1 DNA double digestion protocol

Materials:

- DNA sample(s) in water or TE buffer
- 10x digestion buffer
- Restriction enzyme s (EcoRI or SpeI or XbaI or PstI)
- DNA loading buffer (if electrophoresis is subsequent)
- Agarose gel 1.5% (or different depending on expected band sizes)

Procedure:

1. Test the concentration of the DNA sample(s).

2. Pipet the following into a microfuge tube:

20uL reaction system	50uL reaction system
DNA around 1ug	around 2.5ug
10x Digestion buffer 2uL	5uL
1st Enzyme 1–1.5uL	2.5–4uL
2ndEnzyme 1–1.5uL	2.5–4uL
ddWater Rest of volume	Rest of volume

3. Incubate at recommended temperature (37.0 degrees) for 2 or 4 hours (0.5~2hfor enzymes of NEB, 4h for enzymes of Takara).

4. Take 2 to 5 uLof the digested sample, add loading buffer, and run it on the agarose gel to check the result, or take the entire sample to run to extract a wanted fragment).

Tips:

1. DNA:

- For identification of DNA, use 0.4 ug/uL DNA; (or 2uL from a nice DNA mini prep)
- For cloning, 1ug/uL DNA is enough.

2. Buffer: we'd better use the buffer that comes with the enzyme, which means buffers from other company may cause some abnormal results.

3. Enzyme: the maximum volume that an enzyme can be used is 1/10 of the total reaction volume (example: 2 uL for 20 uL reaction system). If you want to do overnight digestion, add less enzyme (example: 1 uL for 20 uL reaction system). It is necessary to point that too many enzymes will reduce the efficiency of enzyme digestion with glycerol in it.

4. Gel: make sure to run the uncut DNA as a control along with the digested DNA sample(s). And, always run a DNA marker!

References:

*Current protocols in molecular biology (3.1.1–3.1.2)