

## RESTRICTION ENZYME ANALYSIS

Each restriction enzyme has a set of optimal reaction conditions, which are given on the information sheet supplied by the manufacturer. The major variables are the temperature of incubation and the composition of the buffer. Although the temperature requirements are fairly strict, the differences between buffers are often only slight. To avoid the labor involved in making up a separate buffer for every enzyme, it is convenient to divide the enzymes into three groups - those that work best at high ionic strength; those that prefer medium ionic strength; and those that have a preference for buffers of low ionic strength.

By following this scheme, only three stock buffers need be prepared (see table below). Usually all of the buffers are made up as 10 X stock solutions, which may be stored at 4°C for periods of one to two weeks or at -20°C indefinitely.

### Setting Up Digestions with Restriction Enzymes

Reactions typically contain 0.2-1 µg of DNA in a volume of 20 µl or less.

1. Mix water with the DNA solution in a sterile Eppendorf tube to give a volume of 18 µl.
2. Add 2.0 µl of the appropriate 10 X digestion buffer. Mix by tapping tube.
3. Add 1 unit of restriction enzyme, and mix by tapping tube. (One unit of enzyme is usually defined as the amount required to digest 1 µg of DNA to completion in 1 hour in the recommended buffer and at the recommended temperature in a 20-µl reaction. In general, digestion for longer periods of time or with excess enzyme does not cause problems unless there is contamination with DNase or exonuclease. Such contamination is rare in commercial enzyme preparations.)

### Buffers for Restriction Endonuclease Digestion

Buffer	NaCl	Tris ^Cl (pH 7.5)	MgCl <sub>2</sub>	Dithiothreitol
Low	0	10 mM	10 mM	1 mM
Medium	50 mM	10 mM	10 mM	1 mM
High	100 mM	50 mM	10 mM	1 mM

Because the enzyme SmaI will not work well in any of the above buffers, a separate buffer should be made up, consisting of:

20 mM KCl  
10 mM Tris ^Cl (pH 8.0)  
10 mM MgCl<sub>2</sub>  
1 mM Dithiothreitol

4. Incubate at the appropriate temperature for the required period of time.
5. Stop the reaction by the addition of 0.5 M EDTA (pH 7.5) to a final concentration of 10 mM.

If the DNA is to be analyzed directly on a gel, add 6  $\mu$ l of gel-loading dye, mix by vortexing briefly, and load the digest into the gel slot.

If the restricted DNA is to be purified, extract once with phenol/ chloroform, once with chloroform, and precipitate the DNA with ethanol.

Notes:

Restriction enzymes are expensive! Costs can be kept to a minimum by following the advice given below.

- i. Many restriction enzymes are supplied by the manufacturer in concentrated form. Often 1  $\mu$ l of many enzyme preparations is sufficient to digest 10  $\mu$ g of DNA in an hour. To remove small quantities of enzyme from the container, touch the tip of a disposable, glass micropipette (0-5  $\mu$ l) briefly to the surface of the fluid. In this way it is possible to remove as little as 0.1  $\mu$ l of the enzyme preparation. Alternatively, a small piece of plastic tubing (1 cm long) can be attached to a 1- $\mu$ l volumes. The plastic tubing is discarded after each sample is pipetted.
- ii. Restriction enzymes are stable when they are stored at -20°C in buffer containing 50% glycerol. When carrying out restriction enzyme digestions, prepare the reactions to the point where all reagents except the enzyme have been mixed. Take the enzyme from the freezer and immediately put it into ice. Use a fresh, sterile pipette every time you dispense enzyme. Contamination of an enzyme with DNA or another enzyme can be costly and time-consuming. Work as quickly as possible, so that the enzyme is out of the freezer for as short a time as possible. Return the enzyme to the freezer immediately after use.
- iii. Keep reaction volumes to a minimum by reducing the amount of water in the reaction as much as possible. However, make sure that the restriction enzyme contributes less than 1/10 volume of the final reaction mixture, otherwise the enzyme activity may be inhibited by glycerol.
- iv. Often the amount of enzyme can be reduced if the digestion time is increased. This can result in considerable savings when large quantities of DNA are cleaved. Small aliquots can be removed during the course of the reaction and analyzed on a minigel to monitor the progress of the digestion.
- v. When digesting many DNA samples with the same enzyme, calculate the total amount of enzyme that is needed. Remove the correct amount of enzyme solution from the container and mix it with the appropriate volume of water and 10 X restriction buffer. Dispense aliquots of the enzyme/buffer mixture into the reaction mixtures.

- vi. When DNA is to be cleaved with two or more restriction enzymes, the digestions can be carried out simultaneously if both enzymes work in the same buffer. Alternatively, the enzyme that works in the buffer of lower ionic strength should be used first. The appropriate amount of salt and the second enzyme(s) can then be added and the incubation continued.
  
- vii. If the volume of the restriction enzyme reaction is too large to fit into the slot of a gel, the DNA may be concentrated by the following simple procedure: After the reaction has been stopped by the addition of EDTA, add 2/3 volumes of 5 M ammonium acetate and 2 volumes of ethanol. Chill in a dry-ice/methanol bath for 5 minutes, then centrifuge for 5 minutes in an Eppendorf centrifuge. Discard the supernatant, which contains most of the protein. Dry the pellet briefly under vacuum. Dissolve the DNA in the appropriate volume of TE (pH 7.6).

Procedure from Maniatis cloning manual.