CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

Materials

Chemicals were purchased from Sigma unless otherwise noted

- Dynabeads M-280 Streptavidin (Cat. No. 112.05) from Dynal
- magnetic particle concentrator (MPC) from Dynal
- immobilization activator from Dynal
- Biotin-21-dUTP (Cat. No. 5201-1) was obtained from Clontech
- Biotin-14-dATP (Cat. No. 520-95245A) from GibcoBRL
- Thio-dCTP (Cat. No. 27-7360) from Pharmacia
- thio-dGTP (27-7370) from Pharmacia
- G-50 gel filtration (NICK) columns (17-0855-01) from Pharmacia
- Restriction enzymes from Boehringer Mannheim
- klenow (Cat. No. M2201) from Promega.

Solutions

TE

10 mM Tris, 1 mM EDTA, pH 8. To prepare 500 ml, dissolve 0.61 g Tris base and 0.15 g EDTA in 450 ml distilled water. Adjust pH to 8.0 with 2 M HCl and volume to 500 ml. Autoclave and store at room temperature.

2X bead buffer

4 M NaCl, 20 mM Tris, 2 mM EDTA, pH 7.6. To prepare 250 ml, dissolve 58.44 g NaCl, 0.61 g Tris base, and 0.15 g EDTA in 200 ml distilled water. Adjust pH to 7.6 with 2 M HCl and volume to 250 ml. Store at room temperature.

Coupling mix

biotinylated DNA solution containing 1X bead buffer and 1X immobilization activator. Combine 400 ml biotinylated DNA, 300 ml 5X immobilization activator, 750 ml 2X bead buffer and 50 ml distilled water. Prepare just before use.

Steps

1. Prepare plasmid DNA by Qiagen column purification, and cut 50 _g with two restriction enzymes in the polylinker to produce one short and one long DNA fragment. One end of the long fragment should terminate in an overhang containing Gs and Cs , the other should contain only As and Ts (e.g. Not1, BamH1).

2. Ethanol precipitate the DNA, and resuspend in 25 _l TE. Quantify recovery by OD260 measurement.

3. Prepare fill-in reaction in 70 _l, containing 1X klenow buffer, 30 mg DNA, 50 mM nucleotides (biotin-dATP, biotin-dUTP, thio-dCTP and thio-dGTP) and 20 units klenow. Incubate for 2 hours at 37°C.

4. Remove unincorporated nucleotides, following instructions supplied with Pharmacia Nick columns. The DNA is eluted in a large volume, but the recovery is better than with spin columns. Quantify recovery by OD260 measurement.

5. Prepare 5 _l of streptavidin beads for each mg of DNA recovered, so 150 _l for 30 _g. Wash beads three times with bead buffer, using the MPC (magnet) to retrieve the beads. After the final wash, resuspend beads in coupling mix containing DNA.

6. Prepare coupling mix and take a 25 _l sample for later evaluation of coupling efficiency.

7. Incubate bead/coupling mixture overnight on a rotator at 20°C.

8. Retrieve beads with a magnet and save supernatant. Compare OD260 to before coupling sample to determine the amount of DNA immobilized. Typically two thirds of the DNA is coupled.

9. Wash beads four times with bead buffer. After the last wash, resuspend the beads in bead buffer so that the final concentration of immobilized DNA is 1 µg/5 µl of beads. Store at 4°C.