USER GUIDE

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Gateway® Vector Conversion System with One Shot® *ccd*B Survival™ 2 T1R Competent Cells

For conversion of any vector of choice into a Gateway® destination vector

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Contents

Kit Contents and Storage

Shipping and Storage

The Gateway® Vector Conversion System with One Shot® *ccd*B Survival™ 2 T1^R Competent Cells is shipped on dry ice. Upon receipt, store each box as detailed in the following table.

Box	Item	Storage
1	Reading Frame Cassettes	–30°C to –10°C
2–3	One Shot® *ccd*B Survival™ 2 T1^R Competent Cells	–85°C to –68°C

Contents

The Reading Frame Cassettes box contains the following items. Store components at –30°C to –10°C.

Item	Composition	Amount
Reading Frame Cassette A (RfA)	5 ng/µL linear fragment in TE buffer, pH 8.0	40 µL
Reading Frame Cassette B (RfB)	5 ng/µL linear fragment in TE buffer, pH 8.0	40 µL
Reading Frame Cassette C.1 (RfC.1)	5 ng/µL linear fragment in TE buffer, pH 8.0	40 µL
pENTR™-gus Positive Control	50 ng/µL in TE buffer, pH 8.0	20 µL

Prodcut Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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*ccd*B Survival™ 2 T1^R Cells

Each box of One Shot® *ccd*B Survival™ 2 T1 Phage-Resistant Cells contains the following items. The transformation efficiency is >1 × 10^9 cfu/µg pUC19 Control DNA. Store competent cells at −85°C to −68°C.

Item	Composition	Amount
One Shot® *ccd*B Survival™ 2 T1^R Competent Cells	—	11 × 50 µL
S.O.C. Medium (may be stored at room temperature at 15°C to 30°C, or in a cold room at 2°C to 8°C)	2% tryptone 0.5% yeast extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 mL
pUC19 Control DNA	10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µL

Genotype of *ccd*B Survival™ 2 T1^R Cells

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*Δ139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G *fhuA*::*IS2*

Introduction

Overview

Description

The Gateway® Vector Conversion System allows you to easily convert any vector of choice to a Gateway® destination vector. By ligating a blunt-ended cassette containing *att*R sites flanking the *ccd*B gene and the chloramphenicol resistance gene into the multiple cloning site of your vector, you can create your own destination vector for expression of native, N-, or C-terminally-tagged proteins.

Features of the Gateway® Vector Conversion System

The Gateway® Vector Conversion System contains the following features:

- Three reading frame cassettes that differ by 1 nucleotide each, allowing generation of *att*R sites in all 3 reading frames.
- Blunt-ended reading frame cassettes that can be ligated into any blunt-ended restriction site
- Reading frame cassettes that can be identified by a unique restriction site
- pENTR™-gus plasmid for use as a positive control for recombination and expression

Important

Most entry vectors contain the kanamycin resistance gene for selection. For maximal compatibility within the Gateway® Technology, we recommend that your vector **not** contain a kanamycin resistance marker. If this is unavoidable, you will need to perform the LR recombination reaction with an entry clone that carries a selection marker other than the kanamycin resistance gene.

You may use pDONR™/Zeo and your *att*B-flanked gene of interest in a BP recombination reaction to generate an entry clone that confers Zeocin™ resistance. See page 18 for ordering information.

Continued on next page

The Gateway® Technology

The Gateway® Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest using Gateway® Technology, simply:

1. Clone your gene of interest into a Gateway® entry vector to create an entry clone.
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and your converted Gateway® destination vector.
3. Introduce your expression clone into the appropriate host and express your recombinant protein.

For more information on the Gateway® Technology, refer to the Gateway® Technology with Clonase® II manual. This manual is available from **www.lifetechnologies.com/manuals** or by contacting Technical Support (page 20).

Experimental outline

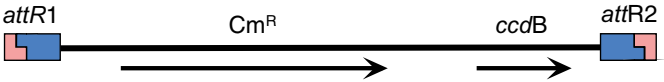
To convert your vector to a destination vector, you will:

1. Choose the appropriate reading frame cassette to use depending on your needs.
2. Linearize the vector you wish to convert with a restriction enzyme of choice. If you use a restriction enzyme that generates an overhang, you will need to blunt the ends.
3. Remove the 5′ phosphates from the vector using calf intestinal alkaline phosphatase.
4. Ligate the reading frame cassette into your vector using T4 DNA ligase.
5. Transform the ligation reaction into One Shot® *ccd*B Survival™ 2 T1^R Competent *E. coli* and select for transformants.
6. Analyze transformants.

