DNA double digestion protocol

Materials:

- DNA sample(s) in water or TE buffer
- 10x digestion buffer
- Restriction enzymes (EcoRI or SpeI or XbaI or PstI)
- DNA loading buffer (if electrophoresis is subsequent)
- Agarose gel 0.8% (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 (2h for 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).
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)