

Purpose: To hybridize, stain, and scan 100K Affymetrix chips..

Procedures: This procedure is taken from the Affymetrix 100K genotyping manual.
The manual should also be consulted for any questions about the protocol.

A. Reagents needed

- 100K chips
- Water, Molecular Biology Grade, BioWhittaker Molecular Applications / Cambrex, P/N 51200
- Distilled water, Invitrogen Life Technologies, P/N 15230147
- 20X SSPE (3M NaCl, 0.2M NaH₂PO₄, 0.02 M EDTA), BioWhittaker Molecular Applications / Cambrex, P/N 51214
- Streptavidin Pierce P/N 21122, 1 mg; reconstitute according to product instructions
- Anti-streptavidin antibody (goat), biotinylated, Vector Laboratories, P/N BA-0500; reconstitute according to product instructions
- R-Phycoerythrin Streptavidin, Molecular Probes, P/N S-866
- 10% surfact-Amps20 (Tween-20), Pierce Chemical, P/N 28320
- Denhardt's Solution, 50X concentrate: Sigma; P/N D2532

B. Equipment needed

- 1.5 mL Eppendorf tubes
- Tough Spots, Label Dots, USA Scientific, P/N 9185

C. Hybridization

1. Defrost the CHIPLO hybridization mix tubes if previously frozen.
2. Heat the 260 μ L of CHIPLO hybridization mix and labeled DNA at 95°C in a heat block for 10 minutes to denature.
3. While it's heating, label the appropriate chips with the sample name. Make sure you use the correct chips – Xba or Hind.
4. Cool down on crushed ice for 10 seconds. **DO NOT LEAVE ON ICE FOR MORE THAN 10 SECONDS.**
5. Spin briefly in a microfuge to collect any condensate.
6. Insert a fresh, P10 pipette tip into the upper septum on the chip to vent it.
7. Inject 200 μ L denatured hybridization into the array using a pipettor.
8. Hybridize overnight at 48°C for 16 to 18 hours at 60 rpm.
9. Store the CHIPLO tubes in the freezer for access tomorrow.

D. Washing and Staining

For reagent preparation (Wash A, Wash B, and labels, see Affy protocol. For now, we are using the DRAFT protocol from 3/04 that does not use the holding buffer and does use Strep.)