Features of the Reading Frame Cassettes

Features

Each reading frame cassette contains the chloramphenicol resistance gene (Cm^R) and the *ccd*B gene flanked by *att*R1 and *att*R2 sites. Each reading frame cassette also differs by 1 nucleotide to allow generation of *att*R sites in all 3 reading frames. For more information on the features of the cassettes, see page 16.



Distinguishing between cassettes

Each reading frame cassette contains a unique restriction site to allow you to distinguish between them (see the following table).

Cassette	Restriction Site	Location (bp)
RfA	*Mlu* I	898
RfB	*Bgl* II	899
RfC.1	*Xba* I	899

Important

The sequences of the *att* sites as well as other elements may vary between the reading frame cassettes. These sequence variations do not affect the functionality of the cassettes nor do they affect the efficiency and specificity of the Gateway® recombination reactions. For the complete sequence of a particular reading frame cassette, refer to **www.lifetechnologies.com** or contact Technical Support (page 20).

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Features of the Reading Frame Cassettes, Continued

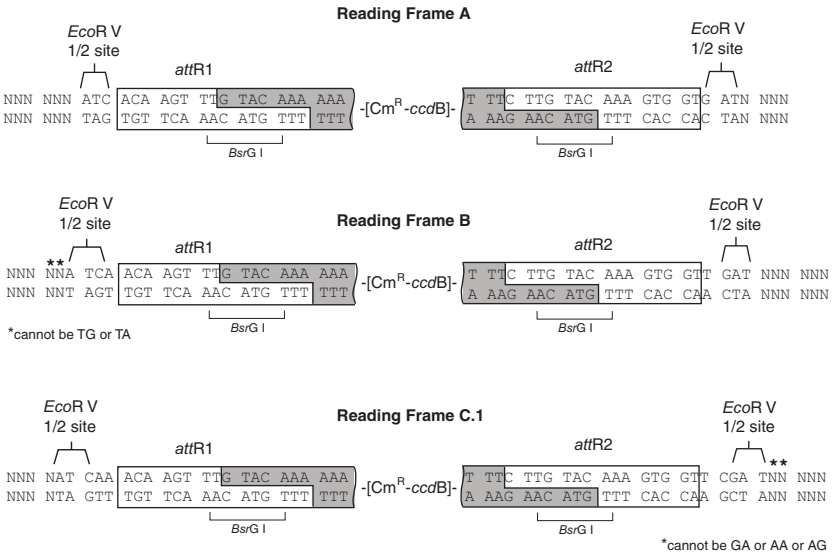
Sequences of the reading frame cassettes

The sequences of the ends of each reading frame cassette are shown below. The complete sequence of each cassette is available from **www.lifetechnologies.com** or by contacting Technical Support (page 20).

Features of the reading frame cassettes:

- Non-shaded regions correspond to DNA sequence that is transferred to the *att*B expression clone following the LR recombination reaction.
- The *Eco*R V half-site present on the 5′ and 3′ ends of each cassette is labeled.
- Sequences contributed by your vector are denoted by Ns.

  Note: If you are using RfB to create an N-terminal fusion vector, the 2 nucleotides next to the 5′ *Eco*R V half-site cannot be TG or TA as this will generate a stop codon. Similarly, if you are using RfC.1 to create a C-terminal fusion vector, the 2 nucleotides next to the 3′ *Eco*R V half-site cannot be GA, AA, or AG.
- The *Bsr*G I restriction site common to all *att*1 and *att*2 sites is indicated.



Methods

Choose a Reading Frame Cassette

Factors to consider

To determine which reading frame cassette to use when converting your vector, consider the following:

- If you plan to express a fusion protein from the destination vector, use a reading frame cassette with the correct translation reading frame (see **N-terminal fusions** and page 6 to help you choose the correct reading frame cassette)
- If you plan to linearize your vector using a restriction enzyme that generates an overhang, choose the correct reading frame cassette based on what the sequence of the ends will be after the vector has been made blunt (i.e. after filling in a protruding 5′ end or polishing a protruding 3′ end)

N-terminal fusions

If you intend to create a destination vector to express N-terminal fusion proteins, use the following table and the diagram on page 4 to help you determine which reading frame cassette to use.

