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Choosing Products to Build GATEWAY™ Expression Clones

Step 1: Construct or Select an Entry Clone starting from:

- **PCR Fragment**
  - Design primers with attB sites
  - Amplify PCR product
  - Clone attB-PCR product into pDONR™201 with BP CLONASE™ Enzyme Mix
  - PCR Cloning System (BP CLONASE Mix included)
  - Cat. No. 11821-014
  - Analyze / Propagate Entry Clone

- **Restriction Fragment**
  - Choose Entry Vector
  - Individual Entry Vectors Cat. Nos. 11813-011; 11816-014; 11817-012; 11818-010; 11819-018
  - Ligation of restriction fragment into Entry Vector
  - Analyze / Propagate Entry Clone

- **Library Screening**
  - Isolate clone from pCMV+SPORT 6; pSPORT-P; or pEXP-AD502 library
  - See Catalog or web site for list of GATEWAY Libraries and Custom Libraries
  - Transfer insert with BP CLONASE Mix into pDONR201
  - BP CLONASE Cat. No. 11789-013
  - pDONR201 Cat. No. 11798-014
  - Analyze / Propagate Entry Clone

Step 2: Construct an Expression Clone

A. **E. coli Expression System with BL21-SI™ Cells**
   - Native: pDEST™14
   - N-GST: pDEST15
   - N-His: pDEST17
   - E. Coli Expression System
     - Cat. No. 11823-010 (LR CLONASE Mix included)
   - Analyze / Propagate Entry Clone
   - **OR**
   - Mammalian Expression System
     - Native: pDEST12.2
     - N-GST: pDEST27
     - N-His: pDEST26
   - Mammalian Expression System
     - Cat. No. 11826-013 (LR CLONASE Mix included)
   - Analyze / Propagate Entry Clone
   - **OR**
   - Baculovirus Expression System*
     - Native: pDEST8
     - N-GST: pDEST20
     - N-His: pDEST10
   - Baculovirus Expression System*
     - Cat. No. 11827-011 (LR CLONASE Mix included)
   - Analyze / Propagate Entry Clone

B. **Use Your Own Vector**
   - Convert existing vector by cloning Conversion Cassette into MCS

Choose a complete Expression System(s) OR purchase Destination Vector(s) individually

- **Destination Vector**
  - OR
  - Use Your Own Vector

- **E. coli**
  - OR
  - Use Your Own Vector

- **Mammalian**
  - OR
  - Use Your Own Vector

- **Baculovirus**
  - OR
  - Use Your Own Vector

B. **Transfer gene from Entry Clone into Destination Vector with LR CLONASE Enzyme Mix to make Expression Clone**

- LR CLONASE Enzyme Mix
  - Cat. No. 11791-019
  - Analyze / Propagate Expression Clone - Express Protein

---

*Baculovirus Expression Systems provide components to construct a transfer vector. User must also purchase MAX EFFICIENCY® DH10Bac™ Competent Cells, Cat. No. 10361-012, and CELLFECTIN® Reagent, Cat. No. 10362-010, included in BAC-TO-BAC® Baculovirus Expression System.

**A second E. Coli Expression System is available with DH5α™ competent cells (Cat. No. 11822-012), suitable for construction of Expression Clone but not for protein expression with pDEST 14, 15, 17.
# Table of Contents

1. Notices to Customer .............................................................................1
   1.1 Important Information .................................................................3
   1.2 Precautions ................................................................................3
   1.3 Limited Label Licenses .................................................................3

2. Overview ........................................................................................4
   2.1 Recombination Reactions of the GATEWAY™ Cloning System ....5
      2.1.1 The GATEWAY LR Cloning Reaction .......................................5
      2.1.2 The GATEWAY BP Cloning Reaction .......................................7
   2.2 Generating Entry Clones ................................................................8
   2.3 Designing Entry Clones for Protein Expression ..........................9
      2.3.1 Location of Translation Start Sequences ...............................10
      2.3.2 Reading Frame .....................................................................12
      2.3.3 Examples of Protein Expression Constructs ..........................12
   2.4 Destination Vectors .....................................................................13
   2.5 GATEWAY Nomenclature ..........................................................15

3. Methods ..........................................................................................16
   3.1 Components ..............................................................................16
   3.2 Creating Entry Clones Using Restriction Endonucleases and Ligase 17
      3.2.1 Preparing the Entry Vector ....................................................17
      3.2.2 Preparing the Insert DNA ....................................................18
      3.2.3 Ligation of Entry Vectors and Restriction Fragments ............19
   3.3 Creating Entry Clones from attB-flanked PCR Products via the BP Reaction .........................................................20
      3.3.1 Preparation of attB-PCR Products .......................................20
      3.3.2 Purification of attB-PCR Products .......................................21
   3.4 Creating Entry Clones via the BP Reaction ...................................21
      3.4.1 Preparation of Expression Clone DNA .................................21
      3.4.2 The BP Reaction ..................................................................22
      3.4.3 Sequencing of pENTR Clones Generated by Recombination with Donor Vectors .........................................................22
   3.5 Creating Expression Clones via the LR Reaction .........................23
      3.5.1 Protein Expression from GATEWAY Expression Clones ..........24
   3.6 Converting a Vector into a GATEWAY Destination Vector ..........24
      3.6.1 Protocol for Constructing a GATEWAY Destination Vector ....25
      3.6.2 Analysis of Destination Vector .............................................28
      3.6.3 Preparing the Destination Vector for Cloning .......................29

4. Troubleshooting Guide .....................................................................30

5. Additional Information ...................................................................33
   5.1 “One-Tube” Protocol: A Protocol for Cloning attB-PCR Products Directly into Destination Vectors ..........................33
Notices to Customer

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Ni-NTA resin may be purchased from QIAGEN, Inc., 9600 De Soto Ave., Chatsworth, CA 91311. (800-426-8157).
Overview

**Overview**

**GATEWAY™ Cloning Technology** is a novel universal system for cloning and subcloning DNA sequences, facilitating gene functional analysis, and protein expression (Figure 1). Once in this versatile operating system, DNA segments are transferred between vectors using site-specific recombination. This powerful system can easily transfer one or more DNA sequences into multiple vectors in parallel reactions, while maintaining orientation and reading frame.

