invitrogen™

Gateway® pENTR™ Dual Selection Vectors

Catalog nos. A10462, A10463, A10464, A10465, A10467

Version A
6 Jun 2008
A10609

User Manual

Table of Contents

Important Information

pENTR™ Dual Selection Vectors

This manual is supplied with the following products.

Product	Catalog no.
pENTR™1A Dual Selection Vector	A10462
pENTR™2B Dual Selection Vector	A10463
pENTR™3C Dual Selection Vector	A10464
pENTR™4 Dual Selection Vector	A10465
pENTR™11 Dual Selection Vector	A10467

Shipping and Storage

pENTR™ Dual Selection Vectors are supplied in TE buffer and shipped on wet ice. Upon receipt, store at -20°C. Products are guaranteed for six months from date of shipment when properly stored.

Contents

20 µl of pENTR™ Dual Selection Vector at 0.5 µg/µl, in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Product Qualification

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

Accessory Products

Additional Products

Additional products that may be used with the pENTR™ Dual Selection Vectors are available from Invitrogen. Ordering information is provided below.

Item	Quantity	Catalog no.
LR Clonase™ II Enzyme Mix	20 reactions 100 reactions	11791-020 11791-100
One Shot® *ccd*B Survival™ 2 T1^R Chemically Competent Cells	10 reactions	A10460
One Shot® TOP10 Chemically Competent Cells	10 reactions 20 reactions	C4040-10 C4040-03
One Shot® TOP10 Electrocompetent Cells	10 reactions 20 reactions	C4040-50 C4040-52
One Shot® MAX Efficiency® DH5α™-T1® Chemically Competent Cells	20 reactions	12297-016
Kanamycin Sulfate	5 g	11815-024
PureLink™ PCR Purification Kit	50 reactions	K3100-01
PureLink™ HQ Mini Plasmid Purification Kit	100 preps	K2100-01

Gateway® Destination Vectors

A large selection of Gateway® destination vectors is available from Invitrogen to facilitate expression of your gene of interest in virtually any protein expression system. For more information about the vectors available, refer to the Gateway® Technology Central application portal on our website at www.invitrogen.com/gateway or contact Technical Support (see page 24).

Methods

Overview

Introduction

The pENTR™ Dual Selection Vectors allow restriction cloning of a gene of interest into a vector for entry into the Gateway® System available from Invitrogen. A choice of pENTR™ Dual Selection Vectors is available (see table below) for optimal expression of your gene of interest after recombination with the Gateway® destination vector of choice. For more information about the Gateway® Technology, see the next page.

Product	Benefit
pENTR™1A Dual Selection Vector	• Three reading frames available • Kozak sequence for efficient initiation of translation in eukaryotic cells • *E. coli* ribosome binding site for efficient initiation of translation in prokaryotic cells **(pENTR™1A and pENTR™3C only)**
pENTR™2B Dual Selection Vector	
pENTR™3C Dual Selection Vector	
pENTR™4 Dual Selection Vector	• Same multiple cloning site as pENTR™1A except that first restriction enzyme site is *Nco* I • Kozak sequence for efficient initiation of translation in eukaryotic cells
pENTR™11 Dual Selection Vector	• Kozak sequence for efficient initiation of translation in eukaryotic cells • Two *E. coli* ribosome binding sites for efficient initiation of translation in prokaryotic cells

continued on next page

Overview, Continued

Features of the pENTR™ Dual Selection Vectors

The pENTR™ Dual Selection Vectors contain the following elements:

- *rrn*B transcription termination sequences to prevent basal expression of the PCR product of interest in *E. coli*
- *att*L1 and *att*L2 sites for site-specific recombination of the entry clone with a Gateway® destination vector (for more information, refer to the Gateway® Technology with Clonase™ II manual or Landy, 1989)
- Kozak consensus sequence for efficient translation initiation in eukaryotic systems
- Ribosome binding site for efficient translation initiation in prokaryotic systems **(pENTR™1A, pENTR™3C, and pENTR™11 only)**
- Dual selection cassette containing the Chloramphenicol resistance gene (Cm^R) and the ccdB gene located between the two *att*L sites for counterselection
- Kanamycin resistance gene for selection in *E. coli*
- pUC origin behaves as medium to low-copy origin of replication for maintenance of the plasmid in *E. coli*

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 the Gateway® Technology, simply:

1. Clone your gene of interest into one of the pENTR™ Dual Selection Vectors to generate an entry clone.
2. Generate an expression clone by performing a recombination reaction between the entry clone and a Gateway® destination vector of choice.
3. Introduce your expression clone into the appropriate host (*e.g.* bacterial, mammalian, yeast, insect) and express your recombinant protein.