Tip: Keep the -AAA-AAA- triplets in the *att*R1 site in frame with the translation reading frame of the fusion protein.

If the coding sequence of the blunt end…	Then use…
terminates after a complete codon triplet	RfA
encodes 2 bases of a complete codon triplet	RfB
encodes 1 base of a complete codon triplet	RfC.1

Continued on next page

Choose a Reading Frame Cassette, Continued

C-terminal fusions

If you intend to create a destination vector to express C-terminal fusion proteins, use the following table and the diagram on page 4 to help you determine which reading frame cassette to use.

Tip: Keep the -TAC-AAA- triplets in the *att*R2 site in frame with the translation reading frame of the fusion protein.

If the coding sequence of the blunt end…	Then use…
terminates after a complete codon triplet	RfB
encodes 2 bases of a complete codon triplet	RfC.1
encodes 1 base of a complete codon triplet	RfA

If you intend to create a destination vector to express both N-terminal and C-terminal fusions, choose a restriction enzyme that will produce blunt-ends that allow in-frame cloning with the *att*R sites in 1 of the 3 cassettes.

Construct a Gateway® Destination Vector

Ligate the reading frame cassette to your vector

Each reading frame cassette is supplied as a blunt-ended DNA fragment that can be ligated into any blunt-ended restriction site. It is possible to linearize your vector using a restriction enzyme that generates 5′ overhangs, however, the ends of the vector must first be made blunt (using a Klenow fill-in reaction) before the blunt-ended reading frame cassette may be ligated into the vector.

To linearize your vector, we recommend that you choose restriction enzymes that will remove as many of the MCS restriction sites as possible. This will minimize the number of additional amino acids added to the fusion and will increase the number of unique restriction sites in the destination vector, which is important if you wish to linearize the vector for the LR recombination reaction.

Required materials

Components required but not supplied:

- Your vector of choice
- Appropriate restriction enzymes to linearize your vector at the position where you wish your gene (flanked by *att* sites) to be after recombination (see the preceding Recommendation)
- T4 DNA polymerase or Klenow fragment (if necessary to create blunt ends in your vector)
- Calf intestinal alkaline phosphatase
- 10X CIAP Buffer
- Sterile water (autoclaved, distilled)
- TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA)
- T4 DNA ligase
- 5X T4 DNA ligase buffer

Components supplied with the kit:

- Appropriate Gateway® reading frame cassette (5 ng/µL)

Continued on next page

Construct a Gateway® Destination Vector, Continued

Converting your vector

1. Digest 1–5 µg of your plasmid vector with the appropriate restriction enzyme(s).
2. If necessary, convert the ends of the vector to blunt double-stranded DNA using T4 DNA polymerase or Klenow fragment according to the manufacturer's recommendations.
3. Remove the 5′ phosphates with calf intestinal alkaline phosphatase (CIAP) to decrease the background associated with self-ligation of the vector.
   a. Determine the mass of DNA required for 1 pmol of the DNA 5′ end.
   b. Add the following reagents to a 1.5-mL microcentrifuge tube:

10X CIAP Buffer	4 µL
DNA	1 pmol of 5′ ends
Sterile water	to a final volume of 39 µL

   c. Dilute the CIAP in dilution buffer such that 1 µL contains the amount of enzyme required to dephosphorylate the appropriate 5′ end (i.e. 1 unit for blunt ends). Add 1 µL of CIAP and incubate for 1 hour at 50°C.
   d. Heat-inactivate CIAP for 15 minutes at 65°C.
4. Adjust the DNA to a final concentration of 20–50 ng/µL in TE buffer, pH 8.0. Run 20–100 ng of DNA on an agarose gel to verify digestion and recovery.
5. To set up the ligation reaction, add the following reagents to a 1.5-mL microcentrifuge tube:

Dephosphorylated vector (20–50 ng)	1–5 µL
5X T4 DNA ligase buffer	2 µL
Gateway® reading frame cassette (10 ng)	2 µL
T4 DNA ligase	1 unit (in 1 µL)
Sterile water	to a final volume of 10 µL

6. Incubate the reaction at room temperature for 1 hour. Proceed to **Transform *ccd*B Survival™ 2 T1^R Competent Cells**.

   Note: Overnight incubation at 16°C is also suitable.

