EZShuttle™ Recombination Cloning System

EZRecombinase™ LR Mix

Cat. No. ER001 (Old Cat. No. RCBM-1001-020, 20 reactions)

Cat. No. ER002 (Old Cat. No. RCBM-1001-100, 100 reactions)

EZRecombinase™ BP Mix

Cat. No. ER003 (Old Cat. No. RCBM-1002-020, 20 reactions)

Cat. No. ER004 (Old Cat. No. RCBM-1002-100, 100 reactions)

User Manual

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USER MANUAL

EZShuttle™ Recombination Cloning System

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 EZRecombinase™ BP Mix, which catalyzes attB x attP cloning reactions, contains Int (λ Integrase) and IHF (*E.coli* Integration Host Factor) and is used to create new EZShuttle™ or Gateway® Entry clones. An attB-containing fragments or expression clone is combined with an attP-containing pShuttle™ vector and EZRecombinase™ BP Mix, incubated at room temperature (25^0C) for one hour, and used to transform *E. coli.* competent cells (Figure 1).

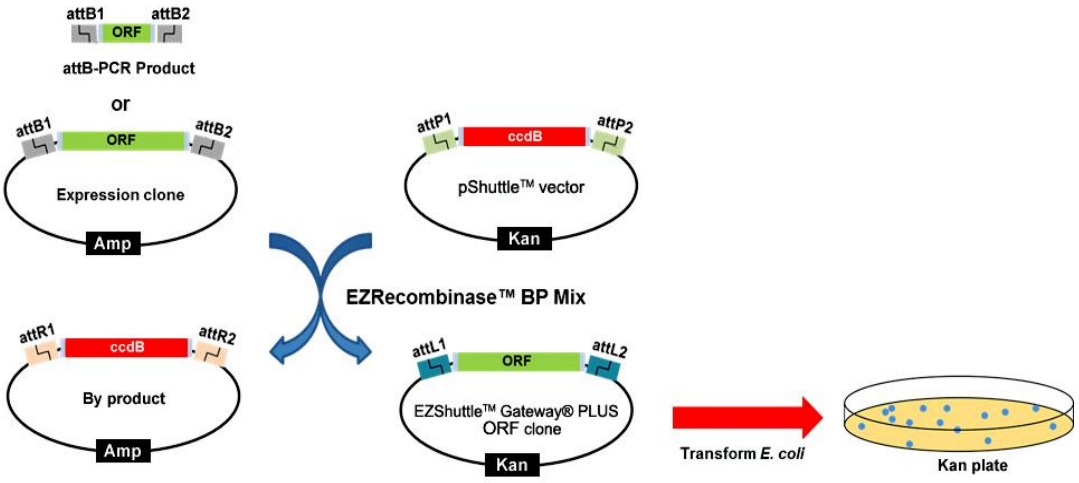

Figure 1. Principle for transferring a DNA fragment from an attB-containing PCR product or expression clone to an attP-containing pShuttle™ vector using EZShuttle™ BP recombination cloning.

The EZRecombinase™ LR Mix, which catalyzes attL x attR cloning reactions, contains Int (λ Integrase) and IHF (*E.coli* Integration Host Factor) and Excisionase (Xis) and is used to create new attB-containing expression clones. 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 2).

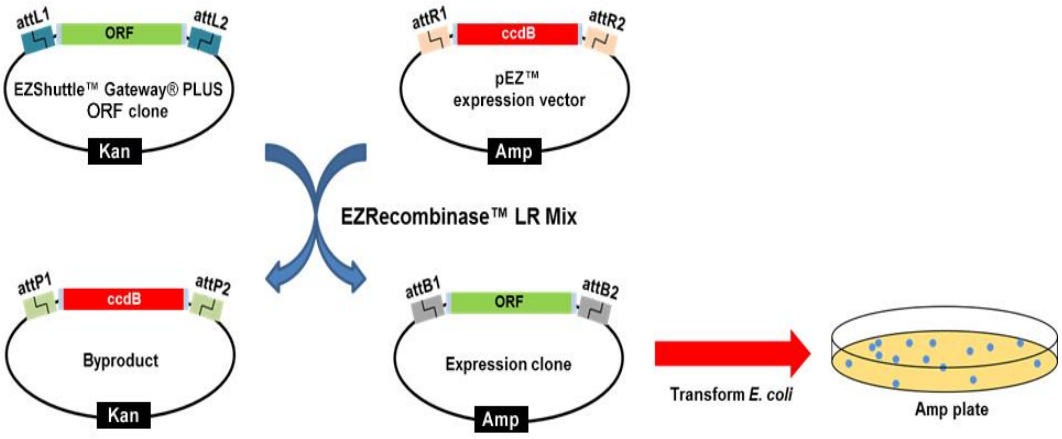

Figure 2. Principle for transferring a DNA fragment from an EZShuttle™ or Gateway® Entry clone to a pEZ™ expression or Gateway® pDEST vector using EZShuttle™ LR recombination cloning.

