

Gateway BP reaction

Reagents needed:

- attB-flanked PCR product
- pDONR vector
- BP Clonase II Enzyme (contains reaction buffer)
- TE
- One-shot Omni-Max Cells (or other high competence DH5a/XL1 strain)
- Proteinase K solution
- LB-plates with appropriate antibiotic (pDONR221 = Kanamycin)
- SOC media

Protocol

1. Add the following components to a 1.5 ml tube at room temperature and mix:
attB-PCR product (=10 ng/μl; final amount ~15-150 ng) 1-7 μl
Donor vector (150 ng/μl) 1 μl
TE buffer, pH 8.0 to 8 μl
2. Thaw on ice the BP Clonase™ II enzyme mix for about 2 minutes. Vortex the BP Clonase™ II enzyme mix briefly twice (2 seconds each time).
3. To each sample (Step 1, above), add 2 μl of BP Clonase™ II enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
4. Return BP Clonase™ II enzyme mix to -20°C or -80°C storage.
5. Incubate reactions at 25°C for 1 hour.
6. Add 1 μl of the Proteinase K solution to each sample to terminate the reaction. Vortex briefly. Incubate samples at 37°C for 10 minutes.

Transformation

1. Transform 1 μl of each LR reaction into 50 μl of One Shot® OmniMAX™ 2 T1 Phage-Resistant Cells (Catalog no. C8540-03). Incubate on ice for 30 minutes. Heat-shock cells by incubating at 42°C for 30 seconds. Add 250 μl of S.O.C. Medium and incubate at 37°C for 1 hour with shaking. Plate 20 μl and 100 μl of each transformation onto selective plates. Note: Any competent cells with a transformation efficiency of $>1.0 \times 10^8$ transformants/μg may be used.
2. Transform 1 μl of pUC19 DNA (10 ng/ml) into 50 μl of One Shot® OmniMAX™ 2 T1 Phage-Resistant Cells as described above. Plate 20 μl and 100 μl on LB plates containing 100 μg/ml kanamycin, or the appropriate selection marker for your donor vector.

Expected Results

An efficient BP recombination reaction will produce >1500 colonies if the entire BP reaction is transformed and plated.