Transform *ccd*B Survival™ 2 T1^R Competent Cells

Introduction

After ligating the reading frame cassette to your vector, you will transform the ligation reaction into the One Shot® *ccd*B Survival™ 2 T1^R Competent Cells included with the kit.

Important

To select, propagate, and maintain your destination vector, you must use the One Shot® *ccd*B Survival™ 2 T1^R Cells included with the kit. The *ccd*B Survival™ 2 T1^R *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene.

Note: Do not use general *E. coli* cloning strains including TOP10 or DH5α™ for propagation and maintenance as these strains are sensitive to CcdB effects.

Required materials

Components required but not supplied:

- Ligation reaction from step 6, page 8
- TE buffer, pH 7.5 (10 mM Tris-HCl, 1 mM EDTA)
- LB plates containing 15–30 µg/mL chloramphenicol **and** an additional antibiotic appropriate for selection of your vector
- LB plates containing 100 µg/mL ampicillin (if using pUC19 positive control)
- 42°C water bath
- 37°C shaking and non-shaking incubator
- *Optional:* LB Medium

Components supplied with the kit:

- One Shot® *ccd*B Survival™ 2 T1^R Cells (thaw on ice before use)
- S.O.C. Medium (supplied with the kit)
- *Optional:* pUC19 positive control

Continued on next page

Transform *ccd*B Survival™ 2 T1^R Competent Cells, Continued

Prepare for transformation

For each transformation, you will need 1 vial of One Shot® *ccd*B Survival™ 2 T1^R competent cells and 2 selective plates.

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
- Warm selective plates at 37°C for 30 minutes.

One Shot® Transformation Protocol

Use the following protocol to transform the One Shot® *ccd*B Survival™ 2 T1^R competent cells.

1. Make a 5-fold dilution of the ligation reaction (from step 6, page 8) in TE buffer.
2. Add 1 µL of the diluted ligation reaction into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down**. For the pUC19 control, add 1 µl (10 pg) of DNA into a separate vial of One Shot® cells and mix gently.
3. Incubate the vials on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove the vials from the 42°C water bath and place them on ice for 2 minutes.
6. Add 250 µL of room temperature S.O.C. Medium to each vial.
7. Cap the vials tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Spread 25–100 µL from each transformation on a prewarmed selective plate. We recommend that you plate 2 different volumes to ensure that at least 1 plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:10 in LB Medium and plate 25–100 µL.
9. Store the remaining transformation mix at 4ºC. Additional cells may be plated out the next day, if desired.
10. Incubate plates overnight at 37ºC.

Analyze Transformants

Important

Because the reading frame cassettes are blunt-ended, they will ligate into your vector in both orientations. You will need to screen transformants to identify plasmids containing the reading frame cassette in the proper orientation.

Analyze transformants

1. Pick 10 colonies and culture them overnight in 3–5 mL of LB medium containing 30 µg/mL chloramphenicol.
2. Isolate plasmid DNA using your method of choice (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989). We recommend the PureLink® HQ Mini Plasmid Purification Kit (see page 18 for ordering information).
3. Analyze the plasmids by restriction analysis to confirm the presence and correct orientation of the cassette. The *Bsr*G I restriction enzyme can be used to identify clones containing the reading frame cassette (see the diagram on page 4).

Verify the functionality of the *ccd*B gene

It is important to verify the functionality of the *ccd*B gene **and** check for the presence of contaminating antibiotic-resistant plasmids (e.g. no contaminating ampicillin-resistant plasmids if your destination vector is ampicillin-resistant). The presence of an inactive *ccd*B gene or contamination with other antibiotic-resistant plasmids can result in high backgrounds in the LR recombination reaction.

Required materials

Components required but not supplied:

- TOP10 competent *E. coli* (or any other strain sensitive to CcdB effects)
- Selective plates (e.g. LB + ampicillin)

Components supplied with the kit:

- One Shot® *ccd*B Survival™ 2 T1^R Competent Cells
- pUC19 Control DNA

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Procedure to verify the functionality of the *ccd*B gene

1. Transform equal amounts (10–50 pg) of your destination vector into competent TOP10 (or equivalent) and One Shot® *ccd*B Survival™ 2 T1^R cells using the protocol provided with the cells. Also transform each strain with 50 pg (5 µL) of the pUC19 Control DNA.
2. Plate onto selective plates and incubate overnight at 37ºC.
3. Use the pUC19 Control DNA to verify that the transformation efficiency is as expected for each strain.
4. Determine the number of colonies obtained in both strains transformed with the destination vector.

