

DNA digestion protocol & hints

Overview: Although it is pretty standard to digest DNA with restriction enzymes, here are a standardized protocol and some hints...

References:

*Current protocols in molecular biology (3.1.1-3.1.2)

Materials:

- DNA sample in water or TE buffer
- 10x digestion buffer
- restriction enzyme
- DNA loading buffer
- Agarose gel 0.8% (or different depending on expected band sizes)

Procedure:

1. Pipet the following into a microfuge tube:

	20 μ l reaction	50 μ l reaction
DNA	0.1 to 4 μ g	0.1 to 4 μ g
10x Digestion buffer	2 μ l	5 μ l
Enzyme	?	?
Water	Rest of volume	Rest of volume

2. Add the enzyme (1-5u/ μ g DNA)
3. Incubate at recommended temperature for an hour.
4. Take 2 to 5 μ l of the digested sample, add loading buffer, and run on agarose gel to check the result.

Tips:

1. DNA:
 - for checking DNA, use 0.1 μ g; (or 4-8 μ l from a good DNA mini prep)
 - For cloning, 4 μ g DNA is enough.
2. Buffer: besides the buffer that comes with the enzyme, buffers from other company can be used, too (as long as the contents are the same)
3. Enzyme: the maximum volume that an enzyme can be used is 1/10 of the total reaction volume (example: 2 μ l for 10 μ l reaction)
4. Incubation time: can be longer than 1 hr, but don't do overnight digestion if you don't have to (genomic DNA requires overnight digestion).
5. Gel: make sure to run the uncut DNA along with the digested DNA. And, always run a DNA marker!