For more information about the Gateway® Technology, refer to the Gateway® Technology with Clonase™ II manual. You may download the manual from www.invitrogen.com or contact Technical Support (page 24).

Using the pENTR™ Dual Selection Vectors

Introduction

This section provides general guidelines for using the pENTR™ Dual Selection Vectors. Diagrams are provided on pages 7-11 to help you ligate your gene of interest into the appropriate pENTR™ Dual Selection Vector.

Propagating the pENTR™ Dual Selection Vectors

If you wish to propagate and maintain the pENTR™ Dual Selection Vectors, we recommend using One Shot® *ccd*B Survival™ 2 T1^R Chemically Competent Cells (Catalog no. A10460) from Invitrogen for transformation. 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.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook et al., 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Important

Your gene of interest must replace the dual selection cassette located between the two *att*L sites. Before cloning your gene of interest into a pENTR™ Dual Selection Vector, we recommend that you:

- Digest the pENTR™ Dual Selection Vector on each side of the dual selection cassette
- Dephosphorylate and gel purify the pENTR™ Dual Selection Vector

This will minimize the competition between the *ccd*B fragment and your gene of interest during the ligation process.

For more guidelines to help you develop your cloning strategy, see **Cloning Considerations** on page 5.

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Using the pENTR™ Dual Selection Vectors, Continued

Kozak Sequence for Mammalian Expression

If you will be recombining your entry clone with a destination vector for mammalian expression, your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

(G/A)NNATG**G**

Note: Cloning a blunt-ended fragment containing a 5′ ATGG (where ATG is the initiation codon) into the *Xmn* I site of any of the pENTR™ Dual Selection Vectors will constitute a Kozak consensus sequence (see diagrams on pages 7-11).

Ribosome Binding Site for Prokaryotic Expression

If you will be recombining your entry clone with a destination vector for prokaryotic expression, your insert should contain an *E. coli* ribosome binding site [AAGGA(A/G)] approximately 9–10 base pairs upstream of the ATG initiation codon (Gold, 1988; Miller, 1992). This will ensure the optimal spacing for proper translation.

Note: Ribosome binding sites are provided in pENTR™1A, pENTR™3C, and pENTR™11 (see diagrams on pages 7-11). If your insert will not be properly spaced from the vector-encoded ribosome binding site, you will need to include your own ribosome binding site for proper initiation of translation.

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Using the pENTR™ Dual Selection Vectors, Continued

Cloning Considerations

Consider the following factors when cloning into the pENTR™ Dual Selection vectors.

If you wish to....	Then your insert...
express your native protein without an N-terminal or C-terminal tag	• should contain a Kozak consensus sequence for mammalian expression or an *E. coli* ribosome binding site for prokaryotic expression (see previous page for more information) • should contain a stop codon if one is not provided in the destination vector
include an N-terminal tag (following recombination of the entry clone with a Gateway® destination vector)	• does not need a Kozak consensus sequence, *E. coli* ribosome binding site, or an ATG initiation codon (these will be provided by the appropriate destination vector) • should be in frame with the tag after recombination (see diagrams on pages 7-11) • should contain a stop codon if one is not provided in the destination vector
include a C-terminal tag (following recombination of the entry clone with a Gateway® destination vector)	• should contain a Kozak consensus sequence for mammalian expression or an *E. coli* ribosome binding site for prokaryotic expression (see previous page for more information) • should be in frame with the tag after recombination (see diagrams on pages 7-11) • should not contain a stop codon
include an N-terminal and C-terminal tag (following recombination of the entry clone with a Gateway® destination vector)	• does not need a Kozak consensus sequence, *E. coli* ribosome binding site, or an ATG initiation codon (these will be provided by the appropriate destination vector) • should be in frame with both the N-terminal and C-terminal tags after recombination (see diagrams on pages 7-11) • should not contain a stop codon

Cloning PCR Products

Note

If you include an N-terminal tag following recombination with a destination vector, and your insert contains an ATG initiation codon, note that translation initiation may also occur at this site. This may result in a small amount of native, untagged protein being expressed along with your tagged fusion protein.

If you wish to clone a PCR product made using primers containing restriction enzyme sites, we recommend the following to ensure efficient cloning:

- Inactivate or remove the DNA polymerase (*Taq* DNA polymerase can fill in sticky ends and add bases to blunt ends of PCR products) using phenol extraction or the PureLink™ PCR Purification Kit (Catalog no. K3100-01).
- Remove small DNA fragments such as primers, primer-dimers, and excess dNTP's. Refer to the Gateway® Technology with Clonase™ II manual for a purification protocol using PEG/$MgCl_2$ precipitation.