What You Should See:

The destination vector should give 10,000 times **more** colonies in *ccd*B Survival™ 2 T1^R cells than in TOP10 cells. Any ratio less than 10,000 indicates either an inactive *ccd*B gene or contamination of the plasmid prep with another antibiotic-resistant plasmid.

Sequence your destination vector

You may sequence your destination vector to verify that the reading frame cassette is in the proper orientation and is correctly in-frame. Digest your destination vector with restriction enzymes that cleave in the middle of the reading frame cassette and in the backbone of your vector. Make sure to avoid digesting where the sequencing primers will anneal (see the map on page 16).

Sequencing primers

Use the sequencing primers listed in the following table with primers that hybridize within your vector to sequence through the *att*R junctions and verify proper ligation of the reading frame cassette to your vector. See the map on page 16 for the location of the primer binding sites.

Primer 1	5′-CAC ATT ATA CGA GCC GGA AGC AT-3′
Primer 2	5′-CAG TGT GCC GGT CTC CGT TAT CG-3′

Perform the LR Recombination Reaction

Introduction

After analyzing your destination vector, you may perform the LR recombination reaction with your destination vector and an entry clone containing the gene of interest. We recommend that you include the pENTR™-gus positive control plasmid supplied with the kit in your experiment to help you evaluate your results.

Entry clone

A number of entry vectors are available to facilitate creation of an entry clone (see page 19 for ordering information). For detailed information on constructing an entry clone, refer to the manual for the entry vector that you are using.

Positive control

The pENTR™-gus plasmid is included in the Gateway® Vector Conversion System for use as a positive control for recombination and expression. Use of the pENTR™-gus entry clone in an LR recombination reaction with your destination vector will allow you to generate an expression clone containing the gene encoding β-glucuronidase (*gus*).

Prepare the destination vector

Once purified, you may use your supercoiled destination vector directly in the LR recombination reaction. If your destination vector is large (>10 kb), you may increase the efficiency of the LR reaction by linearizing the destination vector with a restriction enzyme or relaxing the DNA with topoisomerase I, if desired.

To linearize the destination vector, use a unique restriction enzyme that cuts within the Gateway® reading frame cassette but not within the *ccd*B gene (see below for a list of possible restriction enzymes). Be sure to choose a restriction enzyme that does not cut within your vector sequence.

*Alw*N I	*Nco* I	*Sal* I
*Bss*H II	*Not* I	*Sca* I
*Eco*R I	*Pvu* II	*Sfc* I

Continued on next page

Perform the LR Recombination Reaction, Continued

E. coli host

You may use any *rec*A, *end*A *E. coli* strain including TOP10, OmniMAX™ 2-T1^R, or equivalent for transformation. **Do not** transform the LR reaction mixture into *E. coli* strains that contain the F′ episome (e.g. TOP10F′). These strains contain the *ccd*A gene and will prevent negative selection with the *ccd*B gene.

Gateway® LR Clonase® II Enzyme Mix

Gateway® LR Clonase® II enzyme mix (see page 18 for ordering information) combines the proprietary enzyme formulation and 5X LR Reaction Buffer previously supplied as separate components in Gateway® LR Clonase® enzyme mix into an optimized single tube format to allow easier set-up of the LR recombination reaction. Use the protocol provided on the page 15 to perform the LR recombination reaction using LR Clonase® II enzyme mix.

Note: You may perform the LR recombination reaction using Gateway® LR Clonase® enzyme mix, if desired. To use Gateway® LR Clonase® enzyme mix, follow the protocol provided with the product. **Do not** use the protocol for Gateway® LR Clonase® II enzyme mix provided on page 15.

Required materials

Components required but not supplied:

- Your destination vector (150 ng/µL in TE, pH 8.0)
- Gateway® LR Clonase® II enzyme mix (see page 18 for ordering information; keep at –20°C or –80°C until immediately before use)
- TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA)
- 2 µg/µL Proteinase K solution (supplied with the Gateway® LR Clonase® II enzyme mix; thaw and keep on ice until use)
- Appropriate competent *E. coli* host for expression
- S.O.C. Medium
- LB agar plates with appropriate antibiotic for selection of expression clones

Components supplied with the kit:

- pENTR™-gus positive control (50 ng/µL in TE, pH 8.0)

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Perform the LR Recombination Reaction, Continued

