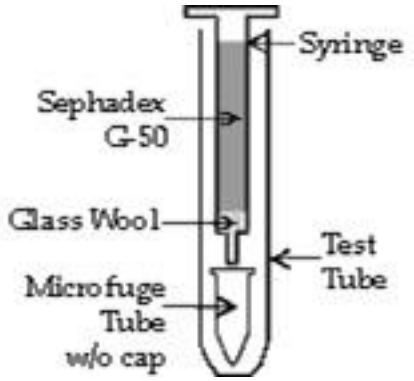
SPIN-COLUMN PROCEDURE

This method is useful when several preparations of DNA are labeled simultaneously or when it is necessary to change the buffer in which DNA is dissolved.

1) Plug the bottom of a 1 ml disposable syringe with a small amount of sterile glass wool. Make sure you use enough that the Sephadex will not leak out. In the syringe, prepare a column (0.9 ml bed volume) of Sephadex G-50 equilibrated in TE (pH 8.0), containing0.1 M NaCl (STE). Add a Sephadex suspension, and allow the liquid to drain out by gravity. Add more until the column is full of Sephadex.

2) Insert the syringe into a glass centrifuge tube, as shown in figure. Centrifuge at 1600g for 4 minutes in a bench centrifuge. Do not be alarmed by the appearance of the column. Usually the Sephadex packs down during centrifugation. Continue to add Sephadex until the packed column volume is 0.9 ml.



Maniatis, p. 466.

3) Add 0.1 ml of STE and recentrifuge at exactly the same speed and for exactly the same time as before.

4) Repeat step 3.

5) Apply the DNA sample to the column in a total volume of 0.1 ml (use STE to make up the volume).

6) Recentrifuge at exactly the same speed and for exactly the same time as before, collecting the 100 ml of effluent from the syringe in a decapped microfuge tube (see figure). Save cap for sealing tube later.

Preparing Sephadex

1) Slowly add sephadex of desired grade (usually G–50) to distilled, sterile water in a 500 ml beaker or bottle. 10 g sephadex G–50 yields 160 ml of slurry. Wash the swollen resin with distilled, sterile water several times to remove soluble dextran, which can create problems by precipitating during ethanol precipitation.

2) Equilibrate the resin in TE pH 8.0 with 0.1 M NaCl (STE), and add sodium azide to a concentration of 0.02%