The GeneCopoeia EZShuttle™ Recombination Cloning System is a complete system with the following major components:

• **EZRecombinase™ Mix:** EZRecombinase™ BP Mix catalyzes attB x attP cloning reactions; EZRecombinase™ LR Mix catalyzes attB x attP cloning reactions.

• **pShuttle™ cloning vector:** pShuttle™ cloning vectors allow simple and fast transfer of DNA fragments from EZShuttle™ expression clones or attB- containing fragments using EZRecombinase™ BP Mix, and create new EZShuttle™ Gateway® PLUS ORF clone or Gateway® Entry clones. They are functionally equivalent to Gateway® pDONR™ vectors.

• **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:** More than 35,000 sequence-verified human and mouse ORFs, available with or without stop codons, in pShuttle™ 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.

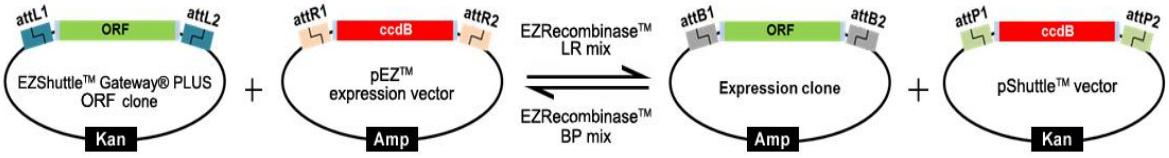

Figure 3. EZShuttle™ recombination cloning system as an operating system for cloning and subcloning DNA. DNA fragments are transferred between vectors using EZRecombinase™ LR or BP recombination reactions.

II. Contents and Storage

1. EZRecombinase™ LR Cloning Kit

Catalog No.ER001 (20 reactions), ER002 (100 reactions)

Catalog No	Component	Quantity 20 reactions 100 reactions	Shipping temperatur	Storage temperature
ER001-01	EZRecombinase™ LR Mix	80 μl 80 μlx 5	Dry ice	-80°C Stable for at least 1 year
ER001-02	5×Reaction Buffer	80 μl 80 μlx 5	Dry ice	-80°C Stable for at least 6 months
ER001-03	Positive control (Shuttle clone eGFPp，100 ng/μl)	20 μl 20 μlx 5	Dry ice	-20°C Stable for at least 6 months

Materials required but not supplied

The following materials are required but not supplied:

- ✓ EZShuttle™ clone or Entry clone DNA.
- ✓ pEZ™ vector or Destination vector DNA.
- ✓ TE buffer, pH 8.0.
- ✓ Competent *E. coli* cells (GeneCopoeia Catalog No. STK200-10, STK200-20, STK3010, STK300-20).
- ✓ SOC medium.

2. EZRecombinase™ BP Cloning Kit

Catalog No.ER003 (20 reactions), ER004 (100 reactions)

Catalog No	Component	Quantity 20 reactions 100 reactions	Shipping temperatur	Storage temperature
ER003-01	EZRecombinase™ BP Mix	80 µl 80 µlx 5	Dry ice	-80°C Stable for at least 1 year
ER003-02	5×Reaction Buffer	80 µl 80 µlx 5	Dry ice	-80°C Stable for at least 6 months
ER003-03	Positive control (attB-PCR Product, ~1.9kb, 100 ng/µl)	20 µl 20 µlx 5	Dry ice	-20°C Stable for at least 6 months

Materials required but not supplied

The following materials are required but not supplied:

- ✓ attB-PCR Product or Expression clone DNA .
- ✓ attP-containing plasmid vector DNA (pShuttle™ cloning vector or Gateway® pDONR™ vector).
- ✓ Competent *E. coli* cells .
- ✓ TE buffer, pH 8.0.
- ✓ SOC medium.

III. Experimental Procedures

1. LR Recombination reaction

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

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.

2. BP Recombination reaction

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

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

Item	Amount
attB-PCR product or clone (≥10 ng/µl, 100-300 ng)	1-10 µl
pShuttle™ Vector or Gateway® pDONR vector (150 ng/ul)	1 µl
5x Reaction Buffer	4 µl
TE buffer, pH 8.0	to 16 µl
Final	**16** µl

2. Remove EZRecombinase™ BP Mix from -80°C and thaw on ice for 2-3 minutes.
3. Centrifuge briefly.
4. Add 4 µl of EZRecombinase™ BP 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™ BP 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 BP 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 EZShuttle™ Recombination Cloning System. 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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