Perform the LR reaction

1. Add the following components to 1.5-mL microcentrifuge tubes at room temperature and mix.

Component	Sample	Negative Control	Positive Control
Entry Clone (50–150 ng/reaction)	1–7 µL	1–7 µL	—
Your Destination Vector (150 ng/µL)	1 µL	1 µL	1 µL
pENTR™-gus (50 ng/µL)	—	—	2 µL
TE Buffer, pH 8.0	to 8 µL	to 10 µL	5 µL

2. Thaw, on ice, the Gateway® LR Clonase® II enzyme mix for about 2 minutes.
3. Vortex the Gateway® LR Clonase® II enzyme mix briefly twice (2 seconds each time).
4. Add 2 µL of the Gateway® LR Clonase® II enzyme mix to each sample and the positive control. **Do not** add Gateway® LR Clonase® II enzyme mix to the negative control. Mix well by vortexing briefly twice (2 seconds each time).

   Reminder: Return Gateway® LR Clonase® II enzyme mix to –20°C or –80°C immediately after use.
5. Incubate the reactions at 25°C for 1 hour.

   Note: For most applications, 1 hour will yield a sufficient number of colonies for analysis, however, the length of the recombination reaction can be extended up to 18 hours. For large plasmids (≥ 10 kb), longer incubation times (e.g. overnight incubation) are recommended as they will yield more colonies.
6. Add 1 µl of the Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Transform 1 µL of the LR recombination reaction into a suitable *E. coli* host and select for expression clones.

   Note: You may store the LR reaction at –20°C for up to 1 week before transformation, if desired.

What you should see

If you use *E. coli* cells with a transformation efficiency of 1×10^8 cfu/µg, the LR reaction should give >5000 colonies if the entire LR reaction is transformed and plated.

Appendix

Map and Features of the Reading Frame Cassettes

Map

The following map shows the elements of the Gateway® reading frame cassettes. **The complete sequence of each reading frame cassette is available from www.lifetechnologies.com or by contacting Technical Support (page 20).**

Reading Frame Cassette:	A (1711 bp)	B (1713bp)	C.1 (1714 bp)
*att*R1 site	4-128	5-129	6-130
Primer 1	163-185	164-186	165-187
Chloramphenicol resistance gene	237-896	238-897	239-919
*ccd*B gene	1238-1543	1239-1544	1239-1544
Primer 2	1444-1466	1445-1467	1445-1467
*att*R2 site	1584-1708	1585-1709	1585-1709

Features

Reading Frame Cassette A (1711 bp), B (1713 bp), and C.1 (1714 bp) contain the following elements. All features have been functionally tested.

Feature	Function
*att*R1 and *att*R2 sites	Allows recombinational cloning of the gene of interest from an entry clone (Landy, 1989)
Primer 1 site	Allows sequencing through the *att*R1 junction
Chloramphenicol resistance gene	Allows counterselection of plasmid
*ccd*B gene	Allows negative selection of plasmid
Primer 2 site	Allows sequencing through the *att*R2 junction

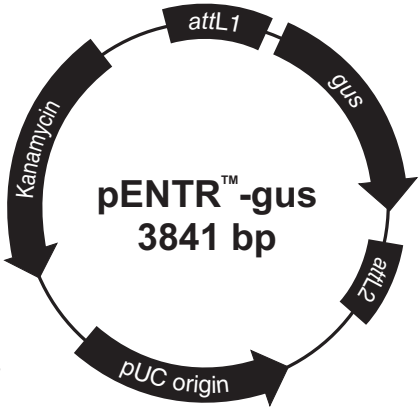
Map of pENTR™-gus

Description

pENTR™-gus is a 3841 bp entry clone containing the *Arabidopsis thaliana* gene for β-glucuronidase (*gus*) (Kertbundit *et al.*, 1991). The *gus* gene was amplified using PCR primers containing *att*B recombination sites. The amplified PCR product was then used in a BP recombination reaction with pDONR™201 to generate the entry clone. For more information about the BP recombination reaction, refer to the Gateway® Technology with Clonase® II manual.

Map

The following map below shows the elements of the pENTR™-gus vector. **The complete sequence for pENTR™-gus is available from www.lifetechnologies.com or by contacting Technical Support (page 20).**



Comments for pENTR™-gus 3841 nucleotides

*att*L1: bases 99-198
gus gene: bases 228-2039
*att*L2: bases 2041-2140
pUC origin: bases 2200-2873 (C)
Kanamycin resistance gene: bases 2990-3805 (C)

C = complementary strand

Accessory Products

Introduction

The products listed in this section may be used with the Gateway® Vector Conversion System. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (page 20).

