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LR Clonase

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Invitrogen[1]

[1]Thermofisher

Robert Roden

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

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 bufferǰ 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 brefl¢ 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 brefl¢ twice. Microcentrifuge brefl¢ǯ

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 electroboration protocol.

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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.