Preparation of Oligolabeled Probes

Reference:
Fienberg and Vogelstein, 1983. Anal. Biochem. 132:6-13.

Materials:
RadPrime DNA Labeling System from Invitrogen Life Technologies
DNA - 25 ng in water or TE
[α-^{32}P]dCTP (3000 Ci/mmol)
TE, pH 8.0
Sephadex G-50: Slowly add 5 grams of Sephadex G-50 (Fine, DNA grade) to 100 ml of ddH_2O in a 250 ml beaker while mixing. Make sure the powder is well dispersed. Pour the resin into a 0.45 µm filtration unit. Wash the resin with 2 volumes of ddH_2O while filtering. Resuspend the resin in TE (pH 7.4). Transfer the resin to a screw capped bottle. Autoclave for 15-30 minutes at 15 lbs/in^2. Allow it to cool to room temperature and settle. Decant the supernatant and replace it with an equal volume of TE (pH 7.4). Store at 4°C.

Protocol:

Part II: Preparation of Oligolabelled probes

1. Denature 25 ng of DNA in 5-20 µl by heating in a boiling water bath for 5 minutes. Immediately cool on ice. Centrifuge the tube briefly.

2. Add the following to the microcentrifuge tube on ice:

Distilled dH_2O	21 µl - X µl *
500 µM dATP**	1 µl
500 µM dGTP**	1 µl
500 µM dTTP**	1 µl
2.5 X Random Primers Solution**	20 µl
[α-^{32}P]dCTP (3000 Ci/mmol)	5 µl
Total volume	49 µl

* X µl is the volume of the DNA (step1).
** Supplied with the RadPrime DNA Labeling System.

3. Mix.
4. Add 1 µl of Klenow fragment (supplied with the RadPrime DNA Labeling System). Mix gently, then centrifuge 15 - 30 sec.
5. Incubate at 37°C for 10 minutes to 1 hour (we get optimal labeling with 1 hour incubations).
6. Add 5 µl of Stop Buffer (supplied with the RadPrime DNA Labeling System) and 45 µl TE, pH 8.0.

Part III: Removal of unincorporated radionucleotides by spun column chromatography

1. Preparation of spun columns:

- Plug the bottom of a 1 ml disposable syringe with a small amount of sterile glass wool. This is best accomplished by using the plunger of the syringe from which the rubber end has been cut off to tamp down the glass wool.
- Fill the syringe with Sephadex G-50. Add more resin until the syringe is completely filled.
- Insert the syringe into a 15 ml corex tube. Centrifuge at 1,600 g for 4 minutes. Do not be alarmed by the appearance of the column. The resin packs down and becomes partially dehydrated during centrifugation. Continue to add more resin and centrifuge until the volume of the packed column is approximately 0.9 ml.
- Add 0.1 ml TE (pH 8.0) to the columns, and centrifuge at 1,600 g for 4 minutes. Repeat.
- Spun columns may be stored at this stage if desired. Fill the syringes with TE (pH 8.0) and wrap with Parafilm to prevent evaporation. Store upright at 4°C. Immediately before use centrifuge the columns at 1,600 g for 4 minutes, then wash once with 0.1 ml of TE (pH 8.0).

2. Apply the probe (100 µl) to the column. Place the column in a 15 ml corex tube that has a decapped microcentrifuge tube in the bottom.

3. Centrifuge at 1,600 g for 4 minutes. The effluent will collect in the decapped microcentrifuge tube.

4. Remove the syringe, which contains the unincorporated radiolabeled dNTPs and other small components (<16 nt). Transfer the contents of the decapped microcentrifuge tube to a capped, labeled microcentrifuge tube.

5. Count 1 µl in a liquid scintillation counter.

6. Store the radiolabeled probe at -20°C until it is needed.

NOTE: probes labeled to high specific activity are rapidly damaged by radiochemical decay and should be used without delay.