Figure 1. The power of GATEWAY Cloning Technology. The gene of interest can be moved into an Entry Vector via PCR, restriction endonuclease digestion and ligation, or site-specific recombination from a cDNA library constructed in a GATEWAY-compatible vector. A gene in the Entry Clone can then be transferred simultaneously into Destination Vectors. This is done by combining the Entry Clone with a GATEWAY Destination Vector and CLONASE Enzyme Mix in a single tube, incubating for 1 h, transforming *E. coli*, and plating.

The GATEWAY Cloning System uses phage lambda-based site-specific recombination instead of restriction endonucleases and ligase. This recombination system is used by λ during the switch between the lytic and lysogenic pathways (1). The key DNA recombination sequences (att sites) and proteins that mediate the recombination reactions are the foundation of GATEWAY Cloning Technology. For a general review of λ recombination, see reference 2.
2.1 Recombination Reactions of the GATEWAY Cloning System

Two reactions constitute the GATEWAY Cloning Technology (Figure 2, Table 1). The LR Reaction is a recombination reaction between an Entry Clone and a Destination (pDEST™) Vector, mediated by a cocktail of recombination proteins, to create an Expression Clone. It is used to move the sequence of interest to one or more Destination Vectors in parallel reactions. The BP Reaction is a recombination reaction between an Expression Clone (or an attB-flanked PCR product) and a Donor (pDONR™) Vector to create an Entry Clone.

Table 1. Summary of reactions and nomenclature.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reacting Sites</th>
<th>Catalyzed by</th>
<th>Product</th>
<th>Structure of Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR Reaction</td>
<td>attL x attR</td>
<td>LR CLONASE™ Enzyme Mix</td>
<td>Expression Clone</td>
<td>attB1-gene-attB2</td>
</tr>
<tr>
<td>BP Reaction</td>
<td>attB x attP</td>
<td>BP CLONASE™ Enzyme Mix</td>
<td>Entry Clone</td>
<td>attL1-gene-attL2</td>
</tr>
</tbody>
</table>

The recombination reactions are equivalent to concerted, highly specific, cutting and ligation reactions. The reactions are conservative, i.e., there is no net synthesis or loss of nucleotides. The DNA segments that flank the recombination sites are merely switched. The recombination (att) sites of each vector comprise a hybrid sequence, donated by the sites on the parental vectors. The recombination can occur between DNAs of any topology (supercoiled, linear, or relaxed), although efficiency varies.

2.1.1 The GATEWAY LR Cloning Reaction

The LR reaction is used to create an Expression Clone (Figure 3). The recombination proteins cut to the left and right of the gene within the attL sites in the Entry Clone and ligate it to the corresponding attR site in the Destination Vector, creating an Expression Clone. The resultant 25-bp attB sites [attB1 on the left (N-
Overview

terminus) and attB2 on the right (C-terminus) created by the LR reaction are
derived from the attL sites (adjacent to the gene), whereas the distal sequences are
derived from the attR sites. The LR CLONASE Enzyme Mix mediates the GATEWAY LR
Reaction and contains λ recombination proteins Int, Xis, and the E. coli-encoded
protein IHF.

The wild type λ attL and attR recombination sites have been modified in the
following manner to improve the GATEWAY Reactions.

- Mutations have been made to the core regions of the att sites to eliminate stop
codonts and to ensure specificity of the recombination reactions to maintain
orientation and reading frame (i.e., attL1 reacts only with attR1, attL2 reacts only
with attR2). The attL sites are 100 bp.

- A part (43 bp) of attR has been removed to make the in vitro attL × attR reaction
irreversible and more efficient (3). The attR sites in the Destination Vectors are
125 bp.

![Diagram of the LR reaction]

**Figure 3. The LR reaction.** attL1 and attR1 (or attL2 and attR2) recombine to form a co-
integrate. The co-integrate resolves to form two daughter molecules by a second
recombination reaction. The two daughter molecules have the same structure regardless of
which pair of sites, attL1 and attR1 or attL2 and attR2, react first to form the co-integrate.
Selection of the Expression Clone is achieved by introduction of the mixture into E. coli by
transformation. Only plasmids without the ccdB gene that are ampicillin-resistant (Ap') yield
colonies.
2.1.2 The Gateway BP Cloning Reaction

The BP reaction is used to create an Entry Clone from Expression Clones (Figure 4) or PCR products (Figure 6). Once a gene is flanked by attL sites (Entry Clone), it can be transferred into any number of Destination Vectors to generate new Expression Clones. The BP CLONASE Enzyme Mix mediates the BP Reaction and contains λ recombination protein Int and the E. coli-encoded protein IHF.

The wild type λ attB and attP recombination sites have been modified to improve the Gateway Reactions.

- Mutations have been made to the core regions of the att sites to eliminate stop codons and to ensure specificity of the recombination reactions to maintain orientation and reading frame (i.e., attB1 reacts only with attP1, attB2 reacts only with attP2). The attP sites are 200 bp.
- Mutations have been introduced into the short (5 bp) regions flanking the 15-bp core regions of the attB sites to minimize secondary structure formation in single-stranded forms of attB plasmids, e.g., in phagemid ssDNA or in mRNA. The attB sites are 25 bp.

The BP Reaction permits rapid, directional cloning of PCR products synthesized with primers containing terminal 25-bp attB sites (+4 Gs). The result is an Entry Clone containing the PCR fragment (Figure 6). Similarly, DNA segments flanked by attB sites in Expression Clones can be transferred to generate Entry Clones (Figure 4).

![Diagram of the BP cloning reaction](image.png)

**Figure 4. The BP cloning reaction.** Only plasmids without the ccdB gene that are kanamycin resistant (Km') yield colonies.
2.2 Generating Entry Clones

The design of an Entry Clone is dictated by the particular DNA and what is to be done with it. Because the DNA sequence between the attL sites transfers as a unit, all the sequences included between these sites transfer into the Destination Vectors. A variety of Destination Vectors (permitting native or fusion protein expression) can be used, making the choice of whether to include translation start and stop signals an important decision in the planning of Entry Clones. For example, expression of native proteins requires that translation initiation signals (ribosome recognition site and ATG) be included between the attL1 sites, whereas Entry Clones used to make N-terminal fusion proteins typically lack these elements since they are donated by the Destination Vector. Note that Entry Clones used to transfer DNA into Destination Vectors for expression require that the encoded N-terminus be oriented proximal to the attL1 site. For a more thorough discussion see Section 1.3.

