RESTRICTION DIGESTION OF DNA

wisdom from Howard Judelson

Read the "rules" at the end of this document!

Setting up the digest

1. Combine the following in a microfuge tube in order (this is for a 20 µl digest; this can be scaled up or down):

2 µl	10x Buffer
13 µl	water (to 20 µl final volume)
0.2 µl	100X BSA (optional**)
2 µl	DNA (0.5-3 µg depending on application)
XX µl	Enzyme*

*In general, aim for about 10-fold overdigestion in a 2 hour digest. By definition, 1 unit of enzyme cuts 1 µg of DNA per hour. So if you are cutting 1 µg of DNA for two hours, this means a 10-fold excess would be 5 units of enzyme. Most digests use about 0.5 µl of enzyme per reaction, which is usually 5 units.

** In nearly all cases, enzyme performance is enhanced by the presence of acetylated bovine serum albumin (BSA) in the reaction. Sometimes the effect is minor, sometimes major. Rather than try to remember which enzymes require BSA, I always add it!

2. Gently flick the tubes (don't vortex, this denatures proteins) and spin down in microfuge for a few seconds.

3. Place in heat block, water bath, or air incubator. *If you are digesting at a high temperature (50-60C), be aware that the liquid will evaporate, leading to a dried sample at the bottom of the tube and water on the lid. Either spin down the liquid every 15 min, or cover with sterile mineral oil.*

A few words about buffers

a. You can check in the enzyme company catalogue where there is a page devoted to enzyme/buffer compatibility. Salt concentration, Mg concentration, and pH are the typical variables.
b. You may be digesting your DNA with two (or more) enzymes. Make sure to use a buffer that will be most compatible with all the enzymes. There is usually a page in the back of an enzyme catalogue devoted to this problem. Often, you can find an intermediate enzyme that will allow simultaneous digestion with two enzymes, even if that is not optimal for one of the enzymes. Sometimes you have to first cut with one enzyme in one buffer, and then add more salt before adding a second enzyme.

Star activity

This is when the enzyme cuts at sites other than its cognate element. eg. *Eco*RI is supposed to only cut GAATTC but, under extreme conditions, it might possibly cut CAATTC also.

This happens when the salt or glycerol concentrations are wrong. The latter can especially be an issue when you are digesting simultaneously with multiple enzymes. Using the correct buffer, and keeping glycerol low, help reduce this problem. Also: know your enzyme!! Most enzymes don't ever show star activity. But some do, such as:

Acc B7 I	*Eco* 72 I	*Nde* I	*Sgf* I[a]
Bam H I	*Eco* R I	*Ngo* M IV	*Sph* I
Bcl I	*Hin* d III	*Pst* I	*Tth* 111 I
Bsr BR I	*Kpn* I	*Pvu* II	*Xmn* I
Bst 71 I	*Msp* A1 I	*Sal* I	
Bst E II	*Nci* I	*Sca* I	

Howard's rules about using restriction enzymes

Rule#1. Know your enzyme. Most enzymes can be digested at 37C in a simple salt buffer. However, a few enzymes require special conditions, which will be noted in the vendor's catalogue.

a. Some require BSA (bovine serum albumin). I always add BSA—it never hurts, and often helps.

b. Some require weak detergents (eg. triton-X-100) to reduce surface tension.

c. Some require to be incubated at temperatures other than 37C (eg. 50C).

d. Some don't cut methylated DNA, or only cut at methylated DNA.

Rule #2. Know your DNA and application.

a. How much DNA do you need to digest? Typically 500 ng is enough for checking most restriction digests on a gel, while a few micrograms are used for genomic DNA blots. For restriction digests, plan on having at least 100 ng per band, since this is the minimum visible.

Be aware that too much DNA loaded onto a gel is a bad thing: the band appears to run fast (implying that it is smaller than it really is) and in extreme cases can mess up the electrical field for the other bands, making them appear the wrong size also.

b. Is your DNA "clean" or "dirty"? Some DNAs digest poorly, and will require more enzyme than others.

c. Please be aware of the prices of enzymes. Some are very expensive, and should be used sparingly.

Rule #3. No digest is DNAse-free.

If your DNA is dirty, it may contain DNAses. Beware of long digestions, especially if you plan on cloning the reaction products. Most restriction enzymes die after about 3 hours at 37C, but many DNAses keep on working!!

Rule #4. Manufacturer's catalogues have a lot more information that I can provide here! Read them.