

Restriction Enzyme Digestion



To check if the two selected restriction enzymes can perform effective catalysis in the same solution

1. Mix DNA solution with the suitable amount of the master mix.

- a. **25.0µL reaction system**

20.0µL DNA sample

0.25µL BSA

2.5µL 10x NEB Buffer

1.0µL of each restriction enzyme

1.25µL ddH₂O

-----**25.0µL** Total

- b. **50.0µL reaction system**

40.0µL DNA sample

0. 5µL BSA

5.0µL 10x NEB Buffer

1.5µL of each restriction enzyme

2.0µL ddH₂O

-----**50.0µL** Total

2. Pipette up and down in the EP tube.
3. Incubate: 37°C for 3 hours.
4. Enzyme inactivation: 80°C for 30 minutes.