Entry Clones can be made in one of several ways (Figure 5).

- A PCR product made with modified primers can be used to generate and Entry Clone using the BP reaction (Figure 6). Primers consist of the structure GGGG[25 bp attB][gene-specific sequence] (see Section 2.3.3).
- An Expression Clone (generated by the LR reaction) can be converted to an Entry Clone using the BP reaction (Figure 4). In addition, clones from cDNA libraries made in vectors in which attB sites flank the cDNA (such as pCMV•SPORT6 or pEXP-AD502) can be transferred to generate an Entry Clone.

Considerations in Designing an Entry Clone

The DNA:
- Does it contain a gene?
- Is the sequence known?
- Is the reading frame known?
- Are there 5' and 3' untranslated regions?
- Do these regions contain stop codons?
- Does the gene fragment carry its own promoter and/or translation signals?
- Is the DNA a restriction fragment, or a PCR product?
- Are there unique restriction endonuclease sites at the amino and carboxy ends?

How is the gene to be expressed?
- In eukaryotes or in E. coli?
- As native protein, or as a fusion protein?
- With or without a protease cleavage site?

Figure 5. Ways to make Entry Clones. Approaches 3 and 4 utilize recombination with a Donor Vector that provides the Entry Vector backbone carrying Km'.
A gene can be cloned between the attL1 and attL2 sites of an Entry Vector using restriction endonucleases and ligase. The starting DNA segment can be generated by restriction digestion or as a PCR product containing restriction sites on the ends. Several Entry Vectors are available (Figure 7, Table 2). These differ as to the translation signals and multiple cloning sites (MCS) available. Detailed vector maps can be found in Section 5.6. Note: Entry Clones made in pENTR1A, 2B, 3C, 4 or 11 are transcriptionally silent and cannot be screened with antibodies.

2.3 Designing Entry Clones for Protein Expression

Protein expression consists of transcription (DNA into RNA) and translation (RNA into protein). (For information on protein synthesis see references 4-7.) Both have signal sequences that determine the start sites. In GATEWAY Technology, the promoters typically are provided on the Destination Vectors outside of the att sites. The translational start site for nearly all proteins is the AUG (methionine) codon. Ribosomes must be able to distinguish between AUG codons in the middle of proteins from those at the start. Most often ribosomes choose an AUG that is first in the RNA (toward the 5’ end) following the proper sequence context. In E. coli, the favored context (8) is a run of purines (As and Gs) from 5 to 12 bases upstream of the initiating AUG, especially AGGAGG or some variant (known as a Shine-Dalgarno sequence). In eukaryotes, the preferred sequence context is --GCC ACC ATG G-- around the initiating methionine, with the A at -3 being most important, and a purine at +4 (where the A of the ATG is +1), preferably a guanine (G), being next most influential (9). Having an A at -3 is enough to make most ribosomes choose the first AUG of an mRNA in plants, insects, yeast, and mammals (known as a Kozak sequence). Shine-Dalgarno and Kozak sequences are referred to here as ribosome recognition sequences (RRS). For a review of initiation of protein synthesis in eukaryotic cells, see reference 10.
Overview

In GATEWAY Cloning, the placement of translation signals is determined by whether the protein being expressed is native, or a fusion protein (Figure 8). For native proteins and C-terminal fusions, the translation signals are included downstream of the attB1 site. Therefore, these signals must be present in the Entry Clone. In this case the attB1 sequence will reside in the 5’ untranslated region of the mRNA. (Note: For C-terminal fusions, the stop codon is provided by the Destination Vector and must be absent from the 3’-end of the gene.) In N-terminal or N+C-terminal fusions, the translational signals and the fusion protein sequences are provided by the Destination Vector and will be upstream of the attB1 site. Consequently, the 25-bp attB1 site becomes part of the coding sequence and inserts 8 amino acids between the fusion domain and the protein encoded by a gene. The att B1 sequence has not been observed to affect protein yield in E. coli, insect, or mammalian cells.

2.3.1 Location of Translation Start Sequences

For native protein expression, the RRS and the ATG needs to be downstream of the attL1 site in the Entry Clone. If the Destination Vector provides a promoter without any N-terminal fusion sequence, protein synthesis will initiate exclusively at the translation start signals of the native open reading frame (ORF). An Entry Clone containing the RRS and ATG downstream of the attL site can be used with a Destination Vector providing an N-terminal fusion peptide if the ATG is in frame with the att site. However, protein synthesis will result in production of both N-terminal fusion protein plus some native protein. Even though ribosomes most often initiate protein synthesis at the 5’-most ATG, internal ATGs can serve to initiate protein synthesis. The better the translation context around the internal ATG, the more internal initiation of translation will be seen. Also, the production of native protein can be more pronounced with short N-terminal fusion tags, such as the 6X histidine affinity tag. If the amount of native protein is large or interferes with your applications, construction of different Entry Clones to express native protein may be necessary.

Table 2. Entry Vectors. All Entry Vectors carry the kanamycin resistance gene.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Cloning Features</th>
<th>Shine-Dalgarno</th>
<th>Kozak</th>
<th>Expression Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>pENTR1A</td>
<td>Represent the 3 reading frames for N-terminal fusions.</td>
<td></td>
<td></td>
<td>N-terminal or C-terminal fusions in E. coli or eukaryotic cells.</td>
</tr>
<tr>
<td></td>
<td>pENTR2B</td>
<td></td>
<td></td>
<td>Native expression and C-terminal fusions require addition of ribosome recognition sequence and ATG translation initiation codon.</td>
</tr>
<tr>
<td></td>
<td>pENTR3C</td>
<td></td>
<td></td>
<td>C-terminal fusions require that no stop codons precede attL2.</td>
</tr>
<tr>
<td>pENTR4</td>
<td>Same as pENTR1A, except that the first restriction endonuclease site after attL1 is Nco l.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pENTR11</td>
<td>E. coli and eukaryotic ribosome binding sites (Shine-Dalgarno and Kozak) downstream of attL1. Blunt (Xmn I) and Nco I sites each preceded by Shine-Dalgarno and Kozak.</td>
<td>•</td>
<td>•</td>
<td>Native, N-terminal or C-terminal fusions in E. coli or eukaryotic cells. (ATG also needed for native and C-terminal.) C-terminal fusions require that no stop codons precede attL2.</td>
</tr>
</tbody>
</table>
Variable Region N Options:
- Blunt sites \([Dra\ I, \ Xmn\ I; \text{pENTR1A,3C}) (Ehe\ I, \ Xmn\ I; \text{pENTR2B})]\) close to \(attL1\) site, in the 3 reading frames
- \(Nco\ I\) site close to \(attL1\) site (pENTR4)
- Blunt \(Xmn\ I\) and \(Nco\ I\) sites each preceded by \(E.\ coli\) and eukaryotic ribosome recognition site (pENTR11)

