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life technologies

Gateway® LR Clonase™ Enzyme Mix

Cat. No. 11791-019 **Size: 20 reactions**
Cat. No. 11791-043 **Size: 100 reactions**
Store at -80°C

Gateway® Technology

Gateway® is a universal cloning technology based on the site-specific recombination properties of bacteriophage lambda (1). The Gateway® Technology provides a rapid and highly efficient way to move DNA sequences into multiple vector systems for functional analysis and protein expression, and can be schematically represented as follows:

*att*B1-gene-*att*B2 × *att*P1-*ccd*B-*att*P2 ⇔ *att*L1-gene-*att*L2 × *att*R1-*ccd*B-*att*R2
(expression clone) (pDONR™) (entry clone) (destination vector)

The *att*B × *att*P reaction is mediated by Gateway® BP Clonase™ enzyme mix; the *att*L × *att*R reaction is mediated by Gateway® LR Clonase™ enzyme mix. *ccd*B is the F plasmid-encoded gene that inhibits growth of *E. coli* (2,3) and "gene" represents any DNA segment of interest (*e.g.* PCR product, cDNA, genomic DNA).

Description

Gateway® LR Clonase™ enzyme mix is a proprietary enzyme formulation containing the bacteriophage lambda recombination proteins Integrase (Int) and Excisionase (Xis), and the *E. coli*-encoded protein Integration Host Factor (IHF) (1). Gateway® LR Clonase™ enzyme mix promotes *in vitro* recombination between an entry clone (*att*L-flanked "gene") and any number of *att*R-containing destination vectors to generate *att*B-containing expression clones. "Genes" present in expression clones can be transferred back into an entry vector by mixing with an *att*P Vector (*e.g.*, pDONR™221) and adding Gateway® BP Clonase™ enzyme mix.

Components Supplied	20 rxns	100 rxns
Gateway® LR Clonase™ Enzyme Mix	80 µl	400 µl
5X LR Clonase™ Reaction Buffer	200 µl	1 ml
2 µg/µl Proteinase K Solution	40 µl	200 µl
50 ng/µl pENTR™-gus Positive Control	20 µl	20 µl

For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

General Recommendations and Guidelines

- pENTR™-gus is provided for use as a positive control in the LR reaction, and is an entry clone containing the *Arabidopsis thaliana* β-glucuronidase (*gus*) gene (4). 1 µg of supercoiled pENTR™-gus plasmid is supplied in TE Buffer, pH 8.0 at a concentration of 50 ng/µl. **Note:** See our Web site (www.invitrogen.com) for a map and the sequence of pENTR™-gus.
- We recommend using plasmid DNA purified with the S.N.A.P.™ MiniPrep Kit (Catalog no. K1900-01). Mini-prep (alkaline lysis) DNA preparations are adequate for Gateway® cloning reactions; however, in general, such DNA cannot be quantitated by UV absorbance due to contaminating RNA and nucleotides. Estimate concentrations by gel electrophoresis in comparison with standard DNA, *i.e.*, DNA Mass Ladder (Cat. Nos. 10068-013 or 10496-016).
- For LR reactions, the most efficient substrates are supercoiled *att*L-containing entry vectors and supercoiled *att*R-containing destination vectors. For large (>10 kb) entry clones or destination vectors, linearizing the entry clone or destination vector may increase the efficiency by up to 2-fold.
- To increase the number of colonies containing the desired expression clone, increase the incubation time from the recommended 1 hour to 2 hours or overnight. Longer incubations are recommended for plasmids ≥10 kb to increase the yield of colonies.
- We recommend using 100-300 ng entry clone per 20 µl reaction. Highest colony yields are typically obtained using 300 ng entry clone and 300 ng destination vector. Do not use >300 ng entry clone as you may obtain colonies containing multiple DNA molecules (often with an associated "small colony" phenotype). Using <100 ng entry clone will generate fewer colonies.

Procedures

LR Reaction

Use the following procedure to perform an LR recombination reaction. For a positive control, use 100 ng (2 µl) of pENTR™-gus.

1. Add the following components to a 1.5 ml microcentrifuge tube at room temperature and mix:

Entry clone (100-300 ng)	1-10 µl
Destination vector (150 ng/µl)	2 µl
5X LR Clonase™ Reaction Buffer	4 µl
TE buffer, pH 8.0	to 16 µl

2. Remove LR Clonase™ enzyme mix from -80°C and thaw on ice for about 2 minutes. Vortex the LR Clonase™ enzyme mix briefly twice (2 seconds each time).
3. To each sample (Step 1, above), add 4 µl of LR Clonase™ enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
4. Return LR Clonase™ enzyme mix to -80°C storage immediately after use.
5. Incubate reactions at 25°C for 60 minutes.
6. Add 2 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 Library Efficiency® DH5α™ Competent Cells (Cat. No. 18263-012). Incubate on ice for 30 minutes. Heat-shock cells by incubating at 42°C for 30 seconds. Add 450 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 Library Efficiency® DH5α™ Competent Cells as described above. Plate 20 µl and 100 µl on LB plates containing 100 µg/ml ampicillin.

Expected Results

An efficient LR recombination reaction will produce >5000 colonies if the entire transformation is plated.

Quality Control

LR Clonase™ enzyme mix is functionally tested in a 1 hour recombination reaction followed by a transformation assay.

References

1. Landy, A. (1989) *Ann. Rev. Biochem. 58*, 913.
2. Bernard, P. and Couturier, M. (1992) *J. Mol. Biol. 226*, 735.
3. Miki, T., Park, J.A., Nagao, K., Murayama, N., and Horiuchi, T. (1992) *J. Mol. Biol. 225*, 39.
4. Kertbundit, S., Greve, H.D., Deboeck, F., Montagu, M.V., and Hernalsteens, J.P. (1991) *Proc. Natl. Acad. Sci. USA, 88*, 5212.

Limited Use Label License No. 19: Gateway® Cloning Products

Limited Use Label License No. 23: GUS Control Vector