

# ELUTION OF DNA FROM AGAROSE GELS

## OBJECTIVE:

To isolate specific bands or regions of agarose-separated DNA for use in subsequent experiments and/or procedures.

## INTRODUCTION:

It is sometimes necessary to isolate a particular DNA fragment from others. For example, you may want to use a specific fragment as a probe for a blot or you may want to clone or subclone a particular fragment. This method can save a great deal of time when attempting to clone a fragment from a genomic digest. For example, you may know the location on a gel of a particular fragment that you want to clone, having located it previously by Southern hybridization. You can digest total DNA and clone all of it, then later identify the clones that contain the fragment of interest, or you can enrich for the fragment of interest prior to cloning. In the latter case, you can save yourself a great deal of time and effort since you will decrease the number of clones that you have to search through to find the one that you want.

Enrichment for the fragment of interest is generally accomplished by elution of the fragment from a gel. While this sounds simple, it is often a messy and impurities in some agaroses can cause inhibition of the enzymes used later in the process of cloning.

There are many methods for eluting DNA from a piece of agarose, and different types of agarose for this purpose. One method is called electroelution. The gel fragment containing the DNA of interest is cut out of the gel and placed into a dialysis bag with some buffer. The bag is then placed into a gel box (also containing the same buffer) and an electric current is applied. After a period of time (usually from 5-30 minutes at from 50-200 V in a small gel box, depending on the size of the gel fragment and the molecular weight of the DNA) the current is reversed for 15-60 seconds to remove any DNA that is stuck to the dialysis bag. The solution inside the bag is then removed and the DNA is ethanol precipitated from the solution. There are also several commercially available electroelution devices.

Another method is to cut the gel directly in front of the band of interest and slide into the slit a piece of paper (DEAE cellulose or DE-81 paper) that will bind the DNA. A piece of dialysis membrane is also placed behind (side away from the DNA band) the paper to stop any DNA from passing completely through. An electric current is then applied until the band moves into the paper. the paper is removed and then, by changing the salt concentration, the DNA is washed off of the paper and precipitated with ethanol. This method yields inconsistent results. A method similar to this is to cut a well in front of the band of interest and fill it with buffer. A piece of dialysis membrane is placed on the far side (positive electrode side) of the well to act as a barrier to the DNA (but not to the electric current). Then an electric current is applied so that the DNA moves into the well. Any DNA passing through the well is stopped by the dialysis membrane. After the band has all moved out of the gel and into the well and membrane, the current is reversed for 15-60 seconds to remove any DNA from the membrane and the solution in the well is removed. The DNA is then precipitated from this solution.

Other methods employ glass beads or columns that either bind DNA or contaminants to purify

the DNA. The main problem with the glass bead methods is that there is often degradation of the DNA, especially if the fragment is larger than 5 kb. Many column purification methods are available, including the ones described in the DNA sequencing section (pages 50 - 61)

The method we will be using, known as the "optimized freeze-squeeze method," (Thuring, et al., 1975; Tautz and Renz, 1983) has several advantages over these others. First, it is not as messy as the others, which is desirable when you are working with small amounts of DNA or where ethidium bromide is used. Second, it is very simple and fast to perform. Third, the yields of DNA are excellent. When small fragments are eluted (less than 5 kb) from 80-95% can be recovered. Even lambda DNA (at 50 kb) can be recovered in yields up to 50%. Fourth, the DNA is excellent shape after precipitation. With some methods of elution, there is a significant amount of degradation of the DNA. With this method, even lambda-sized pieces can be obtained with little or no apparent damage.

### STEPS IN THE PROCEDURE:

1. Since we will be using the DNA for ligations and then for transformations, we will first digest 1 µg of pBR325, and 1-2 µg of each DNA that will be ligated to the pBR325, each separately with EcoRI as follows:

