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LR Clonase Reaction for Multisite Gateway Cloning

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ABSTRACT

Gateway® LR Clonase™ II Plus

Gateway® LR Clonase™ II Plus enzyme mix is a proprietary enzyme formulation specifically designed for MultiSite Gateway® and MultiSite Gateway® Pro. Gateway® LR Clonase™ II Plus enzyme mix contains the bacteriophage lambda recombination proteins Integrase (Int) and Excisionase (Xis), and the E. coli encoded protein Integration Host Factor IHF) (1), and reaction buffer provided in a single mix for convenient reaction set up. Gateway® LR Clonase™ II Plus enzyme mix promotes in vitro recombination between attL- and attR-flanked regions on entry clones and destination vectors to generate attB-containing expression clones.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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GUIDELINES

General Recommendations and Guidelines

- We recommend using plasmid DNA purified with the PureLink™ HiPure Plasmid Midiprep Kit (Catalog no. K2100-04). Mini-prep (alkaline lysis) DNA preparations are not recommended for MultiSite Gateway® cloning reactions.
- DNA cannot be quantitated by UV absorbance due to contaminating RNA and nucleotides, estimate concentration by gel electrophoresis (e.g., DNA Mass Ladder, Cat. no. 10068-013 or 10496-016).
- For LR reactions, supercoiled entry vectors and destination vectors provide efficient substrates.
- For large (>10 kb) entry clones or destination vectors, linearizing the entry clone or destination vector may increase the efficiency by up to two fold.

MATERIALS TEXT

Components:

Gateway® LR Clonase™ II Plus Enzyme Mix (40 µl)
2 µg/µl Proteinase K Solution (40 µl)

Storage:

Store Gateway® LR Clonase™ II Plus at -20°C (in a non-frost-free freezer) for up to 6 months. For long term storage, store at -80°C.

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Reaction Setup

1 For multi-fragment (i.e. 2-, 3-, or 4-fragment recombination) reactions, use an equimolar amount of each entry clone. We recommend 10 fmol of each entry clone and 10 fmol of DEST vector per 5 µl reaction.

Plasmid mixture:

Add the following components to a 1.5-ml microcentrifuge tube at room temperature and mix. Use this reaction mixture in the following procedure.

1. Entry clones (10 fmoles)*
2. Destination vector (10 fmoles)
3. **at least 1 µl 1X TE buffer, pH 8.0 for a total volume of 4µl**

*All entry clones (two, three or four, depending on the type of reaction) must be included. The total of all plasmids and buffer combined should not exceed 4 µl.

Use a calculator to determine the amount of each plasmid to add to the reaction. For example, you would need to add 29 ng of a 4.25 kb plasmid to get 10 fmol. https://www.promega.com/resources/tools/biomath/

Procedure 16h 12m

2 Remove LR Clonase™ II Plus enzyme mix from freezer and thaw on ice for about 00:02:00 . Vortex the enzyme mix 2m briefly twice (2 seconds each).

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3 To each MultiSite or MultiSite Pro LR reaction mixture, add 1 µl of LR Clonase™ II Plus and mix well by vortexing briefly twice. Microcentrifuge briefly.

4 Return enzyme mix to freezer immediately after use. The enzyme mix can be stored at -20°C for up to 6 months or at -80°C for long-term storage.

5 Incubate recombination reaction at 25°C for **16:00:00** . 16h

6 Add 0.5 µl of the Proteinase K solution to each sample to terminate the reaction. Vortex briefly. Incubate samples at 37°C for **00:10:00** . 10m

Transformation 16h 12m

7 For 2- or 3-fragment recombination reactions, add 2-3 µl to 50 µl of Mix & Go Chemically Competent *E. coli* and incubate on ice for 5-30 minutes.

For 4-fragment recombination reactions, add 4-5 µl to 50 µl of Mix & Go Chemically Competent *E. coli* and incubate on ice for 5-30 minutes.

8 Add 200 µl of SOC medium and incubate at 37°C for 1-1.5 hour with shaking at 225-250 RPM. Can be omitted with Ampicillin resistance.

9 Plate entire reaction on selective media.

Typical Numbers of Colonies (per 10 µl reaction):

2-fragment recombination reaction: 2,000-15,000
3-fragment recombination reaction: 1,000-5,000
4-fragment recombination reaction: 50-500

Some labs have found that clear colonies contain the correct clone >99% of the time, while opaque colonies never contain the correct clone. A reaction that has worked well will have a clear to opaque colony ratio of at least 3:1. However, as long as clear colonies can be identified, the correct clone will be isolated. Clones can be tested via restriction digest or colony PCR.

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