Figure 7. Schematic of Entry Vectors. The \(mB\) transcriptional terminator sequence (11) makes clones transcriptionally silent in contrast to standard \(lac\) promoter systems. The \(ccdB\) gene inhibits growth in most \(E.\ coli\) strains which facilitates recovery of only the desired clones. The \(Xmn\ I\) site has 4 of the 6 most favored bases for the Kozak sequence involved in eukaryotic expression. The restriction sites shown on the figure are in all Entry Vectors. Unique enzymes in the variable Region N are shown below the circle map.

Figure 8. GATEWAY Protein Expression Clones. RRS refers to a ribosome recognition sequence.
Overview

2.3.2 Reading Frame

For native expression, reading frame is determined by the translation start site located between the two att sites so the reading frame in the Entry Clone relative to that of attB1 or attB2 is not typically an issue (see example below). For fusion proteins, it is essential to establish the correct reading frame. For N-terminal fusions, construct the Entry Clone so that the DNA sequence is in frame with the Tyr-Lys (TAC-AAA) in attL2. (See below and the Entry Vector maps in Section 5) Destination Vectors that make amino-terminal fusions have been constructed with the attR1 site in this (AAA - AAA) reading frame, so amino terminal fusions will automatically be correctly phased, for N-terminal fusion tags. For C-terminal fusion Destination Vectors, align the fusion in phase with the -TAC-AAA- (Tyr-Lys) sequence so C-terminal fusions will automatically be in frame.

2.3.3 Examples of Protein Expression Constructs

The following examples of Expression Clone sequences and attB-PCR primer sequences (for preparing Entry Clones) have been successfully to express both native and fusion proteins in E. coli, insect, and mammalian cells using GATEWAY Cloning. Other sequence options and motif combinations are possible, and may be preferable in some situations. These examples are a starting point for recombinant protein expression in the GATEWAY Cloning System.

Native Expression

A. Expression clone structure:

```
<table>
<thead>
<tr>
<th>RRS</th>
<th>ATG</th>
<th>Open Reading Frame</th>
<th>Stop</th>
</tr>
</thead>
</table>
```

From Destination Vector From Entry Clone From Destination Vector

B. Expression clone sequence (for E. coli and eukaryotic expression):

```
5' - ACA AGT TTG TAC AAA AAA GCA GGC TTC GAA GGA GAT AGA ACC ATG* NNN NNN NNN ---
3' - TGT TCA AAC ATG TTT TTT CGT CCG AAG GTT CCT CTA TCT TGG TAC
```

Note: The ATG in this example is in frame with the attB1 sequence so this construction can be used in both native and N-terminal fusion Destination Vectors.

C. Oligonucleotides for attB-PCR cloning of gene for native expression:

- **attB1 forward oligo**: (attB1 sequence bold; translation start codon underlined; sequence includes Shine-Dalgarno and Kozak)
  ```
  attB1
  GGGG ACAAGTTTGTACAAAAAAGCAGGCT TCGAAGGAGATAGAACCATG (18-25 gene-specific nucleotides in frame with start codon) - 3'
  ```

- **attB2 reverse oligo**: (attB2 sequence bold; translation stop codon [complement strand] underlined)
  ```
  attB2
  GGGG ACCACCTTTGATACAAAAAGCGAGCT TCGAAGGAGATAGAACCATG (18-25 gene-specific nucleotides [complement strand] in frame with stop codon) - 3'
  ```

Note: See section 5.2 for adapter-primer method primer design.
2.4 Destination Vectors

Once a gene is configured as an Entry Clone, it can easily be moved into any Destination Vector using the LR Reaction. The currently available Destination Vectors concentrate on protein expression applications (Table 3). However, it is possible to convert any vector (for maximal compatibility, the Destination Vector should not be kanamycin-resistant) into a GATEWAY Destination Vector using the GATEWAY Vector Conversion System. To convert a vector, a DNA cassette (Figure 10) containing the ccdB gene and a chloramphenicol resistance gene flanked by attR sites is cloned into your vector (at the multiple cloning site) using restriction endonucleases that generate blunt ends and ligase. The ccdB protein interferes with E. coli DNA gyrase and thereby inhibits growth of most E. coli strains. Since the Destination Vector contains the ccdB gene, it must be propagated in the E. coli DB3.1 strain (parent strain RR1) containing a gyrase mutation (gyrA462) (12-14) Strains of E. coli that contain an F’ episome also carry the ccdA gene which is

Fusion Protein Expression

A. Expression clone structure:

<table>
<thead>
<tr>
<th>(promoter)</th>
<th>RRS</th>
<th>ATG</th>
<th>Open Reading Frame</th>
<th>Stop</th>
</tr>
</thead>
<tbody>
<tr>
<td>From Destination Vector</td>
<td>N-tag</td>
<td>attB1</td>
<td>From Entry Clone</td>
<td>attB2</td>
</tr>
</tbody>
</table>

Note: Here the Destination Vector provides the ATG and Stop codon.

