EZShuttle™ Recombination Cloning System

EZRecombinase™ LR II Enzyme Mix

Cat. No. ER011

Cat. No. ER012

EZRecombinase™ BP II Enzyme Mix

Cat. No. ER013

Cat. No. ER014

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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.

EZRecombinase™ BP II Enzyme Mix is a mix of proprietary enzymes and buffers, 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 II Enzyme Mix, incubated at room temperature (25℃) for one hour, and used to transform *E. coli.* competent cells (Figure 1).

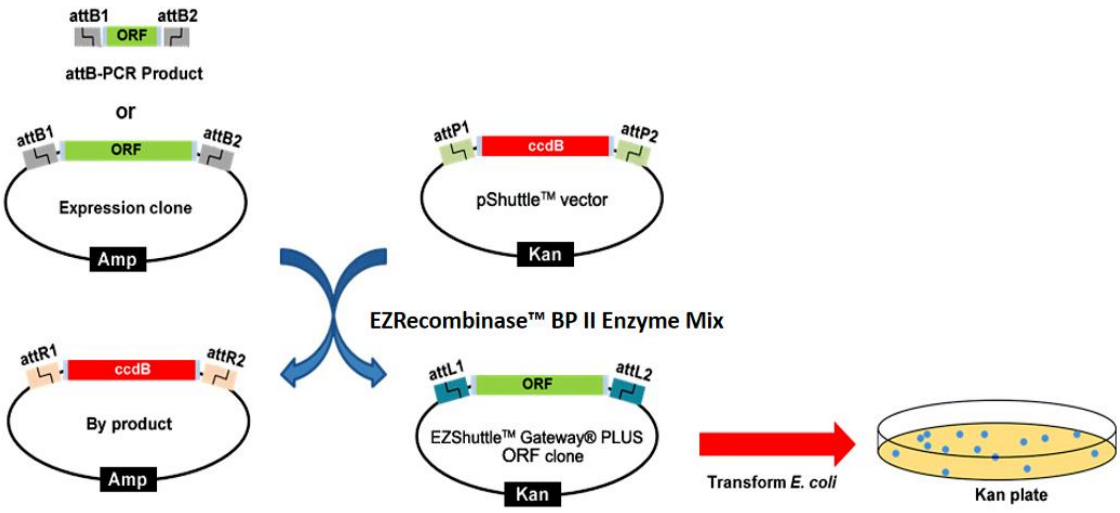

Figure 1. Principle for transferring a DNA fragment from an attB-containing PCR product or expression clone to an attP-containing pShuttle™ vector using EZShuttle™ BP recombination cloning.

EZRecombinase™ LR II Enzyme Mix is a mix of proprietary enzymes and buffers, 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 II Enzyme Mix are incubated at room temperature (25°C) for one hour and used to transform *E. coli.* competent cells (Figure 2).

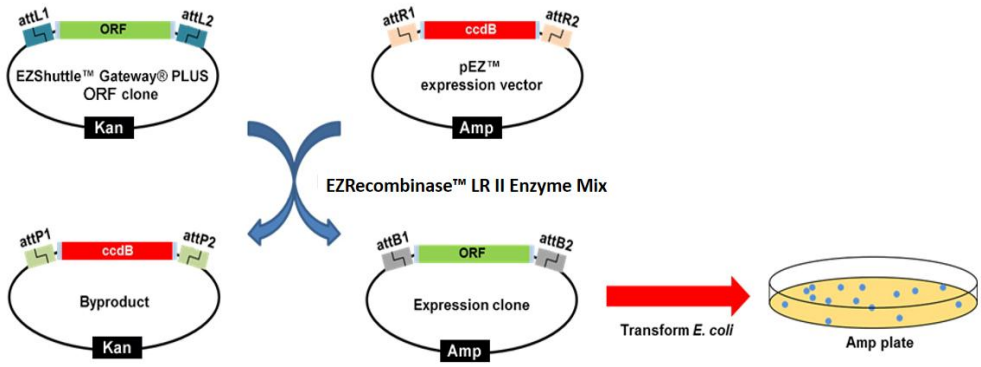

Figure 2. Principle for transferring a DNA fragment from an EZShuttle™ or Gateway® Entry clone to a pEZ™ expression or Gateway® pDEST vector using EZShuttle™ LR recombination cloning.

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

• **EZRecombinase™ Mix：** Is a mix of proprietary enzymes and buffers.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 II Enzyme 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 II Enzyme 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.

