Random primer DNA labeling

Experimental Considerations

- We typically employ the T7 QuickPrime Kit from Pharmacia (cat# 27-952-01), which is based on the technique described by Feinberg and Volgstein (Anal Biochem,1983,132:6)
- This kit can be used to label purified DNA samples as well as samples in low-melting point agarose pieces.
- It contains pre-mixed reagents, making the process easier.

Protocol

- Preparation of DNA

  - For LMP probes (i.e. for labeling DNA samples contained in Low Melting Point Agarose gel pieces):
    - Excise DNA fragment that is to be labeled from a Low Melting Point Agarose gel.
    - Melt agarose piece at 65°C for 5 minutes.
    - Add 2 volumes of ddH_2O.
    - Aliquot 25ul into labeling tube (you may freeze the remainder for subsequent labeling reactions).
    - Bring volume in labeling tube up to 49ul with ddH_2O.
    - Heat at 95°C for 10 minutes to denature DNA.
    - Transfer solution to 37°C bath for 10 minutes.
    - Spin down briefly.
    - Start reaction immediately (see below).

  - For purified probes:
    - Aliquot probe into labeling tube (50-200ng DNA).
    - Bring volume up to 49ul with ddH_2O.
    - Incubate probe at 95°C for 5 minutes to denature DNA.
    - Spin down briefly and place immediately on ice.
    - Start reaction (see below).

- To start reaction, add the following to labeling tube:

10ul	Reagent mix**
5ul	^{32}P-dCTP (600-3000 Ci/mM)
1ul	T7 DNA polymerase**

- Incubate for 5 to 15 minutes at 37°C.
- Run spun column to separate probe from free nucleotides.

Solutions

**T7 QuickPrime Kit - 40 reactions - (Pharmacia Biotech Cat# 27-952-01)