B. Expression clone sequence:

```
5' - ATG NNN --- --- NNN ACA AGT TTG TAC AAA AAA GCA GGC TTC NNN NNN NNN ---
3' - TAC NNN --- --- NNN TGT TCA AAC ATG TTT TTT CGT CCG AAG NNN NNN NNN ---
```

Open reading frame (amino end)

```
--- NNN NNN NNN GAC CCA GCT TTC TTG TAC AAA GTG TGN NNN --- --- NNN (Stop) - 3'
--- NNN NNN NNN CTG GGT CGA AAG AAC ATG TTT TTT CAC CA N NNN --- --- NNN NNN - 5'
```

Open reading frame (carboxy end)

attB1

attB2

C. Suggested oligonucleotides for attB-PCR cloning of gene for N-terminal and C-terminal fusion expression:

**attB1 forward oligo:** (attB1 sequence bold)

```
5' - GGGG ACAAGTTTGACAAAAAGCAGGCT TC+ (18-25 gene-specific nucleotides in frame with attB1) - 3'
```

**attB2 reverse oligo:** (attB2 sequence bold)

```
5' - GGGG ACCACTTTGTACAAAGAAAGCTGGGT C+ (18-25 gene-specific nucleotides [complement strand] in frame with attB2) - 3'
```

+ Other nucleotides may be substituted for the underlined sequences. For attB1, maintain the reading frame and do not create a stop codon. For N-terminal fusion proteins, the attB2 primer must contain a stop codon in the gene-specific region. For C-terminal or N-terminal plus C-terminal fusion proteins, the attB2 primer must not contain any in-frame stop codons.

2.4 Destination Vectors

Once a gene is configured as an Entry Clone, it can easily be moved into any Destination Vector using the LR Reaction. The currently available Destination Vectors concentrate on protein expression applications (Table 3). However, it is possible to convert any vector (for maximal compatibility, the Destination Vector should not be kanamycin-resistant) into a GATEWAY Destination Vector using the GATEWAY Vector Conversion System. To convert a vector, a DNA cassette (Figure 10) containing the ccdB gene and a chloramphenicol resistance gene flanked by attR sites is cloned into your vector (at the multiple cloning site) using restriction endonucleases that generate blunt ends and ligase. The ccdB protein interferes with E. coli DNA gyrase and thereby inhibits growth of most E. coli strains. Since the Destination Vector contains the ccdB gene, it must be propagated in the E. coli DB3.1 strain (parent strain RR1) containing a gyrase mutation (gyrA462) (12-14) Strains of E. coli that contain an F’ episome also carry the ccdA gene which is
an antidote to ccdB protein toxicity. Therefore, do not use strains with F episomes for selection following BP or LR Reactions.

DB3.1 strain genotype:  
\[ \text{F}^{-} \text{ gyrA462 endA1 D(sr1-recA) mcrB mrr hsdS20(R_{R}, m_{R}) supE44 ara-14 galK2 lacY1 proA2 rpsL20(Sm^{r}) xyl-5 \omega: leu mtl-1.} \]

### Table 3. Destination Vectors

See vector maps (in section 5).

<table>
<thead>
<tr>
<th>Vector</th>
<th>Expression Host</th>
<th>Protein Expressed</th>
<th>History Tag</th>
<th>GST Affinity Tag</th>
<th>TEV Protease Cleavage Site</th>
<th>Promoter for Expression</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDEST14</td>
<td>E. coli</td>
<td>Native</td>
<td></td>
<td>T7 (E. coli strain must express T7 RNA polymerase)</td>
<td></td>
<td>pBR ori to aid in regulation of expression</td>
<td></td>
</tr>
<tr>
<td>pDEST15</td>
<td>E. coli</td>
<td>N-terminal fusion</td>
<td>•</td>
<td>T7 (E. coli strain must express T7 RNA polymerase)</td>
<td></td>
<td>pBR ori to aid in regulation of expression</td>
<td></td>
</tr>
<tr>
<td>pDEST17</td>
<td>E. coli</td>
<td>N-terminal fusion</td>
<td>•</td>
<td>T7 (E. coli strain must express T7 RNA polymerase)</td>
<td></td>
<td>pBR ori to aid in regulation of expression</td>
<td></td>
</tr>
<tr>
<td>pDEST8</td>
<td>Insect</td>
<td>Native</td>
<td>polyhedrin (baculovirus)</td>
<td>•</td>
<td>•</td>
<td>polyhedrin (baculovirus)</td>
<td></td>
</tr>
<tr>
<td>pDEST10</td>
<td>Insect</td>
<td>N-terminal fusion</td>
<td>•</td>
<td>polyhedrin (baculovirus)</td>
<td>•</td>
<td>polyhedrin (baculovirus)</td>
<td></td>
</tr>
<tr>
<td>pDEST20</td>
<td>Insect</td>
<td>Native fusion</td>
<td>•</td>
<td>polyhedrin (baculovirus)</td>
<td>•</td>
<td>polyhedrin (baculovirus)</td>
<td></td>
</tr>
<tr>
<td>pDEST12.2</td>
<td>Mammalian</td>
<td>Native</td>
<td>CMV</td>
<td>neo resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pDEST26</td>
<td>Mammalian</td>
<td>N-terminal fusion</td>
<td>CMV</td>
<td>neo resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pDEST27</td>
<td>Mammalian</td>
<td>N-terminal fusion</td>
<td>CMV</td>
<td>neo resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

More Destination Vectors will be available soon. Refer to the GATEWAY website for the most up-to-date listing (www.lifetech.com/gateway).
2.5 GATEWAY Nomenclature

For subclones, the following naming convention has been adopted: the name of the vector is placed first, followed by the name of the transferred gene.

<table>
<thead>
<tr>
<th>Plasmid Type</th>
<th>Descriptive Name</th>
<th>Individual Vector or Clone Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>attL Vector</td>
<td>Entry Vector</td>
<td>pENTR1,2,...</td>
</tr>
<tr>
<td>attL subclone</td>
<td>Entry Clone</td>
<td>pENTR3-gus, .. ; pENTR201-gus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The number 3 refers to the Entry Vector.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>201 refers to the Donor Vector used to make the Entry Clone.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gus is the subcloned gene.</td>
</tr>
<tr>
<td>attR Vector</td>
<td>Destination Vector</td>
<td>pDEST1,2,3,...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>These vectors are used to prepare Expression cDNA libraries.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other nomenclature has also been used for cDNA libraries, e.g., pCMV•SPORT6.</td>
</tr>
<tr>
<td>attB subclone</td>
<td>Expression Clone</td>
<td>pEXP3-cat,...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 refers to the Destination Vector (pDEST3) used to make the expression subclone.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cat is the subcloned gene.</td>
</tr>
<tr>
<td>attP Vector</td>
<td>Donor Vector</td>
<td>pDONR™201</td>
</tr>
</tbody>
</table>

Examples:

An LR Reaction:

pENTR201-tet x pDEST10 → pEXP10-tet

Two BP Reactions:

attB-p53 PCR product x pDONR207 → pENTR207-p53
pEXP14-lacZ x pDONR201 → pENTR201-lacZ
Methods

See www.lifetech.com/gateway for current information on additions/modifications to the protocols and an increasing selection of GATEWAY™-compatible vectors and libraries.

