pJET Cloning

Followed Thermo Scientific CloneJET PCR Blunt-End Cloning Protocol

1. Combine the following reagents sequentially on ice:

Component	Volume (µL)
2 X reaction Buffer	10
DNA fragment (50 ng/µL)	1
Water, nuclease free	Up to 17
DNA blunting Enzyme	1
Total volume	18

2. Vortex briefly and centrifuge for 3-5 seconds.
3. Incubate the mixture at 70°C for 5 minutes and chill on ice.
4. Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (µL)
pJET1.2/blunt Cloning Vector (50 ng/µL)	1
T4 DNA ligase	1
Total Volume	20

5. Vortex briefly and centrifuge for 3-5 seconds. Then incubate at room temperature (approximately 22°C) for 5 minutes. (Note for DNA fragments in excess of 3 kb, ligation can be prolonged to 30 mins).
6. Transform ligation mixture into chemical competent cells.