

Protocol for double digestion (20ul system)

Pipette the following into a 0.2ml microfuge tube:

Enzyme A 1ul

Enzyme B 1ul

10×buffer 2ul

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/Spel/ PstI), and buffer4 is suitable for most of double digestion;

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

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