3.1 Components

GATEWAY Cloning Technology is the basis for several systems whose components are listed below. Most of the components are also available separately (see section 7). Store the BP CLONASE™ Enzyme Mix, LR CLONASE Enzyme Mix and the competent cells at -70°C. All other components can be stored at -20°C or -70°C.

PCR Cloning System with GATEWAY Technology
(Cat. No. 11821-014; Size: 20 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP CLONASE Enzyme Mix</td>
<td>80 µl</td>
</tr>
<tr>
<td>BP CLONASE Reaction Buffer</td>
<td>100 µl</td>
</tr>
<tr>
<td>GATEWAY pDONR™201 Vector (150 ng/µl)</td>
<td>40 µl</td>
</tr>
<tr>
<td>pEXP7-tet Positive Control (50 ng/µl)</td>
<td>20 µl</td>
</tr>
<tr>
<td>proteinase K solution (2 µg/µl)</td>
<td>40 µl</td>
</tr>
<tr>
<td>30% PEG/Mg Solution</td>
<td>1 ml</td>
</tr>
<tr>
<td>LIBRARY EFFICIENCY® DH5α™ Competent Cells</td>
<td>1 ml</td>
</tr>
<tr>
<td>pUC19 DNA</td>
<td>100 µl</td>
</tr>
<tr>
<td>manual</td>
<td>one</td>
</tr>
</tbody>
</table>

E. coli Expression Systems with GATEWAY Technology (Size: 20 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR CLONASE Enzyme Mix</td>
<td>80 µl</td>
</tr>
<tr>
<td>LR CLONASE Reaction Buffer</td>
<td>100 µl</td>
</tr>
<tr>
<td>GATEWAY pDEST™14 Vector (150 ng/µl)</td>
<td>40 µl</td>
</tr>
<tr>
<td>GATEWAY pDEST15 Vector (150 ng/µl)</td>
<td>40 µl</td>
</tr>
<tr>
<td>GATEWAY pDEST17 Vector (150 ng/µl)</td>
<td>40 µl</td>
</tr>
<tr>
<td>pENTR™-gus Positive Control (50 ng/µl)</td>
<td>20 µl</td>
</tr>
<tr>
<td>proteinase K solution (2 µg/µl)</td>
<td>40 µl</td>
</tr>
<tr>
<td>LIBRARY EFFICIENCY DH5α Competent Cells*</td>
<td>1 ml</td>
</tr>
<tr>
<td>BL21-SI™ Competent Cells**</td>
<td>1 ml</td>
</tr>
<tr>
<td>pUC19 DNA</td>
<td>100 µl</td>
</tr>
<tr>
<td>manual</td>
<td>one</td>
</tr>
</tbody>
</table>

*Included with Cat. No. 11822-012. See section 3.5.1.

**Included with Cat. No. 11823-010. See section 3.5.1.
Baculovirus Expression System with Gateway Technology

(Cat. No. 11827-011; Size: 20 reactions)
(Designed for use with the BAC-TO-BAC® technology.)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR CLONASE Enzyme Mix</td>
<td>80 μl</td>
</tr>
<tr>
<td>LR CLONASE Reaction Buffer</td>
<td>100 μl</td>
</tr>
<tr>
<td>Gateway pDEST8 Vector (150 ng/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>Gateway pDEST10 Vector (150 ng/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>Gateway pDEST20 Vector (150 ng/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>Gateway pENTR-gus Positive Control (50 ng/μl)</td>
<td>20 μl</td>
</tr>
<tr>
<td>Proteinase K solution (2 μg/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>LIBRARY EFFICIENCY DH5x Competent Cells</td>
<td>1 ml</td>
</tr>
<tr>
<td>pUC19 DNA</td>
<td>100 μl</td>
</tr>
</tbody>
</table>

Refer to section 3.5.1 for additional materials required for protein expression in insect cells.

Mammalian Expression System with Gateway Technology

(Cat. No. 11826-013; Size: 20 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR CLONASE Enzyme Mix</td>
<td>80 μl</td>
</tr>
<tr>
<td>LR CLONASE Reaction Buffer</td>
<td>100 μl</td>
</tr>
<tr>
<td>Gateway pDEST12.2 Vector (150 ng/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>Gateway pDEST26 Vector (150 ng/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>Gateway pDEST27 Vector (150 ng/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>pENTR-gus Positive Control (50 ng/μl)</td>
<td>20 μl</td>
</tr>
<tr>
<td>Proteinase K solution (2 μg/μl)</td>
<td>40 μl</td>
</tr>
<tr>
<td>LIBRARY EFFICIENCY DH5x Competent Cells</td>
<td>1 ml</td>
</tr>
<tr>
<td>pUC19 DNA</td>
<td>100 μl</td>
</tr>
</tbody>
</table>

3.2 Creating Entry Clones Using Restriction Endonucleases and Ligase

Materials:
- Entry Vector
- Restriction endonucleases and buffers
- Calf intestinal alkaline phosphatase
- T4 DNA ligase and buffer
- LIBRARY EFFICIENCY DH5x competent cells
- S.O.C. Medium
- LB plates with 50 μg/ml kanamycin
- TE [10 mM Tris-HCl (pH 7.5), 1 mM EDTA]
- CONCERT™ Gel Extraction System (or equivalent)
- Prepared DNA restriction fragment(s)

3.2.1 Preparing the Entry Vector

It is necessary to restriction digest the Entry Vector on each side of the ccdB gene to remove the gene during cloning. It is recommended that the Entry Vector be dephosphorylated and gel purified after restriction digestion so that there is less competition between the ccdB fragment and the DNA of interest for the Entry Vector during ligation.
Methods

