Prep digest for constructions

Use the following gudelines when setting up preparative digests:

In ligation reactions, the optimum situation is to have a 3 to 4 fold molar excess of insert to vector. So, if you start out digesting approximately equal molar amounts of your dna (based on the sizes of the fragments you want) you can set up your ligation reactions to have an excess of insert.

The following table summarizes this principle:

vector (3000bp)	1.5 ug	3 pmol
insert (500 bp)	10 ug	3.4 pmol

If you will be doing a lot of manipulations, such as Klenow, phosphatasing or multiple ethanol precipitations then you will want to start out with more DNA than suggested above.

You can calculate the molar quantities of DNA using the following formula:

$$[\text{pmol}] = \frac{\{\ 680\ (\text{molecular weight of one bp}) \times (\text{size of fragment in bp}) \times (\ \text{ug of DNA digested})\}}{1000000}$$

A typical reaction: (100 ul)

plasmid DNA (1mg/ml)	10 ul
10X enzyme buffer	10 ul
water	79 ul
enzyme	1 ul

- always check the catalogue of the company which made the enzyme for appropriate buffer conditions
- for plasmid DNA use 1 unit of enzyme per ug of DNA
- for PCR products use a 10 fold excess of enzyme
- Transfer to ice, check 1-2ul of digest on gel for completion of digestion, add more enzyme and continue incubation if incomplete.
- Separate desired vector or fragment by agarose gel electrophoresis (1% regular grade agarose for greater than 500 bp, or Nusieve GTG (20%) for very small fragments).
- Cut the desired band out of the gel with a scalpel blade on the UV transilluminator (if this takes a long time be sure to place your gel onto a glass plate to avoid UV damage to your DNA: likewise you should wear safety goggles to prevent UV damage to your eyes)
- Gel purify using glassmilk method OR add 1/10th vol NH4OAc, and precipitate with 2.5 volumes of absolute ethanol.
- Spin 10 min in microfuge, aspirate off supernatant carefully, wash pellet by addition of

0.5 ml 70% ethanol and respin 5 minutes, aspirate off carefully with a pulled pipette, air dry at 37øC for a few minutes or with speed vacuum and dissolve in sterile distilled water (volume depends on the next step). It is generally worth saving a small aliquot for rechecking on a gel in the future.