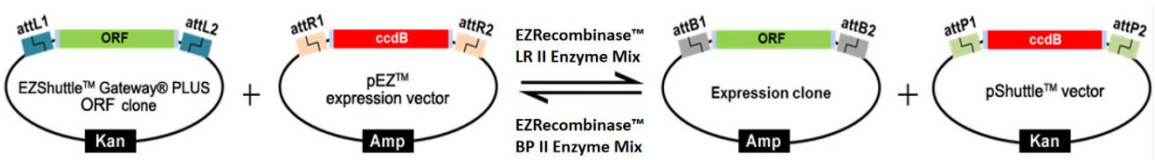

Figure 3. EZShuttle™ recombination cloning system as an operating system for cloning and subcloning DNA. DNA fragments are transferred between vectors using EZRecombinase™ LR or BP recombination reactions.

II. Contents and Storage

1. EZRecombinase™ BP II Enzyme Mix

Cat. No: ER013 (20 reactions)，ER014 (100 reactions)

Product Information	Component	Component Catalog No	Quantity	Storage Temperat
ER013 (20 reactions)	EZRecombinase™ BP II Enzyme Mix	ER013-01	40 μl	-20℃ Stable for at least 6 months
	Positive control (attB-PCR Product, ~1.9kb, 100 ng/μl)	ER013-02	10 μl	

Cat. No: ER014 (100 reactions) is assembled by 5 ER013.

Materials required but not supplied

• attB-PCR Product or Expression clone DNA.

• attP-containing plasmid vector DNA (pShuttle™ cloning vector or Gateway® pDONR™ vector).

• TE buffer, pH 8.0.

• Competent *E. coli* cells: GeneCopoeia Catalog No. CC001 (DH5α Competent *E. coli* cells), CC003 (Stbl3 Competent *E. coli* cells), CC007 (2T1 Competent *E. coli* cells).

• S.O.C medium(See appendix).

2. EZRecombinase™ LR II Enzyme Mix

Cat. No: ER011 (20 reactions)，ER012 (100 reactions)

Product Information	Component	Component Catalog No	Quantity	Storage Temperat
ER011 (20 reactions)	EZRecombinase™ LR II Enzyme Mix	ER011-01	40 μl	-20℃ Stable for at least 6 months
	Positive control (Shuttle clone eGFPp，100 ng/μl)	ER011-02	10 μl	

Cat. No: ER012 (100 reactions) is assembled by 5 ER011.

Materials 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. CC001 (DH5α Competent *E. coli* cells), CC003 (Stbl3 Competent *E. coli* cells), U0104A (2T1 Competent *E. coli* cells).

• S.O.C medium(See appendix).

III. Experimental Procedures

❖ 1. EZRecombinase™ BP II Enzyme Mix (ER013/ER014)

1.1 BP Recombination reaction

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

1） Take out EZRecombinase™ BP II Enzyme Mix (ER013-01) from the -20℃ refrigerator, place it on ice for 2 minutes, and briefly vortex twice, 2 seconds each time.

2） 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)	1-7 μl
pShuttle™ vector or Gateway® pDONR vector (150 ng/μl)	1 μl
TE buffer, pH 8.0	to 8 μl
Final	8 μl

Note: Please use the purified attB-PCR product.

3） Add 2 μl EZRecombinase™ BP II Enzyme Mix (ER013-01) to the reaction tube and mix well.

4） Briefly centrifuge to make sure all the reagents are at the bottom of the reaction tubes.

5） Incubate reactions at 25°C for 60 minutes.

Return EZRecombinase™ BP II Enzyme Mix(ER013-01) to -20°C storage immediately after use.

1.2 Transformation

1） Thaw competent cells on ice.

2） Use a pipette to transfer 2 μl of the reaction product into a sterilized 1.5 ml EP tube, add 50-100 μl of competent cells, and incubate on ice for 30 minutes.

3） Heat shock cells by placing the tubes into a 42°C water bath for 45 seconds, then ice bath for 2-3 minutes.

4） Add 500 μl of SOC medium and incubate at 37°C for 1 hour with shaking at 200 or 220 rpm.

5） Plate 50 μl and 200 μl of each transformation onto antibiotic-containing plates containing the same resistance as pShuttle™ vector (or Gateway® pDONR vector).

**** It is not recommended to plate all the transformations to prevent too many bacteria from affecting subsequent screening work.***

6） Incubate plates for 12 to 16 hr at 37°C.

❖ 2. EZRecombinase™ LR II Enzyme Mix (ER011/ER012)

2.1 LR Recombination reaction

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

1） Take out EZRecombinase™ LR II Enzyme Mix (ER011-01) from the -20℃ refrigerator, place it on ice for 2 minutes, and briefly vortex twice, 2 seconds each time.

2） 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)	1-7 µl
pEZ™ vector (or Gatewayt® pDEST vector) (150 ng/µl)	1 µl
TE buffer, pH 8.0	to 8 µl
Final	8 µl

3） Add 2 µl EZRecombinase™ LR II Enzyme Mix (ER011-01) to the reaction tube and mix well.