Cloning Blunt PCR Products

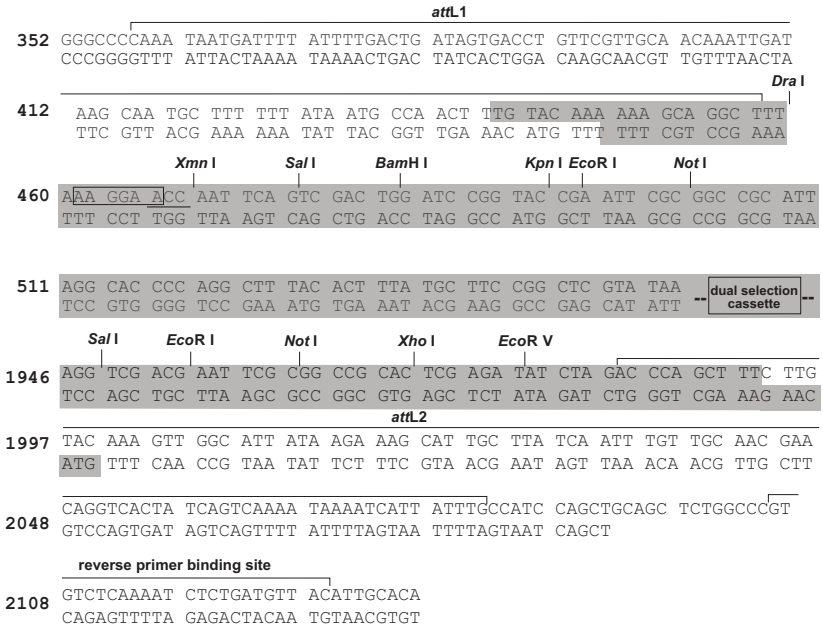
Because primers usually contain a 5′ hydroxy group, PCR products generally do not have 5′ phosphates and are not necessarily blunt. If you wish to clone a blunt PCR product into your entry vector, we recommend you perform the **Blunt Cloning of PCR Products** protocol provided in the **Appendix**, page 16.

Multiple Cloning Site for pENTR™1A Dual Selection Vector

Multiple Cloning Site

Below is the multiple cloning site for pENTR™1A Dual Selection Vector. Restriction sites are labeled to indicate the cleavage site. **Note:** Your gene of interest must replace the dual selection cassette located between the two *att*L sites. Features are indicated as follows:

- The *att*L sites are properly spaced to indicate the correct reading frame for fusion of your gene of interest to an N-terminal or C-terminal tag following recombination with a destination vector.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector following recombination.
- Boxed and underlined sequences correspond to the *E. coli* ribosome binding site [AAGGA(A/G)] and the 5′ end of the Kozak consensus sequence (ACC), respectively.

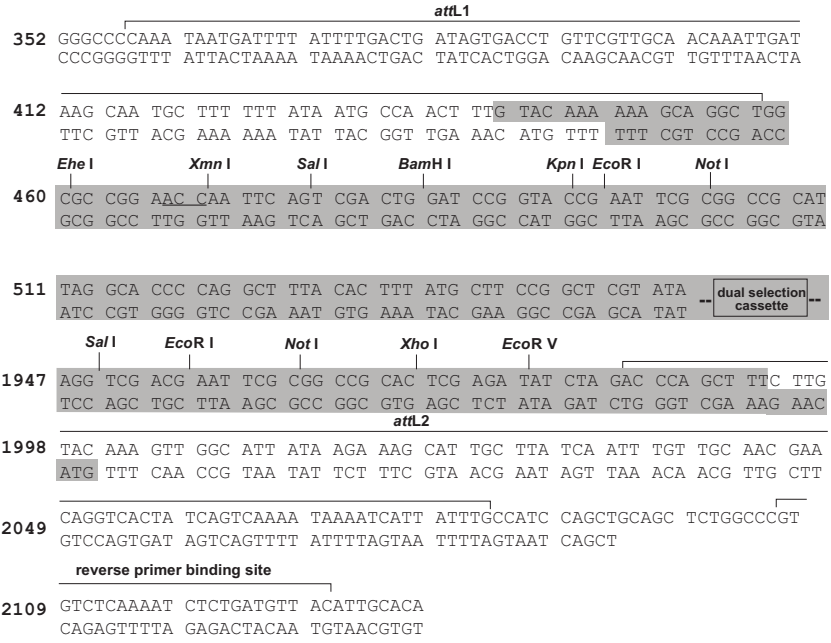


Multiple Cloning Site for pENTR™2B Dual Selection Vector

Multiple Cloning Site

Below is the multiple cloning site for pENTR™2B Dual Selection Vector. Restriction sites are labeled to indicate the cleavage site. **Note:** Your gene of interest must replace the dual selection cassette located between the two *att*L sites. Features are indicated as follows:

- The *att*L sites are properly spaced to indicate the correct reading frame for fusion of your gene of interest to an N-terminal or C-terminal tag following recombination with a destination vector.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector following recombination.
- Underlined sequence corresponds to the 5′ end of the Kozak consensus sequence (ACC).

