



Apr 27, 2020

## LR Clonase

DOI

[dx.doi.org/10.17504/protocols.io.bfm9jk96](https://dx.doi.org/10.17504/protocols.io.bfm9jk96)

Invitrogen<sup>1</sup>

<sup>1</sup>Thermofisher



Robert Roden

### Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bfm9jk96>

**Protocol Citation:** Invitrogen 2020. LR Clonase. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bfm9jk96>

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**We use this protocol and it's working**

**Created:** April 27, 2020

**Last Modified:** April 27, 2020

Protocol Integer ID: 36257

## Troubleshooting

- 1 Add the following components to a 1.5-mL microcentrifuge tube at room temperature and mix: • 1–7  $\mu\text{L}$  entry clone (50–150 ng) • 1  $\mu\text{L}$  destination vector (150 ng/ $\mu\text{L}$ ) • TE buffer pH 8.0, to 8  $\mu\text{L}$
- 2 Thaw on ice the LR Clonase™ II enzyme mix for about 2 minutes. Vortex the LR Clonase™ II enzyme mix briefly twice (2 seconds each time).
- 3 To each sample (step 1), add 2  $\mu\text{L}$  of LR Clonase™ II enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
- 4 Return LR Clonase™ II enzyme mix to  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  storage.
- 5 Incubate reactions at  $25^{\circ}\text{C}$  for 1 hour. (overnight)
- 6 Add 1  $\mu\text{L}$  of the Proteinase K solution to each sample to terminate the reaction. Vortex briefly. Incubate samples at  $37^{\circ}\text{C}$  for 10 minutes. Transform
- 7 Transform 1  $\mu\text{L}$  of each LR reaction into 50  $\mu\text{L}$  of One Shot™ OmniMAX™ 2 T1 Phage-Resistant Cells (Cat. no. C8540-03) (5 alpha e. coli). Incubate on ice for 30 minutes.  
  
Follow electrotransformation protocol.
- 8
- 9 T1 Phage-Resistant Cells as described above. Plate 20  $\mu\text{L}$  and 100  $\mu\text{L}$  on LB plates containing 100  $\mu\text{g}/\text{mL}$  ampicillin. Expected results An efficient LR recombination reaction will produce >5000 colonies if the entire LR reaction is transformed and plated.