APR 27, 2020

LR Clonase

Invitrogen¹

¹Thermofisher

Robert Roden

OPEN ACCESS

DOI:

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: Apr 27, 2020

Last Modified: Apr 27, 2020

PROTOCOL integer ID:

36257

- 1 Add the following components to a 1.5-mL microcentrifuge tube at room temperature and mix: • 1–7 µL entry clone (50–150 ng) • 1 µL destination vector (150 ng/µL) • TE buffer pH 8.0, to 8 µL

- 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 µL 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°C or –80°C storage.
- 5 Incubate reactions at 25°C for 1 hour. (overnight)
- 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. Transform
- 7 Transform 1 µL of each LR reaction into 50 µL 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 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 LR reaction is transformed and plated.