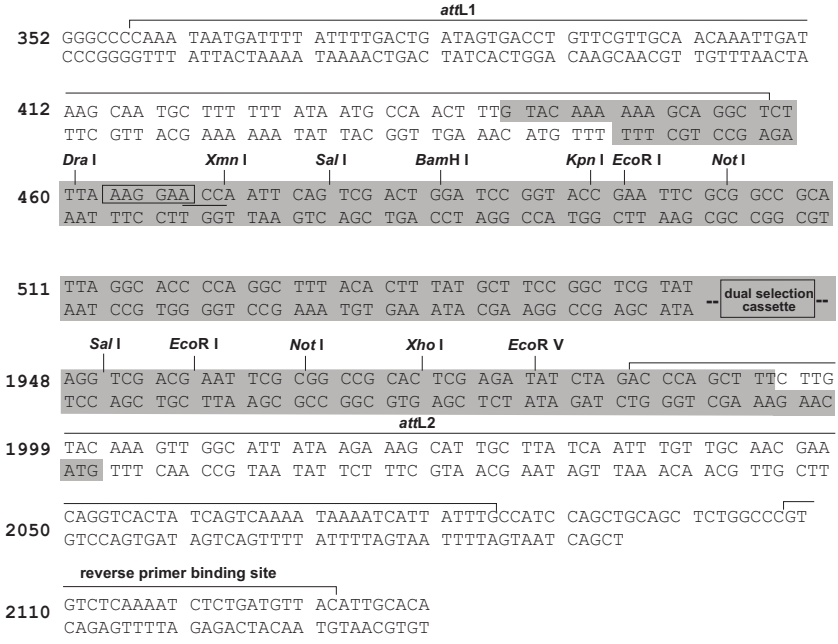


Multiple Cloning Site for pENTR™3C Dual Selection Vector

Multiple Cloning Site

Below is the multiple cloning site for pENTR™3C Dual Selection Vector. Restriction sites are labeled to indicate the cleavage site. **Note:** Your gene of interest must replace the dual selection cassette located between the two *att*L sites. Features are indicated as follows:

- The *att*L sites are properly spaced to indicate the correct reading frame for fusion of your gene of interest to an N-terminal or C-terminal tag following recombination with a destination vector.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector following recombination.
- Boxed and underlined sequences correspond to the *E. coli* ribosome binding site [AAGGA(A/G)] and the 5′ end of the Kozak consensus sequence (ACC), respectively.

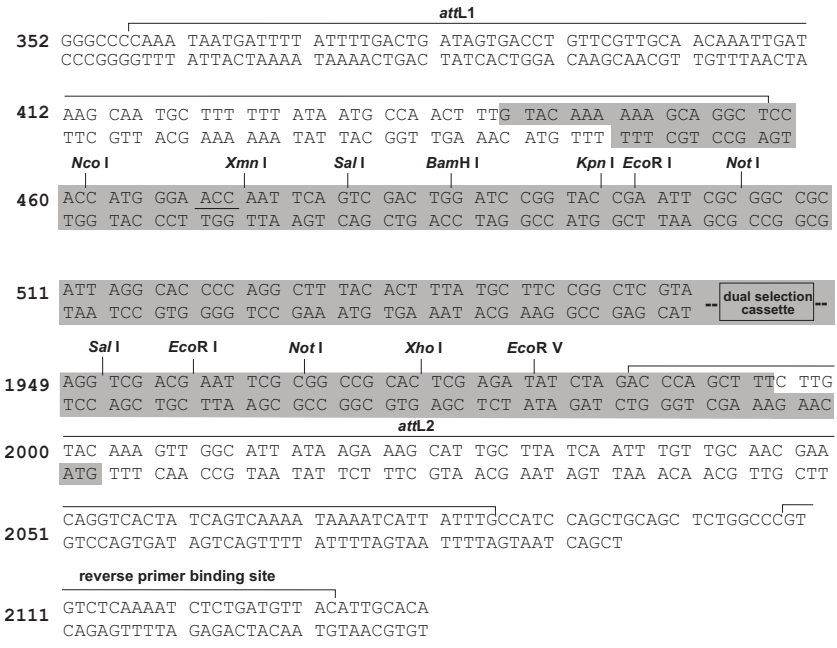


Multiple Cloning Site for pENTR™4 Dual Selection Vector

Multiple Cloning Site

Below is the multiple cloning site for pENTR™4 Dual Selection Vector. Restriction sites are labeled to indicate the cleavage site. **Note:** Your gene of interest must replace the dual selection cassette located between the two *att*L sites. Features are indicated as follows:

- The *att*L sites are properly spaced to indicate the correct reading frame for fusion of your gene of interest to an N-terminal or C-terminal tag following recombination with a destination vector.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector following recombination.
- Underlined sequence corresponds to the 5′ end of the Kozak consensus sequence (ACC).

