Protocol for double digestion (20μl system)

Pipette the following into a 0.2ml microfuge tube:

- Enzyme A: 1μl
- Enzyme B: 1μl
- 10 buffer: 2μl
- DNA: 0.5 - 1ug
- ddwater rest of the volume

incubate at recommended temperature (37°C) for at least 1 hour;

Purify the digestion product;

Notes:

The enzymes used here are NEB enzymes (EcoRI/ XbaI/ SpeI/ PstI), and buffer4 is suitable for most of double digestion;

For 50μl reaction system, the suggested amount of each restriction enzyme is 2μl;

According to personal experience, 50μl reaction system has lower efficiency than 20μl reaction system, so 20μl reaction system is recommended.