Electrophoresis of DNA fragments from a gel onto DEAE-p81 paper (or NA-45) followed by elution

1. Digest your DNA to completion with the appropriate restriction enzymes and run on a preparative agarose gel. Electrophorese until the fragment of interest is well resolved.

2. Stop the electrophoresis and cut slits in the gel (using a clean scalpel or razor blade) immediately before and after the band of interest.

3. Prewet the DEAE paper in whatever buffer is being used in the gel (TBE or TAE).

4. Insert the slices of DEAE paper in the slits that were just cut into the gel (before and after the band). Cut the paper a little longer than the band since all excess will be cut off later.

5. Slide the gel back together so that the agarose is in contact with the paper. This way the current will be able to pass through the DEAE paper freely. Be sure that the paper is pushed down the whole thickness of the gel and that there are no air bubbles caught in the slits.

6. Electrophorese an additional 10 - 15 minutes allowing the band to run onto the DEAE paper. You can monitor this using a hand held UV lamp.

7. Carefully remove the paper containing the DNA fragment with clean forceps. Verify that the fragment is there using a UV lamp and cut off any excess paper not containing any DNA.

8. Pack the paper into a 1 mL syringe with a Pasteur pipet (being careful not to tear the paper). Next pack down the paper with the plunger of the syringe.

9. Add 800 µL TE buffer to wash the paper. Push the liquid through with the syringe plunger. Once all the liquid is through, push the plunger all the way down to repack the paper and to force out any remaining liquid. Now remove the plunger.

10. Add 400 µL elution buffer, push with the plunger until the liquid just begins to emerge from the syringe tip. Cap the bottom of the syringe and leave the plunger in so that top and bottom are sealed.

    Elution Buffer
    10 mM Tris pH 8
    1 mM EDTA
    1 M NaCl

11. Place the syringe into a heat block (equilibrated to 65°C). Incubate at 65°C for 15 minutes (up to 1 hour if > 1500bp).

12. Remove the bottom cap and push the elution buffer into a clean tube (push the plunger all the way to the bottom to repack the paper before pulling it back out).

13. Repeat steps 10-12 for a total of three elutions.

14. Combine the three fractions and extract once with an equal volume of phenol/chloroform/isoamyl alcohol and once with chloroform/isoamyl alcohol.

15. Add 3 mL 100 % Ethanol and precipitate overnight at -20°C.

16. Centrifuge at 20,000 x g for 30 minutes. Wash with 70 % Ethanol.

17. Resuspend the DNA in 10 mM Tris pH 8.5 and quantitate.