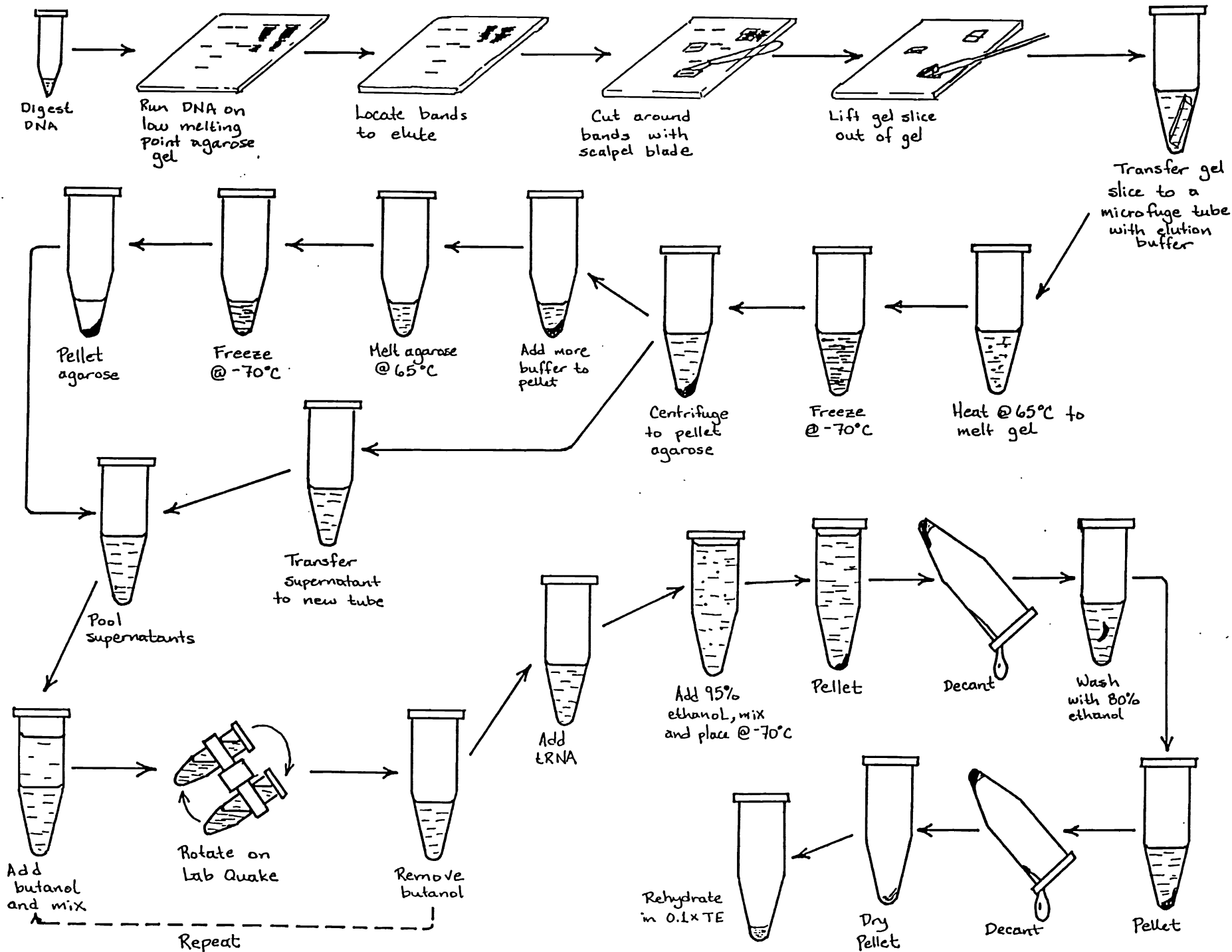
2 µl pBR325 DNA (500 ng/µl)  
6 µl H<sub>2</sub>O  
1 µl 10X RE salts  
1 µl EcoRI

2 µl pGEM3Z DNA (500 ng/µl)  
6 µl H<sub>2</sub>O  
1 µl 10X RE salts  
1 µl EcoRI

8 µl of DNA + H<sub>2</sub>O  
1 µl 10X RE salts  
1 µl EcoRI

[NOTE: This method can be used to recover smaller amounts of DNA from gels, as well. Excellent yields (>50%) can be obtained even when starting with amounts as low as 1-10 ng.]

