

One-Tube BP and LR Gateway reaction

Lamitina Lab Protocols 2007

Reagents needed:

- attB flanked PCR product (50ng/ μ l)
- pDONR vector (150 ng/ μ l)
- BP clonase enzyme mix
- TE, pH 8.0
- Destination vector (150ng/ μ l)
- LR clonase enzyme mix
- Proteinase K solution
- Competent DH5 α cells (competency $>10^8$ / μ g DNA, ie Invitrogen library efficiency DH5)
- LB + carbenicillin plates

Method

1. In a 1.5 ml microcentrifuge tube, prepare the following 15 μ l BP reaction:

attB PCR product (50ng/ μ l)	2.5 μ l
pDONR vector (150ng/ μ l)	1.3 μ l
BP clonase enzyme	3 μ l
TE , pH 8.0	To 15 μ l

2. Mix well by vortexing briefly and incubate at 25°C for 4 hours.
3. Remove 5 μ l of the reaction to a separate tube and use this aliquot to assess the efficiency of the BP reaction (see below).
4. To the remaining 10 μ l reaction, add:

Destination vector (150ng/ μ l)	2.0 μ l
LR clonase enzyme mix	3.0 μ l

5. Mix well by vortexing briefly and incubate at 25°C for 2 hours.
6. Add 2 μ l of proteinase K solution. Incubate at 37°C for 10 minutes.
7. Transform 50 μ l of 'Library Efficiency' DH5a cells with 1 μ l of the reaction
 - Thaw cells on ice
 - Place 50 μ l of cells into pre-chilled 12ml culture tubes
 - Add 1 μ l of LR reaction
 - Incubate on ice for 30 minutes
 - Heat shock cells at 42°
 - Add 250 μ l of SOC medium and incubate at 37° for 1 hour with shaking
 - Plate 20 μ l and 100 μ l onto LB + carbenicillin plates (or other as determined by destination vector
 - A typical LR reaction will produce >5000 colonies if the entire reaction is transformed and plated

Assessing the Efficiency of the BP Reaction

1. To the 5 μ l aliquot obtained from step 3, add 0.5 μ l of proteinase K solution.
2. Incubate at 37°C for 10 minutes.
3. Transform 50 μ l of the appropriate competent E. coli with 1 μ l of the reaction.
4. Plate on LB plates containing the appropriate antibiotic to select for entry clones.

Depending on your needs, the length of both recombination reactions can be extended up to 20 hours. An overnight incubation typically yields 5 times more colonies than a 1 hour incubation. Longer incubation times are recommended for large plasmids (=10 kb) and PCR products (=5 kb).