Additional Products

Additional products that may be used with the Gateway® Vector Conversion System are available. Ordering information is provided in the following table.

Product	Amount	Catalog no.
One Shot® *ccd*B Survival™ 2 T1^R Competent Cells	10 reactions	A104660
pDONR™221	6 µg	12536-017
pDONR™/Zeo	6 µg	12535-035
Calf Intestinal Alkaline Phosphatase (CIAP)	1000 units	18009-019
T4 DNA Ligase	100 units	15224-017
S.O.C. Medium	10 × 10 mL	15544-034
PureLink® HQ Mini Plasmid Purification Kit	100 reactions	K2100-01
Gateway® LR Clonase® II Enzyme Mix	20 reactions	11791-020
	100 reactions	11791-100
Gateway® BP Clonase® II Enzyme Mix	20 reactions	11789-020
	100 reactions	11789-100
One Shot® TOP10 Chemically Competent Cells	10 reactions	C4040-10
	20 reactions	C4040-03
One Shot® TOP10 Electrocompetent Cells	10 reactions	C4040-50
	20 reactions	C4040-52

Continued on next page

Accessory Products, Continued

Gateway® Entry Vectors

A variety of Gateway® entry vectors are available to facilitate creation of entry clones. For rapid TOPO® Cloning of PCR products, we recommend using the one of the pENTR™/D-TOPO® Cloning Kits. For traditional restriction enzyme digestion and ligase-mediated cloning, use one of the other pENTR™ vectors. For more information about the features of each entry vector, refer to **www.lifetechnologies.com** or contact Technical Support (page 20).

Product	Amount	Catalog no.
pENTR™/D-TOPO® Cloning Kit		
with One Shot® TOP10 Competent Cells	20 reactions	K2400-20
with One Shot® Mach1™-T1^R Competent Cells	20 reactions	K2435-20
pENTR™/SD/D-TOPO® Cloning Kit		
with One Shot® TOP10 Competent Cells	20 reactions	K2420-20
with One Shot® Mach1™-T1^R Competent Cells	20 reactions	K2635-20
pENTR™/TEV/D-TOPO® Cloning Kit		
with One Shot® TOP10 Competent Cells	20 reactions	K2525-20
with One Shot® Mach1™-T1^R Competent Cells	20 reactions	K2535-20
pENTR™1A Dual Selection Vector	10 µg	A10462
pENTR™2B Dual Selection Vector	10 µg	A10463
pENTR™3C Dual Selection Vector	10 µg	A10464
pENTR™4 Dual Selection Vector	10 µg	A10465
pENTR™11 Dual Selection Vector	10 µg	A10467

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com/support**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Continued on next page

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ccdB selection technology is described in Bernard et al., "Positive Selection Vectors Using the F Plasmid ccdB Killer Gene" Gene 148 (1994) 71-74. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). For licensing information for use in other than research, please contact: **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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Gateway® Clone Distribution Policy

For additional information about Life Technologies' policy for the use and distribution of Gateway® clones, see the section entitled **Gateway® Clone Distribution Policy**, page 23.

Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning Life Technologies' policy for the use and distribution of cloned nucleic acid fragments, including open reading frames, created using Life Technologies' commercially available Gateway® Technology.

Gateway® Entry Clones

Life Technologies understands that Gateway® entry clones, containing *att*L1 and *att*L2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Life Technologies.

Gateway® Expression Clones

Life Technologies also understands that Gateway® expression clones, containing *att*B1 and *att*B2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Life Technologies. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee ($10 per clone) payable to Life Technologies.

Additional Terms and Conditions

We would ask that such distributors of Gateway® entry and expression clones indicate that such clones may be used only for research purposes, that such clones incorporate the Gateway® Technology, and that the purchase of Gateway® Clonase® from Life Technologies is required for carrying out the Gateway® recombinational cloning reaction. This should allow researchers to readily identify Gateway® containing clones and facilitate their use of this powerful technology in their research. Use of Life Technologies' Gateway® Technology, including Gateway® clones, for purposes other than scientific research may require a license and questions concerning such commercial use should be directed to Life Technologies' licensing department at 760-603-7200.

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