Genomic DNA Labeling Protocol_Version A

Developed for microarray-based comparative genomic hybridization.

Genomic DNA can be labeled with a simple random-priming protocol based on Gibco/BRL's Bioprime DNA Labeling kit, though nick translation protocols work too. I routinely use the BioPrime labeling kit (Gibco/BRL) as a convenient and inexpensive source of random octamers, reaction buffer, and high concentration klenow (do not use the dNTP mix provided in the kit), though other sources of random primers and high concentration klenow work as well.

1. Add 2 µg DNA of the sample to be labeled to an eppindorf tube.

> **Note: For high complexity DNAs (e.g. human genomic DNA), the labeling reaction works more efficiently if the fragment size of the DNA is first reduced. I routinely accomplish this by restriction enzyme digestion (usually DpnII, though other 4-cutters work as well). After digestion, the DNA should be cleaned up by phenol/chloroform extraction / EtOH precipitation (Qiagen PCR purification kit also works well).**

2. Add ddH_2O or TE 8.0 to bring the total volume to 21µl. Then add 20µl of 2.5X random primer / reaction buffer mix. Boil 5 min, then place on ice.

> **<u>2.5X random primer / reaction buffer mix</u>:**
> **125 mM Tris 6.8**
> **12.5 mM $MgCl_2$**
> **25 mM 2-mercaptoethanol**
> **750 ug/ml random octamers**

3. On ice, add 5µl 10X dNTP mix.

> **<u>10X dNTP mix</u>:**
> **1.2 mM each dATP, dGTP, and dTTP**
> **0.6 mM dCTP**
> **10 mM Tris 8.0, 1mM EDTA**

4. Add 3µl Cy5-dCTP or Cy3-dCTP (Amersham, 1 mM stocks)

> **Note: Cy-dCTP and Cy-dUTP work equally well. If using Cy-dUTP, adjust 10X dNTP mix accordingly.**

5. Add 1µl Klenow Fragment.

> **Note: High concentration klenow (40-50 units/µl), available through NEB or Gibco/BRL (as part of the BioPrime labeling kit), produces better labeling.**

6. Incubate 37°C for 1 to 2 hours, then stop reaction by adding 5µl 0.5 M EDTA pH8.0

7. As with RNA probes, I purify the DNA probe using a Microcon-30 filter (Amicon/Millipore):

Add 450 µl TE 7.4 to the stopped labeling reaction.
Lay onto microcon 30 filter. Spin ~10 min at 8000g (10,000 rpm in microcentrifuge).
Invert and spin 1 min 8000g to recover purified probe to new tube (~20-40 µl volume).

8. For two-color array hybridizations, combine purified probes (Cy5 and Cy3 labeled probes) in new eppendorf tube. Then add:

30-50 µg human Cot-1 DNA (Gibco/BRL; 1 mg/ml stock; blocks hybridization to repetitive DNAs if present on array).
100 µg yeast tRNA (Gibco/BRL; make a 5 mg/ml stock; blocks non-specific DNA hybridization).
20 µg poly(dA-dT) (Sigma; make a 5 mg/ml stock; blocks hybridization to polyA tails of cDNA array elements).
450 µl TE 7.4

Concentrate with a Microcon-30 filter as above (8000g, ~15 min, then check volume every 1 min until appropriate). Collect probe mixture in a volume of 12 µl or less.

9. Adjust volume of probe mixture to 12 µl with ddH_2O. Then add 2.55 µl 20X SSC (for a final conc.of 3.4X) and 0.45 µl 10% SDS (for a final conc. of 0.3%).

10. Denature the hybridization mixture (100°C, 1.5 min), incubate for 30 min at 37 °C (Cot-1 preannealing step), then hybridize to the microarray.

If you have any questions, comments, or improvements, please pass them along to:

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