Transferring an ORF from an ORF*EXPRESS*™ Gateway® PLUS Shuttle Clone to a Destination Vector

Reagents

ORFEXPRESS™ Gateway PLUS shuttle clone
LR reaction buffer (Invitrogen)
Linearized destination vector (Invitrogen)
Gateway® enzyme mix (Invitrogen)
50 mM TE
Proteinase K
GCI-5α chemically competent *E.coli* cells (GeneCopoeia Cat. No. STK200-10/20)
S.O.C. Medium
LB plates with 100 µg/ml ampicillin

Equipment

Autoclaved assorted pipet tips
Pipetman pipets: P2, P20, P200, P1000
Autoclaved 1.5-ml microtubes
17 x 100 mm polypropylene tubes (Falcon 2059)
37°C and 42°C incubators

Procedures

1. LR Reaction

Use the following procedure to perform an LR recombination reaction. For a positive control, use 100 ng (2 µl) of pENTR™-eGFP.

1.1 Add the following components to a 1.5-ml microcentrifuge tube at room temperature and mix:

Component	Volume
Entry clone (100–300 ng)	1−10 µl
Destination vector (150 ng/µl)	2 µl
LR Clonase™ reaction buffer 5X	4 µl
TE buffer, pH 8.0	to 16 µl

1.2 Remove LR Clonase enzyme mix from −80°C and thaw on ice for about 2 minutes. Vortex the LR Clonase enzyme mix briefly twice (2 seconds each time).

1.3 To each sample (Step 1.1 above), add 4 µl of LR Clonase enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.

1.4 Return LR Clonase enzyme mix to −80°C storage immediately after use.

1.5 Incubate reactions at 25°C for 60 minutes.

2. Transformation

2.1 Transform 1 µl of each LR reaction into 50 µl of GCI-5α chemically competent *E.coli* cells. Incubate on ice for 30 minutes. Heat shock cells by incubating at 42°C for 30 seconds. Add 450 µl of S.O.C. Medium and incubate at 37°C for 1 hour with shaking. Plate 20 µl and 100 µl of each transformation onto selective plates.

Note: Any competent cells with a transformation efficiency of >1.0 × 10^8 transformants/µg may be used.

2.2 Transform 1 µl of pUC19 DNA (10 ng/ml) or other suitable plasmid for use as a control into 50 µl of GCI-5α chemically competent *E.coli* 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 transformation is plated.

LB plates (per liter)

10.0 g	Bacto-Tryptone
5.0 g	Bacto-Yeast extract
5.0 g	NaCl
15.0 g	Agar

Adjust the pH to 7.0 with NaOH (~200 µl 5M NaOH). Autoclave (keep the top loosened to allow steam to vent) and cool to 50°C before adding antibiotics. Mix well and pour LB media into plates.

For more information, please contact us:

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