Genomic DNA Labeling Protocol

We typically use 0.5ug of E. coli genomic DNA in a labeling reaction for each hybridization. The genomic DNA was fragmented to 500 to 1000bps before labeling. The following protocol should produce enough labeled probe for 8 hybridizations.

For labeling 4ug Genomic DNA:

DNA Mix

Genomic DNA	1.9ug/ul	2.1ul
Random Hexamer	5mg/ml	1ul
H_2O		14.9
Total		20ul

Heat to 95C for 5min, place on ice for 5min

Labeling

DNA Mix		20ul
dAGC	5mM each	5ul
EcoPol Buffer	10x	5ul
CyDye-dUTP	1mM	2ul
H_2O		17ul
Klenow Fragment	50u/ul	1ul
Total		20ul

Incubate at 37°C for 3.5 hours

Add 2.5ul 0.5M EDTA to stop reaction

Clean up Labeled Probes

- Prewash Microcon-30 microfilter by adding 450ml miliQ H_2O and spinning for 10 min. @ 12,000 RPM.
- Add 450ml miliQ H_2O to each of the probe samples (or total 500ul). Mix thoroughly by pipetting up and down. Transfer samples to separate Microcon-30 microfilters. (Amicon)
- Spin at 12,000 RPM in microfuge for 10 minutes or until 20-40 ml remains in the filter.
- Add 450 ml miliQ H_2O to the probe and gently mix by pipetting up and down. Be careful not to touch the filter at the bottom of the filtration unit.

Spin 10 minutes at 12,000 RPM.

- Repeat step 4 , spin 12min to get smaller volume.
- Invert column into a fresh tube and spin 1 minute at maximum speed to recover probe. Carefully measure recovered probe volume if necessary.

Probe can be stored at 4°C or -20°C in dark for further use.

Reagents and Suppliers

Cy3-dUTP	1mM	Perkinelmer	NEL578
Cy5-dUTP	1mM	Perkinelmer	NEL579
Klenow Fragment	50U/ul	NEB	M0210M
100 mM dNTP set*	10X	Amersham	27-2035-01
$pd(N)_6$ Sodium Salt (Hexamer)	50U	Amersham	27-2166-01
Microcon YM-30 column		Amicon	42410

*for 10X stock: 5 mM each of dA, dG, dC.