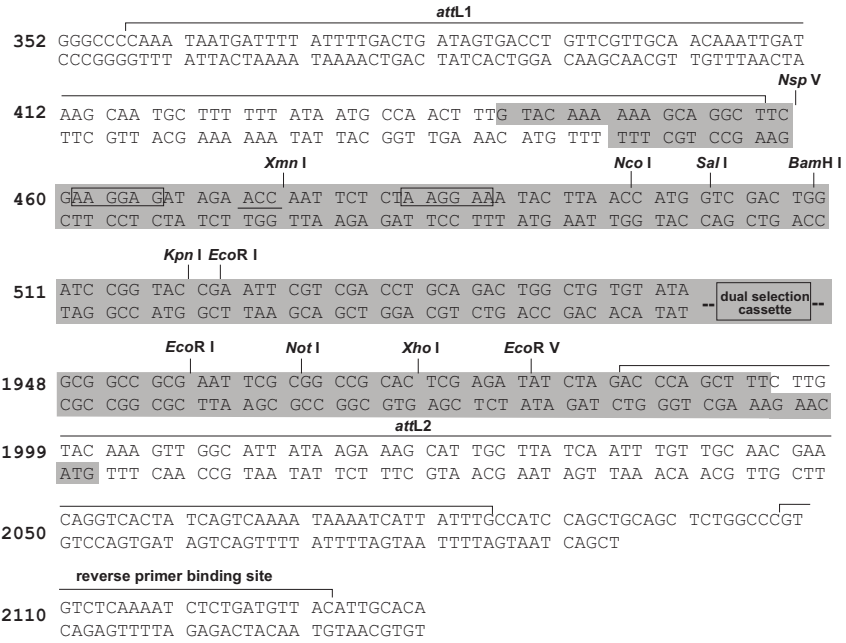


Multiple Cloning Site for pENTR™11 Dual Selection Vector

Multiple Cloning Site

Below is the multiple cloning site for pENTR™11 Dual Selection Vector. Restriction sites are labeled to indicate the cleavage site. **Note:** Your gene of interest must replace the dual selection cassette located between the two *att*L sites. Features are indicated as follows:

- The *att*L sites are properly spaced to indicate the correct reading frame for fusion of your gene of interest to an N-terminal or C-terminal tag following recombination with a destination vector.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector following recombination.
- Boxed and underlined sequences correspond to the *E. coli* ribosome binding site [AAGGA(A/G)] and the 5′ end of the Kozak consensus sequence (ACC), respectively.



Transforming and Analyzing Entry Clones

Introduction

Once you have restriction cloned your gene of interest into your entry vector, you will transform the ligation reaction into competent *E. coli* and select for positive transformants. See below for general guidelines to transform and analyze your entry clones.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook et al., 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Transformation

Transform your ligation mixture into a competent *rec*A, *end*A *E. coli* strain (*e.g.* TOP10, DH5α™) and select on LB plates containing 50 µg/ml kanamycin. For your convenience, competent TOP10 and DH5α™ *E. coli* are available from Invitrogen in a One Shot® format (see page vi for ordering information).

Analyzing Positive Clones

1. Pick 5 colonies for culture in LB or SOB medium containing 50 µg/ml kanamycin. In parallel streak the same five clones on LB plates containing 30 µg/ml chloramphenicol. Check growth after overnight culture at 37°C. True positive clones will be kanamycin-resistant (Km^R) and chloramphenicol-sensitive (Cm^S).
2. Isolate plasmid DNA from clones that are Km^R and Cm^S using your method of choice. We recommend using the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01).
3. Analyze the entry clones by restriction analysis to confirm the presence and correct orientation of the insert. Use a restriction enzyme or a combination of enzymes that cut once in the vector and once in the insert.

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Transforming and Analyzing Entry Clones, Continued

Analyzing Transformants by PCR

You may also analyze positive transformants using PCR. Use a primer that hybridizes within the pENTR™ Dual Selection Vector and one that hybridizes within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, perform restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template. The protocol below is provided for your convenience. Other protocols are suitable.

Materials Needed:

PCR SuperMix High Fidelity (Catalog no. 10790-020)

Appropriate forward and reverse PCR primers, 20 µM each

Protocol:

1. For each sample, aliquot 48 µl of PCR SuperMix High Fidelity into a 0.5 ml microcentrifuge tube. Add 1 µl each of the forward and reverse PCR primer.
2. Pick 5 colonies and resuspend them individually in 50 µl of the PCR SuperMix containing primers (make a patch plate to preserve the colonies for further analysis).
3. Incubate reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at +4°C.
6. Visualize by agarose gel electrophoresis.

