ELECTROELUTION INTO DIALYSIS BAGS

1. Run the gel and localize the band of interest using a long-wavelength (300-360 nm) UV lamp to minimize damage to DNA.

2. Using a plastic ruler, cut out the slice of agarose containing the band. Be sure to cut out the band precisely; if there is a "trailer" of diffuse ethidium staining, DO NOT cut that out! It may include junk that will inhibit your ligation reactions.

3. Photograph the gel after cutting out the band, so you will have a record of which band was eluted.

4. Fill a dialysis bag to overflowing (see Maniatis Cloning Manual for preparation of dialysis tubing) with 0.5x TBE. Holding the neck of the bag, pick up the gel slice with forceps and place it in the fluid-filled bag.

5. Allow the gel slice to sink to the bottom of the bag. Remove most of the buffer, leaving just enough fluid to keep the gel slice in constant contact with the electrophoresis buffer. Then tie the bag just above the gel slice; avoid trapping air bubbles.

6. Immerse the bag in a shallow layer of 0.5x TBE in an electrophoresis tank. Pass electric current through the bag (usually 100V for 2-3 hours). During this time, the DNA is electroeluted out of the gel and onto the inner wall of the dialysis bag.

7. Reverse the polarity of the current for 2 minutes to release the DNA from the wall of the dialysis bag.

8. Open dialysis bag and carefully recover all the buffer surrounding the gel slice. Using a pasteur pipette, wash out the bag with a small quantity of 0.5x TBE.

9. Stain the gel slice with ethidium bromide (for 30 minutes in 0.5x TBE containing 0.5 µg/ml of ethidium bromide). Examine under UV light to check that all the DNA is eluted.

10. Purify the DNA by the following method:
    1. Pass the DNA recovered from the gel in 0.5x TBE over a column of packed siliconized glass wool in a pasteur pipette to remove pieces of gel.
    2. Extract the eluate twice with phenol, once with phenol/chloroform, and once with chloroform.
    3. Recover the DNA by ethanol ppt.
    4. Resuspend the precipitate in 200 µl of H_2O, add 25 µl of 3 M NaOAc (pH 5.2), and ppt. the DNA again w/EtOH.

Reference: McDonnell et al. (1977) J. Mol. Biol. 110:119.