RANDOM PRIMING DNA

(Feinberg and Vogelstein (1983) Anal. Biochem. 137, 266-267.)

Can use purified fragment in which case start at 5.

1. Digest DNA in appropriate restriction buffer. Electrophorese on a 1-2% low melting agarose gel in cold room.

2. Excise fragment of interest and weigh agarose slice in a preweighed eppendorf tube (trim as much agarose as possible).

3. Add H_2O at a ratio of 3 ml H_2O to 1 g agarose.

4. Boil tube for 7' and then measure volume of liquid. Calculate concentration of fragment per ml of solution based on amount of DNA fragment in gel (assume 100% recovery). Store at –20°C in aliquots of 50 µl.

5. Use approximately 20 ng DNA per labeling reaction. Boil DNA for 3' and place on ice if purified fragment or at 37°C if in agarose.

6. Set up labeling reaction as follows:

   15.5 µl DNA and H_2O
   5 µl **OLB**
   1 µl 10 $mgml^{-1}$ BSA
   1 µl Klenow polymerase
   2.5 µCi $^{32}P\alpha dCTP$

7. Incubate at 37°C for 1- 3 hours or overnight at room temperature.

8. Add 300 µl TE and boil for 10' prior to use.

To make **OLB:**

Solution A (Store at –20°C)

625 μl 2M Tris-HCl pH 8
125 μl 1M $MgCl_2$
18 μl β-mercaptotethanol
5 μl 100 mM dATP in TE
5 μl 100 mM dTTP in TE
5 μl 100 mM dGTP in TE
250 μl dH_2O

Solution B (Store at 4°C)
2 M Hepes pH 6.6 with NaOH

Solution C
Hexadeoxyribonucleatides (Pharmacia #2166) evenly suspended in TE at 90 OD unitsml^{-1}.

OLB = A 100 : B 250 : C 150

Store at –20°C in 50 μl aliquots.