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℃) 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.