Sequencing

You may sequence your entry clone using the recommended primer (see table below and the diagrams on pages 7-11) to confirm the presence and orientation of the insert. For your convenience, Invitrogen offers a custom primer synthesis service. See www.invitrogen.com/oligos or contact Technical Support (page 24) for more information.

Primer	Sequence
Reverse	5′-GTAACATCAGAGATTTTGAGACAC-3′

Guidelines to Perform the LR Recombination Reaction

Introduction

Once you have obtained an entry clone containing your gene of interest, you may perform an LR recombination reaction between the entry clone and a destination vector of choice. General guidelines are provided below.

Important

For most applications, we recommend performing the LR recombination reaction using a:

- Supercoiled *att*L-containing entry clone
- Supercoiled *att*R-containing destination vector

Note: If your destination vector or entry clone is large (>10 kb), you may linearize either vector to increase recombinational efficiency. You may also relax the destination vector using topoisomerase I to increase efficiency. For more details, refer to the Gateway® Technology with Clonase™ II manual.

Destination Vectors

A large selection of Gateway® destination vectors is available from Invitrogen to facilitate expression of your gene of interest in virtually any protein expression system. For more information about the vectors available, refer to our website (www.invitrogen.com) or contact Technical Support (page 24).

E. coli Host

Once you have performed the LR recombination reaction, you will transform the reaction mixture into competent *E. coli* and select for expression clones. You may use any *rec*A, *end*A *E. coli* strain including TOP10, DH5α™, DH10B™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.

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Guidelines to Perform the LR Recombination Reaction, Continued

Performing the LR Reaction

To perform the Gateway® LR recombination reaction, you will need:

- Purified plasmid DNA of your entry clone
- A destination vector of choice
- LR Clonase™ II enzyme mix (Catalog no. 11791-020; see below)
- 2 µg/µl proteinase K solution (supplied with the LR Clonase™ II enzyme mix)
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- Appropriate chemically competent *E. coli* host and growth media for expression
- Appropriate selective plates

For instructions to perform the LR recombination reaction, refer to the LR Clonase™ II enzyme mix manual or to the manual for the destination vector you are using.

LR Clonase™ II Enzyme Mix

To catalyze the LR recombination reaction, we recommend using Gateway® LR Clonase™ II enzyme mix. The LR Clonase™ II enzyme mix combines the proprietary enzyme formulation and 5X LR Reaction Buffer previously supplied by Invitrogen as separate components in LR Clonase™ enzyme mix (Catalog no. 11791-019) into an optimized single-tube format for easier set-up of the LR recombination reaction.

Note: You may perform the LR recombination reaction using LR Clonase™ enzyme mix, if desired. To use LR Clonase™ enzyme mix, follow the instructions included with the product. **Do not** use the protocol for LR Clonase™ II enzyme mix as reaction conditions differ.

Appendix

Blunt Cloning of PCR Products

Introduction

Use this protocol to clone blunt-end PCR products into your pENTR™ Dual Selection Vector.

Materials Needed

You should have the following materials on hand before beginning:

- PCR product (~40 ng as judged from an agarose gel)
- 3 M sodium acetate
- 100% ethanol
- 10 mM ATP
- 2 mM dNTP's
- 5X T4 forward reaction buffer (350 mM Tris-HCl, pH 7.6; 50 mM $MgCl_2$; 500 mM KCl; 5 mM 2-mercaptoethanol)
- T4 polynucleotide kinase and buffer (10 units/µl) (Catalog no. 18004-010)
- T4 DNA polymerase (5 units/µl) (Catalog no. 18005-017)
- 30% PEG 8000/30 mM $MgCl_2$
- T4 DNA ligase and buffer (1 unit/µl) (Catalog no. 15224-017)
- Entry vector (blunt, dephosphorylated, ~50 ng)

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Blunt Cloning of PCR Products, Continued

Protocol

1. In a 0.5 ml tube, precipitate approximately 40 ng of PCR product by adding 0.1 volume of 3 M sodium acetate followed by 2.5 volumes of 100% ethanol.
2. Pellet the DNA by centrifugation. Wash the pellet twice with 70% ethanol.
3. Add the following reagents to the DNA:

Distilled H_2O	4 µl
10 mM ATP	1 µl
2 mM of each dNTP (*i.e.* dATP, dCTP, dTTP, dGTP)	1 µl
5X T4 Forward Reaction Buffer	2 µl
T4 polynucleotide kinase	1 µl
T4 DNA polymerase	1 µl
Total Volume	10 µl

