

## Enzyme Digestion and Connecting

### I . Enzyme Digestion( Total volume: 50 $\mu$ L)

#### The fragement

-10*FD Buffer:	5 $\mu$ L
-DNA I :	1 $\mu$ g
- FD Enzyme I :	1 $\mu$ L
-FD Enzyme II :	1 $\mu$ L
-ddH <sub>2</sub> O:	Add to total volume

#### The vector

-10*FD Buffer:	5 $\mu$ L
-DNA II :	1 $\mu$ g
- FD Enzyme I :	1 $\mu$ L
-FD Enzyme II :	1 $\mu$ L
-ddH <sub>2</sub> O:	Add to total volume

### II . Incubate at 37°C for 1h.

### III.Connecting(Total volume: 30)

-10*T4 Ligase buffer:	3 $\mu$ L
-T4 Ligase:	1 $\mu$ L
-Fragemant:	30-60ng
-Vector :	3 times the mass of fragement
-ddH <sub>2</sub> O:	Add to total volume.

### IV. Gel Analysis