2. Pour a 0.7% agarose gel using the low melting point agarose. [I have found the FMC low melting point agaroses, NuSieve GTG and SeaPlaque, to be the best for this method. They are more expensive than others, but well worth it, since time can be saved and frustration avoided.] Use the 8 well comb. This agarose takes longer to harden and appears to be much flimsier than a standard gel of the same percentage. For example, a 0.5% low melting point gel is about as difficult to handle as a 0.3-0.4% standard gel. Allow the gel to harden at least several hours in the refrigerator or overnight in a cool lab. [NOTE: these gels are nearly impossible to use in a lab that is above 80 °F, since they never completely harden.]



3. Since we are mainly trying to purify the DNA away from the *EcoRI* activity and we are not trying to separate bands from one another, electrophoresis of the gel only needs to be for about an hour.
4. Take a picture of the gel. Be sure that you have protective goggles and a face shield on. Then, with the gel on the gel tray and the tray on the UV light box, locate the plasmid band. It should be very large (and probably distorted, since we loaded so much into the lane). Using a scalpel blade (or a razor blade), carefully cut around the band (trying not to scratch the tray beneath the gel). Also, cut around the DNA that will be eluted from the other lanes. The gel pieces should be the width of the lane and no more than about a centimeter wide in the other direction. [If you want to test the difference in ligation and transformation efficiency between larger fragments and smaller fragments, cut some of the lanes in halves (or smaller pieces) according to molecular weight.]
5. Turn off the UV light. Carefully transfer the gel pieces into separate 1.5 ml microfuge tubes using flat spatulas and/or forceps (don't use fine forceps or you will damage the gel slices). Label the tubes accordingly.
6. Add enough elution buffer until the level of the buffer is a few millimeters above the level of the gel slice. Heat in a 65 °C water bath until the agarose melts (about 5 minutes).
7. Fast-freeze by placing in a -70 °C freezer for 10 minutes (or in an alcohol/dry ice bath, liquid nitrogen, or on a block of dry ice).
8. Immediately (i.e., before the solution can thaw) centrifuge for 10 minutes.
9. Transfer the supernatant into a new tube.
10. Add fresh elution buffer to the pellet. This time add about half as much as you added in step 6.
11. Heat in a 65 °C water bath until the pellet melts (about 5 minutes).
12. Fast-freeze and centrifuge as before (steps 7-8).
13. Pool the supernatant with the supernatant from step 9 (discard the tube containing the pellet).
14. Add an equal volume of 1-butanol to the supernatant fraction and mix thoroughly. Then, place the tube onto the Lab Quake for 15 minutes (this will remove the ethidium bromide from the DNA). Discard the top (butanol) phase and repeat with new 1-butanol one or two more times.
15. Add 50 µg of yeast tRNA and mix thoroughly. [Note: If performing PCR or Cycle Sequencing in subsequent steps, DO NOT use the tRNA)

16. Add 2.5 volumes of cold 95% ethanol and mix gently. Allow to precipitate at  $-70^{\circ}\text{C}$  for 30 minutes or more. Overnight is best, and for dilute samples it is absolutely necessary.
17. Centrifuge for 15 minutes.
18. Decant off and discard the supernatant and add 200  $\mu\text{l}$  of cold 80% ethanol to the pellet. Mix until the pellet is dislodged from the bottom of the tube.
19. Centrifuge for 5 minutes. Decant off and discard the supernatant.
20. Dry the pellet in the Speed-Vac.
21. Rehydrate the DNA pellets in 20  $\mu\text{l}$  of 0.1X TE.
22. If there is any remaining debris in the tube, spin for 30 seconds and transfer the supernatant into a new microfuge tube (either 1.5 ml or 0.5 ml).
23. To confirm that you have recovered the DNA you should run 1  $\mu\text{l}$  of each sample on a standard gel (0.6-0.7%). You can then calculate the percent recovered.

### SOLUTIONS:

#### Elution Buffer

0.5 M sodium acetate (pH 7.0)  
1 mM EDTA (pH 8.0)

#### Yeast tRNA (as a carrier)

10 mg/ml

#### 95% Ethanol

#### 80% Ethanol

#### 0.1X TE:

1 mM Tris (pH 8.0)  
0.1 mM EDTA (pH 8.0)

### REFERENCES:

Thuring, et al., 1975, Anal. Biochem. 66:213-220  
Tautz and Renz, 1983, Anal. Biochem. 132:14-19