

## 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. **25.0 $\mu$ L reaction system**

**20.0 $\mu$ L** DNA sample

**0.25 $\mu$ L** BSA

**2.5 $\mu$ L** 10x NEB Buffer

**1.0 $\mu$ L** of each restriction enzyme

**1.25 $\mu$ L** ddH<sub>2</sub>O

-----**25.0 $\mu$ L** Total

- b. **50.0 $\mu$ L reaction system**

**40.0 $\mu$ L** DNA sample

**0.5 $\mu$ L** BSA

**5.0 $\mu$ L** 10x NEB Buffer

**1.5 $\mu$ L** of each restriction enzyme

**2.0 $\mu$ L** ddH<sub>2</sub>O

-----**50.0 $\mu$ L** 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.