BP Reaction for entry clone (total volumn 5 ul) :

1. Add following components to a 1.5 ml microcentrifuge tube, vortex briefly twice. Spin down by a microcentrifuge briefly.

attB-PCR product (50~100 ng)	**1 µl**
pDONR-zeo vector (150ng/ul)	**1 µl**
TE buffer (pH=8.0)	**to 4 µl**
BP clonase II mix	**1 µl**

2. Incubate reactions at 25°C for more than 3 hours; overnight incubation for up to 18 hours typically yields more colonies.
3. Add 1 ul of the Proteinase K solution to each sample to terminate the reaction, vortex briefly and incubate at 37°C for 10 min.
4. Transform 1ul of each sample to E.coli DH5α according to the heat shock protocol for bacterial transformation. Keep the remaining solution until in case a retransformation will be required.