4） Briefly centrifuge to make sure all the reagents are at the bottom of the reaction tubes.

5） Incubate reactions at 25°C for 60 minutes.

Return EZRecombinase™ LR II Enzyme Mix(ER013-01) to -20° C storage immediately after use.

2.2 Transformation

1） Thaw competent cells on ice.

2） Use a pipette to transfer 2 µl of the reaction product into a sterilized 1.5 ml EP tube, add 50-100 µl of competent cells, and incubate on ice for 30 minutes.

3） Heat shock cells by placing the tubes into a 42°C water bath for 45 seconds, then ice bath for 2-3 minutes.

4） Add 500 µl of SOC medium and incubate at 37°C for 1 hour with shaking at 200 or 220 rpm.

5） Plate 50 µl and 200 µl of each transformation onto antibiotic-containing plates containing the same resistance as pShuttle™ vector (or Gateway® pDONR vector).

**** It is not recommended to plate all the transformations to prevent too many bacteria from affecting subsequent screening work.***

6） Incubate plates for 12 to 16 hr at 37°C.

IV. Troubleshooting Guide

Trouble	Cause	Suggestion
The sample has low or no bacterial count, but the positive control is normal.	Antibiotic use errors in transformation experiments.	Check that the antibiotic used is consistent with the entry clone or expression clone.
	att site usage error.	Entry clone (attL) and destination vector (attR) were used for LR reactions; Expression clone (or attB-PCR product) and pShuttle™ vector (attP) are used for BP reaction.
	EZRecombinase™ LR or BP Enzyme Mix is inactivated or is not being used according to the recommended dosage.	Test another portion of EZRecombinase™ LR or BP Enzyme Mix; Confirm that the kit is stored at the temperature indicated on the label; EZRecombinase™ LR or BP Enzyme Mix should be frozen and thawed no more than 10 times; Conduct the experiment strictly in accordance with the recommended dosage in the user manual.
	LR or BP Enzyme Mix usage error.	LR Enzyme Mix is used for LR reaction; BP Enzyme Mix is used for BP reaction.
	Excessive input of attB-PCR product in the BP reaction.	Reduce the amount of attB-PCR product and use equal amounts (fmol) of attB-PCR product and pShuttle™ vector.
	attB-PCR product or linearized attB expression clone ≥5kb.	Increase the dosage of attB-PCR product; BP reaction time extended to overnight.
	Improper design of attB PCR primers.	Verify that attB PCR primers contain attB1 or attB2 sites.
	Over input of entry clone in LR reaction.	Use equal amounts (fmol) of destination vector and entry clone.
	Destination vector or entry clone ≥10kb.	The LR reaction time is extended to overnight; Linearize the destination vector and entry clone; Use topoisomerase I to treat the destination vector.
Colonies of different sizes appear.	LR reaction: Small colonies may be the result of co-transformation of unreacted entry clones and expression clones.	Reduce entry clone usage; Reduce the amount of conversion products; Increase the concentration of destination vector screening resistance (Amp+) to 300μg/ml.
	BP reaction: There is a mutation or deletion in the ccdB	Repurchase pShuttle™ Vector or Donor Vector.

	gene of the pShuttle™ vector or donor vector (similar colonies appear in the negative control group).	
	Plasmid loss during colony growth (usually occurs in experiments with large fragment genes or toxic genes).	The screening plate culture temperature was adjusted to 30°C; Confirm whether the DNA in the colony is deleted; Use Stbl3 competent state to maintain stable replication of large genes.
Positive control has low or no bacterial growth.	Improper storage of competent cells.	Competent cells should be stored at -80°C.
	Improper conversion operation.	Check conversion steps.
	Competent cells are inefficient.	Use >1.0×10^8 transformants/μg competent cells.

V. Appendix

Medium prepare

SOC medium (100ml)

2% (W/V) Bacto Tryptone

0.5% (W/V) Bacto Yeast Extract

0.05% (W/V) NaCl

2.5mM KCl

Adjust the pH to 7.0, and cool to below 60℃ after sterilization. MgCl2 solution (final concentration 10mM) and sterile glucose solution (final concentration 20mM) were added.

LB (Luria-Bertani) medium (1L)

Add the following components to 950mL of deionized water:

10g Tryptone

5g Yeast extract

10g NaCl

Shake the container until the solute is completely dissolved. Adjust pH to 7.0 with 5 mol/L NaOH (about 0.2mL). Fill with deionized water to 1L. High-pressure steam sterilization with liquid circulation under 15psi(1.05kg /cm^2) pressure for 20min.

VI. 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.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.