position so it rests on all 6 spacers.
- 5) Immediately take the slide to the 37°C incubator and place on the shelf until the slide chamber is disassembled. Quickly insert the slide into the slide chamber and reassemble. Incubate at 37°C for 2 nights.

Slide Wash

- 1) Disassemble the slide chamber and carefully slide the coverslip off using tweezers

- 2) Quickly rinse the slide in a room temperature PN solution for ~10 seconds to remove majority of hybridization mixture.
- 3) Place slide in coplan jar with 50% formamide/2xSSC wash solution preheated to 45°C for 15 min.
- 4) Remove slide from wash and place in coplan jar with PN solution at room temperature for 15 min.
- 5) Remove slide from PN, remove the spacers with tweezers, shake off the excess PN, and apply DAPI and a coverslip. Stand the slide on the side on a paper towel to wick away the excess DAPI.