4. Incubate at 37°C for 10 minutes, then at 65°C for 15 minutes. Cool on ice for 5 minutes. Centrifuge briefly to bring any condensate to the bottom of the tube.
5. Add 5 µl of 30% PEG 8000/30 mM $MgCl_2$. Mix and centrifuge immediately at room temperature for 10 minutes.
6. Carefully remove and discard supernatant.
7. Dissolve the invisible pellet in a 10 µl cocktail containing:
   - 2 µl of 5X T4 DNA ligase buffer
   - 0.5 units T4 DNA ligase
   - ~ 50 ng of blunt, dephosphorylated entry vector
   - Sterile water up to 10 µl
8. Incubate at 25°C for 1 hour, then at 65°C for 10 minutes. Add 40 µl TE.
9. Transform competent *E. coli* using your method of choice.

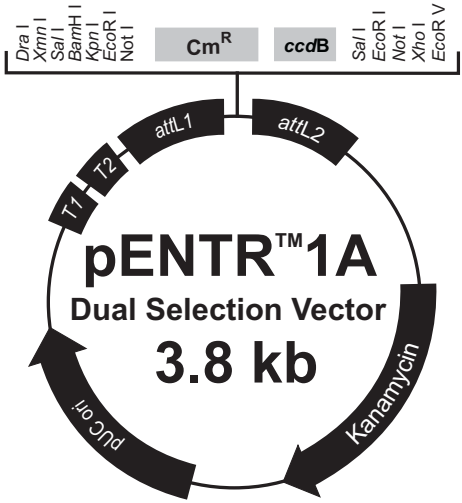
Transformation

Refer to the Gateway® Technology with Clonase™ II manual for instructions to transform the appropriate competent *E. coli* host. Make sure to digest isolated DNA from positive clones with the appropriate restriction enzymes to determine the orientation of the PCR fragment.

Maps and Features of the pENTR™ Dual Selection Vectors

Maps of the pENTR™ Dual Selection Vectors

The following maps show the features of the pENTR™ Dual Selection Vectors. Maps and a complete sequence for each pENTR™ Dual Selection Vector are available for downloading from our website (www.invitrogen.com) or by contacting Technical Support (page 24).



Comments for pENTR™1A
3754 nucleotides

*rrn*B T1 transcription termination sequence: bases 106-149
*rrn*B T2 transcription termination sequence: bases 281-308
*att*L1: bases 358-457 (complementary strand)
Chloramphenicol resistance gene (Cm^R): bases 608-1266
*ccd*B gene: bases 1608-1913
*att*L2: bases 1983-2082
Kanamycin resistance gene: bases 2205-3014
pUC origin: bases 3078-3751

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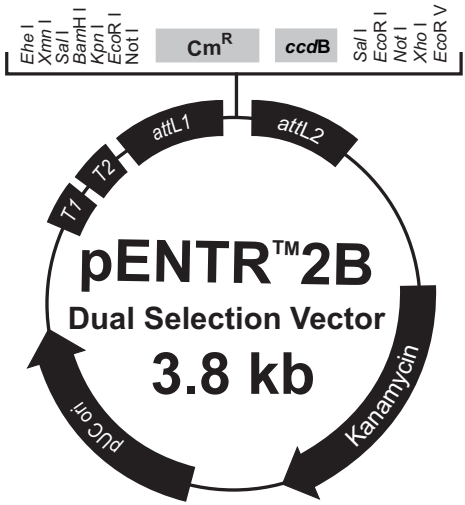

Comments for pENTR™2B
3755 nucleotides

*rrn*B T1 transcription termination sequence: bases 106-149
*rrn*B T2 transcription termination sequence: bases 281-308
*att*L1: bases 358-457 (complementary strand)
Chloramphenicol resistance gene (Cm^R): bases 609-1267
*ccd*B gene: bases 1609-1914
*att*L2: bases 1984-2083
Kanamycin resistance gene: bases 2206-3015
pUC origin: bases 3079-3752

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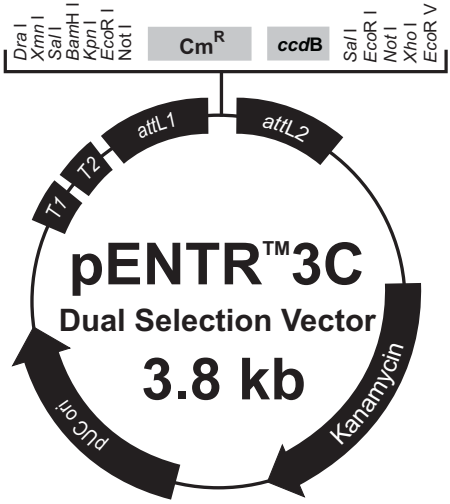