1. Digest 1 μg of the Entry Vector with the selected restriction endonucleases.
2. Ethanol precipitate the DNA by adding 0.1 volume of 3 M sodium acetate followed by 2.5 volumes of 100% ethanol.
3. Dephosphorylate the Entry Vector DNA.
   a. Determine the mass of DNA required for 1 pmol of the type of DNA 5’ end.
   b. To a 1.5-ml microcentrifuge tube, add 4 μl of calf intestinal alkaline phosphatase (CIAP) 10X Buffer [500 mM Tris-HCl (pH 8.5), 1 mM EDTA] and 1 pmol of DNA ends.
   c. Add autoclaved, distilled water to 39 μl.
   d. Dilute CIAP in dilution buffer such that 1 μl contains the amount of enzyme required for the appropriate 5’ end (i.e., 1 unit for 5’-recessed and blunt ends and 0.01 units for a 5’ overhang).
   e. For 5’-recessed and blunt-ended DNA, incubate at 50°C for 60 min. For DNA with a 5’ overhang, incubate at 37°C for 30 min.
   f. Heat-inactivate CIAP at 65°C for 15 min.
4. Purify the DNA fragment by agarose gel electrophoresis and extract the DNA from the gel with a silica-based system like CONCERT™ Gel Extraction System (15) (optional).

3.2.2 Preparing the Insert DNA
Restriction endonuclease fragments, cDNA, or PCR products can be cloned into Entry Vectors using restriction endonucleases and ligase.

A. Restriction fragments:
   Digest DNA (0.5 to 1.0 μg) with selected restriction endonucleases (16,17).
   Purify the DNA fragment by agarose gel electrophoresis and extract the DNA from the gel with a silica-based system like CONCERT™ Gel Extraction System (15) (optional).

B. PCR Products with restriction endonuclease sites in primers:
   Materials:
   • PCR product
   • phenol:chloroform:isoamyl alcohol (25:24:1)
   • Restriction endonuclease and buffer
   • 2-butanol
   • TE [10 mM Tris-HCl (pH 7.5), 1 mM EDTA]
   • 3 M sodium acetate
   • ethanol
   • 30% PEG 8000/30 mM MgCl₂

Efficient cloning of PCR products made using primers containing restriction endonuclease sites on their 5’-ends depends on 3 steps:
1. inactivation or removal of the DNA polymerase (because Taq DNA polymerase can fill in sticky ends and add bases to blunt ends of PCR products),
2. efficient restriction endonuclease digestion, and
3. removal of small DNA fragments such as primers and primer-dimers and dNTPs.

B1. Phenol Extraction of PCR Products to Remove the DNA Polymerase
   The DNA polymerase can be removed before restriction endonuclease digestion by phenol extraction (protocol below) or a silica membrane spin cartridge such as the CONCERT Rapid PCR Purification System. (18). Alternatively, TAQUENCH™ PCR Cloning Enhancer can be used to inactivate the DNA polymerase (19).
1. Add TE to the PCR to 200 µl. Add 200 µl of buffer-saturated phenol:chloroform:isoamyl alcohol (25:24:1). Vortex vigorously for 20 s. Centrifuge for 1 min at 15,000 x g at room temperature. Remove the upper aqueous phase.

2. Add an equal volume of 2-butanol. Vortex briefly. Centrifuge for 15 s at 15,000 x g at room temperature. Remove the lower aqueous phase.

3. Repeat the extraction with 2-butanol. This time the volume of the lower aqueous phase will decrease significantly. Remove the lower aqueous phase.

4. Ethanol precipitate the DNA by adding 0.1 volume of 3 M sodium acetate followed by 2.5 volumes of 100% ethanol.

5. Dissolve in 200 µl of a restriction endonuclease buffer.

B2. Restriction Digestion of PCR Products

The efficiency of restriction endonuclease digestion can be improved by adding extra bases on the 5'-end of each PCR primer (20). Depending on the enzyme, the number of nucleotides recommended varies. Also, use 5 times excess restriction endonuclease to ensure complete digestion.

1. Digest with the appropriate restriction endonuclease(s).

2. Inactivate the restriction endonucleases by heat or phenol extraction, depending on the enzyme.

3. Precipitate the DNA by adding 100 µl of 30% PEG 8000/30 mM MgCl₂ to the 200 µl reaction mix. Mix well and immediately centrifuge at 10,000 x g for 10 min at room temperature. Remove the supernatant (pellet is clear and nearly invisible).

4. Dissolve the pellet in 50 µl TE. Check quality and recovery on a gel.

3.2.3 Ligation of Entry Vectors and Restriction Fragments

1. Ligate the prepared Entry Vector and insert fragments under appropriate conditions.

   For cohesive ends, add the following to a 1.5-ml tube:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X ligase reaction buffer</td>
<td>4 µl</td>
</tr>
<tr>
<td>vector DNA</td>
<td>3 to 30 fmol</td>
</tr>
<tr>
<td>insert DNA</td>
<td>9 to 90 fmol</td>
</tr>
<tr>
<td>autoclaved distilled water</td>
<td>≤15 µl</td>
</tr>
<tr>
<td>T4 DNA ligase</td>
<td>1 unit (in 1 µl)</td>
</tr>
</tbody>
</table>

   Mix gently. Incubate at room temperature for 1 to 2 h.

2. Transform 2 µl of ligation reaction into LIBRARY EFFICIENCY DH5α Competent Cells according to the instructions on the product profile sheet.

3. Plate transformants on LB plates containing 50 µg/ml kanamycin.

4. Isolate miniprep DNA from single colonies (16). Treat the miniprep with RNase A and store in TE. Cut with the appropriate restriction endonuclease to determine the orientation of the PCR fragment. Choose clones with the attL1 site next to the amino end of the open reading frame. Notes: BsrGI I cleaves within all att sites and can be used to help characterize clones. To sequence clones in Entry Clones see section 3.4.3.
3.3 Creating Entry Clones from attB-flanked PCR Products via the BP Reaction

**Materials:**
- attB-modified GATEWAY primers
- DNA polymerase, reaction buffer, and dNTPs for PCR
- PCR Cloning System with GATEWAY Technology
- TE [10 mM Tris-HCl (pH 7.5), 1 mM EDTA]
- S.O.C. Medium
- LB plates with 50 μg/ml kanamycin

Addition of 5’-terminal attB sequences to PCR primers allows synthesis of a PCR product that is an efficient substrate for recombination with a Donor Vector via a GATEWAY reaction. This is typically more efficient than classic restriction endonuclease cloning of PCR products (see section 3.2.2.B.) and results in directionally cloned PCR products flanked by attL1 and attL2 sites. For high throughput applications or unusually long primers (>70 nucleotides), the attB adapter protocol can be used (section 5.2).

