Gateway LR recombination of entry clones in pDONR/zeo into destination plasmid (5 µl assay)

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Abstract

This is a slightly modified version of Thermo Fisher's gateway LR protocol which uses less enzyme and is therefore more economical.

Citation: Johannes Debler Gateway LR recombination of entry clones in pDONR/zeo into destination plasmid (5 µl assay). **protocols.io**
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Protocol

Combine in 0.2 ml PCR tube
Step 1.

75 ng entry clone	x µl
75 ng destination plasmid	x µl
TE buffer pH 8.0	to a total volume of 4 µl

LR Clonase II enzyme mix
Step 2.

Remove from freezer and vortext for 2 seconds

add LR Clonase II
Step 3.

add **0.5 µl** of LR Clonase II enzyme mix and mix well

Incubate at room temperature
Step 4.

Incubate for **3 hours** at **room temperature** (4-6 hours or overnight for more colonies)

DURATION
03:00:00

Stop reaction with Proteinase K
Step 5.

add **0.5 µl** Proteinase K, mix well and incubate for **10 min at 37°C**

DURATION
00:10:00

Transform into Omnimax 2 E.coli

Step 6.

Combine **1 µl** LR product with **50 µl** Omnimax 2 competent *E.coli* cells in 1.5 ml tube.

Incubate on ice

Step 7.

DURATION
00:10:00

Heat shock

Step 8.

30 seconds at **42°C**

Recover on ice

Step 9.

DURATION
00:02:00

Add SOC

Step 10.

add **500 µl** SOC

Incubate

Step 11.

37° C at **250 rpm** on shaking incubator.

DURATION
01:00:00

Concentrate cells

Step 12.

Spin tubes at **1000 x g for 2 minutes.**

Decant most of ther supernatant.

Resuspend cells in **50 - 100 µl** of leftover supernatant.

Plate out

Step 13.

Plate concentrated cells on LB containing **30 µg/ml** kanamycin.