Comments for pENTR™3C
3756 nucleotides

*rrn*B T1 transcription termination sequence: bases 106-149
*rrn*B T2 transcription termination sequence: bases 281-308
*att*L1: bases 358-457 (complementary strand)
Chloramphenicol resistance gene (Cm^R): bases 610-1268
*ccd*B gene: bases 1610-1915
*att*L2: bases 1985-2084
Kanamycin resistance gene: bases 2207-3016
pUC origin: bases 3080-3753

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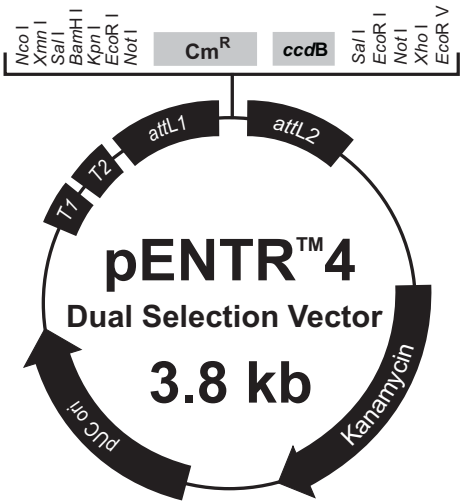

Comments for pENTR™4
3757 nucleotides

*rrn*B T1 transcription termination sequence: bases 106-149
*rrn*B T2 transcription termination sequence: bases 281-308
*att*L1: bases 358-457 (complementary strand)
Chloramphenicol resistance gene (Cm^R): bases 611-1269
*ccd*B gene: bases 1611-1916
*att*L2: bases 1986-2085
Kanamycin resistance gene: bases 2208-3017
pUC origin: bases 3081-3754

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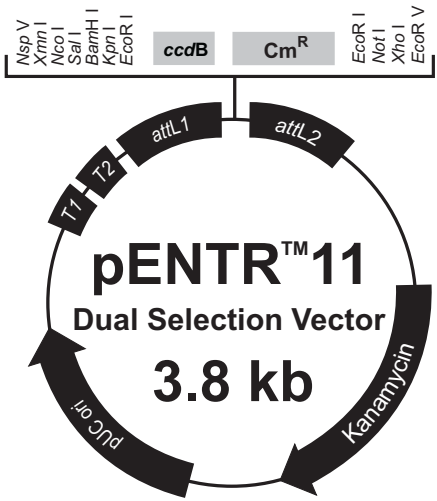

Comments for pENTR™11
3781 nucleotides

*rrn*B T1 transcription termination sequence: bases 106-149
*rrn*B T2 transcription termination sequence: bases 281-308
*att*L1: bases 358-457 (complementary strand)
*ccd*B gene: bases 570-872 (complementary strand)
Chloramphenicol resistance gene (Cm^R): bases 1217-1872 (complementary strand)
*att*L2: bases 2010-2109
Kanamycin resistance gene: bases 2232-3041
pUC origin: bases 3105-3778

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Maps and Features of the pENTR™ Dual Selection Vectors, Continued

Features of the pENTR™ Dual Selection Vectors

pENTR™1A Dual Selection Vector (3754 bp), pENTR™2B Dual Selection Vector (3755 bp), pENTR™3C Dual Selection Vector (3756 bp), pENTR™4 Dual Selection Vector (3757 bp), and pENTR™11 Dual Selection Vector (3781 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
*rrn*B T1 and T2 transcription termination sequences	Protects the cloned gene from expression by vector-encoded promoters, thereby reducing possible toxicity (Orosz *et al.*, 1991)
*att*L1 and *att*L2 sites	Allows site-specific recombination of the entry clone with a Gateway® destination vector (Landy, 1989)
Chloramphenicol resistance gene (Cm^R)	Allows counterselection of expression clones
*ccd*B gene	Allows negative selection of expression clones
Kanamycin resistance gene	Allows selection of the plasmid in *E. coli*
pUC origin	Allows maintenance of the plasmid in *E. coli*

Technical Support

World Wide Web

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
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Technical Support, Continued

MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

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Gateway® Clone Distribution Policy

For additional information about Invitrogen's policy for the use and distribution of Gateway® clones, see the section entitled **Gateway® Clone Distribution Policy**, page 28.

Gateway® Clone Distribution Policy

Introduction

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

Gateway® Entry Clones

Invitrogen 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. Invitrogen agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Invitrogen.

Gateway® Expression Clones

Invitrogen 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. Invitrogen agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Invitrogen. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee ($10 per clone) payable to Invitrogen.

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 Invitrogen 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 Invitrogen's 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 Invitrogen's licensing department at 760-603-7200.

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