

Transferring an ORF from an EZShuttle™ Gateway® PLUS ORF clone or ORFeome Shuttle Clone to a Destination Vector

Reagents

EZShuttle™ Gateway PLUS ORF clone or ORFeome Gateway entry clone
 5X Reaction Buffer (GeneCopoeia Cat. No. RCBM-1001-020)
 EZRecombinase™ LR mix (GeneCopoeia Cat. No. RCBM-1001-020)
 Linearized destination vector
 50 mM TE buffer, pH 8.0
 Proteinase K
 GCI-5α chemically competent *E. coli* cells (GeneCopoeia Cat. No. CC001)
 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
5X Reaction Buffer	4 µl
TE buffer, pH 8.0	to 16 µl

- 1.2 Remove EZRecombinase™ LR mix from –80°C and thaw on ice for about 2 minutes. Vortex briefly twice (2 seconds each time).
- 1.3 To each sample (Step 1.1 above), add 4 µl of EZRecombinase™ LR mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
- 1.4 Return EZRecombinase™ LR mix to –80°C storage immediately after use.
- 1.5 Incubate reactions at 25°C for 60 minutes.

2. Transformation

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

Note: Any competent cells with a transformation efficiency of $>1.0 \times 10^8$ transformants/ μ g may be used.

- 2.2 Transform 1 μ l of pUC19 DNA (10 ng/ml) or other suitable plasmid for use as a control into 50 μ l of GCl-5 α chemically competent *E. coli* cells as described above. Plate 20 μ l and 100 μ l on LB plates containing 100 μ g/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 μ l 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.

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