



EZRecombinase™ LR Cloning Kit

Cat. No. RCBM-1001-020

Cat. No. RCBM-1001-100

User Manual

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USER MANUAL

EZRecombinase™ LR Cloning Kit

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I. Introduction

The EZShuttle™ recombination-based cloning system for DNA fragment transfer among plasmid vectors is based on the site-specific recombination machinery between the *E. coli* and phage lambda genomes, the same principle as Gateway® technology.

The transfer of a DNA fragment occurs between attL sites in an EZShuttle™ or Gateway® Entry clone and attR sites in a destination vector. The EZShuttle™ or Gateway® entry clone, a destination vector, and EZRecombinase™ LR Mix are incubated at room temperature (25° C) for one hour and used to transform *E. coli* competent cells (Figure 1).

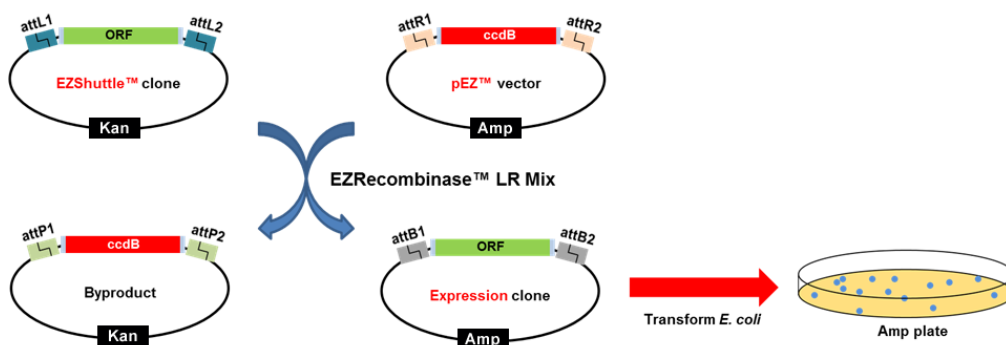


Figure 1. Principle for transferring a DNA fragment from an EZShuttle™ or Gateway® Entry clone to a pEZ™ expression or Gateway® pDEST vector using EZShuttle™ LR recombination cloning.

The EZRecombinase™ LR Cloning Kit is part of the GeneCopoeia EZShuttle™ Cloning System, which is a complete system with the following major components:

- **EZRecombinase™ LR Cloning Kit:** A robust enzyme mixture for catalyzing attL x attR cloning reactions.
- **pEZ™ vectors:** A series of cloning vectors with chemical selection markers different from GeneCopoeia's Shuttle™ Gateway® PLUS ORF clones or Gateway® Entry clones, suitable for creating ready-to-use expression clones by using LR recombination reaction.
- **EZShuttle™ Gateway® PLUS ORF clones.** GeneCopoeia carries more than 35,000 sequence-verified human and mouse ORFs, available with or without stop codons, in EZShuttle™ Gateway® PLUS plasmid backbones. EZShuttle™ Gateway® PLUS ORF clones are similar to Gateway®-based Entry clones. Using the EZRecombinase™ LR Mix, ORFs can be easily transferred from EZShuttle™ Gateway® PLUS ORF clones to any pEZ™ vectors or other Gateway®-compatible destination vectors by the LR reaction. EZShuttle™ Gateway® PLUS ORF clones also carry multiple cloning sites to permit conventional restriction enzyme-based cloning.

II. Contents and Storage

EZRecombinase™ LR Cloning Kit (RCBM-1001-020, RCBM-1001-100)

The EZRecombinase™ LR Cloning Kit is available in two sizes:

20-Reaction Kit (Catalog No.RCBM-1001-020)

100-Reaction Kit (Catalog No. RCBM-1001-100)

Component	Quantity	Shipping temperature	Storage temperature
	20 reactions 100 reactions		
Enzyme reagents			
EZRecombinase™ LR Mix	80 µl 400 µl	Dry ice	-80° C Stable for at least 1 years
5× Reaction Buffer	80 µl 400 µl	Dry ice	-80° C Stable for at least 1 years
Control reagents			
Positive control (150 ng/µl)	20 µl 20 µl	Dry ice	-80° C Stable for at least 1 years

Important note: .

Store all components at –80° C upon receipt.

Materials required but not supplied

The following materials are required but not supplied:

- ☐ **Entry clone DNA.**
- ☐ **Destination vector DNA.**
- ☐ **TE buffer, pH 8.0.**
- ☐ **Competent *E. coli* cells (GeneCopoeia catalog numbers STK200-10, STK200-20, STK3010, STK300-20**
- ☐ **SOC medium**

III. Experimental Procedures

This section provides instructions for performing an attL x attR recombination cloning and transforming *E. coli* cells.

LR Recombination reaction

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

Item	Amount
EZShuttle™ or Gateway® Entry clone (100-300 ng)	1-10 µl
pEZ™ or Gateway® pDEST vector (150 ng/ul)	2 µl
5x Reaction Buffer	4 µl
TE buffer, pH 8.0	to 16 µl
Final	16 µl

2. Remove EZRecombinase™ LR Mix from -80° C and thaw on ice for 2-3 minutes.
3. Centrifuge briefly.
4. Add 4 µl of EZRecombinase™ LR Mix to the reaction tube and mix well.
5. Briefly centrifuge to make sure all the reagents are at the bottom of the reaction tubes.
6. Incubate reactions at 25° C for 60 minutes.
7. Return EZRecombinase™ LR Mix to -80° C storage immediately after use.
8. Proceed to transform *E. coli*.

Transformation

1. Thaw competent cells on ice. Place the required number of microcentrifuge tubes on ice and dispense 50-100µl of competent cells into each tube.
2. Gently mix 4 µl of each LR reaction with the competent cells. Incubate on ice for 30 minutes.
3. Heat shock cells by placing the tubes into a 42° C water bath for 45 seconds.
4. Immediately place the tubes on ice for 2 to 3 minutes.
5. Add 500 µl of SOC medium and incubate at 37° C for 1 hour with shaking at 100 to 200 rpm.
6. Plate 50 µl and 200 µl of each transformation onto antibiotic-containing plates.
7. Incubate plates for 12 to 16 hr at 37° C.

IV. Limited Use License and Warranty

Limited use license

The following terms and conditions apply to use of the EZRecombinase™ LR Cloning Kit. If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. Use of any part of the Product constitutes acceptance of the above terms.

Limited warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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