

Step 1. DNA probe generation

Invitrogen BioPrime® DNA Labeling System

Cat. No.: 18094-011 Size: 30 Reactions. Store at -20°C

DESCRIPTION (derived from Invitrogen product document)

Random primers (octamers) are annealed to the denatured DNA template and extended by Klenow fragment in the presence of biotin-14- dCTP to produce sensitive biotinylated-DNA probes for use in the nonradioactive detection of DNA and RNA. With the BioPrime® DNA Labeling System, considerable net DNA synthesis occurs resulting in an 10-40 fold amplification of the probe.

BioPrime® LABELING PROTOCOL (modified from Invitrogen product document)

Dissolve or dilute 500 ng DNA in 7 µl of dilute buffer in a microcentrifuge tube.

On ice, add 20 µl 2.5X Random Primers Solution.

Denature by heating for 5 min @ 100°C; immediately cool on ice.

Perform the following additions on ice:

- 5 µl 10X dNTP Mixture
- 17 µl Distilled Water [to a total volume of 49 µl]
- Mix briefly.
- Add 1 µl Klenow Fragment. Mix gently but thoroughly.
- Centrifuge 15-30 sec.
- Incubate at 37°C for 60 min.
- Add 5 µl Stop Buffer.

Test yield using 1ul in the Nanodrop spectrophotometer.

Step 2. Production of Hybridisation cocktail

(as per Affymetrix Eukaryotic hyb instructions – <http://affymetrix.com>)

- | | |
|---------------------------------|-----------------------------------|
| • 3ul BSA (50mg/mL) | • 70.3ul DEPC water. |
| • 2.7ul Herring sperm (10mg/mL) | |
| • 5ul B2 Control | - add 54ul of the sample solution |
| • 15ul 20x Euk Control | from above. |
| • 150ul 2x Hyb buffer | - Total volume = 300ul |

Step 3. Normal (as post fragmentation) Affymetrix GeneChip hyb procedure

Wet chip.

- 100ul H₂O
- 100ul 2x Buffer
- 45°C Oven 10 mins @ 60rpm

Specimen - 99°C for 5 min

Specimen - 45°C for 5 min

Centrifuge specimen @1500 for 5 min

Remove wetting solution.

Fill chip with 200ul of specimen.

Hybridise overnight @45°C for 16hrs at 60rpm.