### 3.3.1 Preparation of attB-PCR Products

PCR primers for amplification and subsequent cloning by GATEWAY technology have the structure: 4Gs - 25 bp attB site - 18 to 25 bp gene-specific sequence. 50 nmol of standard purity oligonucleotides are adequate for most applications. Dissolve oligonucleotides to 20-50 mM and verify the concentration by spectrophotometry. For cloning of large PCR products (>5 kb), colony output can be increased if oligonucleotides (>65 bases) are further purified (i.e., HPLC or PAGE).

Design primers to contain the attB1 and attB2 primer sequences (Figure 9). The four guanine (G) residues at the 5’ end are required to make the 25-bp attB sequences an efficient substrate for GATEWAY cloning. The attB1 primer ends with a thymidine (T). To maintain proper reading frame for N-terminal fusions the primer must donate two additional nucleotides. These two nucleotides cannot be AA, AG, or GA, because these additions would create translation termination codons. Similarly, for C-terminal fusions, the attB2 primer requires one nucleotide from the rest of the primer to maintain the proper reading frame into the attB2 region. Also, any in-phase termination codons present between the coding region of the PCR sequence and the attB2 region need to be eliminated if C-terminal fusions will be generated (see section 2.3.3).

**Figure 9. attB Sequences to Add to Primers for PCR Cloning into a pDONR Vector.**

**attB1 forward primer (amino-terminal):**

```plaintext
Lys-Lys
5’-GGGG -ACA-AGT-TTG -TAC-AAA-AAA-GCA-GGC-TNN--(template-specific sequence)-3’
```

**attB2 reverse primer (carboxy terminal):**

```plaintext
Lys-Tyr
5’-GGGG -AC -CAC-TTT- GTA-CAA-GAA-AGC-TGG- GTN--(template-specific sequence)-3’
```
It is possible to install a protease cleavage sequence to permit the removal of N-terminal or C-terminal peptides from the fusion proteins. Include this sequence between the gene-specific and the attB sequences of the primer. For examples of attB-PCR primer sequences for native and fusion protein expression clones, refer to Section 2.3.3.)

Standard PCR conditions can be used to prepare the attB-PCR product. Genomic DNA, mRNA, cDNA libraries, and cloned DNA sequences have been used successfully for amplification with attB-containing primers. In general, the attB sequences have not been observed to affect PCR product yield or specificity. The suggested polymerase, if you are cloning PCR products <5-6 kb for protein expression is PLATINUM® Pfx DNA Polymerase due to its high fidelity and high specificity (21). For all other applications, PLATINUM Taq DNA Polymerase High Fidelity results in high-yield, robust, and high-specificity PCR of products 100 bp to 12 kb.

Following PCR, analyze 1-2 µl on an agarose gel to assess the yield and purity of the product.

### 3.3.2 Purification of attB-PCR Products

Purification of the PCR product is recommended to remove attB primers and any attB primer-dimers which can clone efficiently into the Entry Vector. The following protocol is fast and will remove DNA <300 bp.

1. Add 150 µl of TE to a 50-µl amplification reaction.
2. Add 100 µl of 30% PEG 8000/30 mM MgCl₂. Mix well and centrifuge immediately at 10,000 × g for 15 min at room temperature. Remove the supernatant (pellet is clear and nearly invisible).
3. Dissolve the pellet in 50 µl TE. Check quality and recovery on a gel.
4. Proceed to section 3.4.2.

Note: For some PCR products, agarose gel electrophoresis followed by excision of the PCR product may be needed. Purify the excised product using the CONCERT Rapid Gel Extraction System.

### 3.4 Creating Entry Clones via the BP Reaction

The BP Reaction transfers a gene present in an attB Expression Clone (or attB-flanked PCR products) to generate an attL-flanked Entry Clone. The gene can then be subcloned into any number of new Expression Vectors using the LR Reaction. See section 5.1 for a one-tube protocol to directly go from a PCR product or Expression Clone into Destination Vectors.

Purify plasmid DNA with the CONCERT™ Rapid Plasmid Systems for best results. Alternatively, DNA can be purified using an alkaline lysis protocol, with or without RNase treatment. During alkaline lysis treatment, keep the NaOH ≤0.125 M to minimize irreversible denaturation of the supercoiled plasmid DNA.

The most efficient attB substrates are linear (Expression Clones linearized by restriction endonucleases or attB-flanked PCR products). Supercoiled or relaxed Expression Clones (attB) react less efficiently than linearized Expression Clones. The attP-containing pDONR Vector should be supercoiled.

### 3.4.1 Preparation of Expression Clone DNA

1. Linearize 1 to 2 µg of the Expression Clone with a unique restriction endonuclease that does not digest within the gene of interest and is located outside the attB region.
2. Ethanol precipitate the DNA after digestion by adding 0.1 volume of 3 M sodium acetate followed by 2.5 volumes of 100% ethanol. Dissolve DNA in TE.
Methods

3.4.2 The BP Reaction

1. Add the following to 1.5-ml tubes at room temperature and mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>attB Expression Clone DNA, linearized, ≥10 ng/µl or attB PCR product (use 40-100 fmol; a 1-kb PCR product is ~0.65 ng/fmol)</td>
<td>---</td>
<td>---</td>
<td>1-10 µl</td>
</tr>
<tr>
<td>pEXP7-tet Positive Control, 50 ng/µl</td>
<td>---</td>
<td>2 µl</td>
<td>---</td>
</tr>
<tr>
<td>pDONR201 Vector, 150 ng/µl</td>
<td>2 µl</td>
<td>2 µl</td>
<td>2 µl</td>
</tr>
<tr>
<td>BP Reaction Buffer (5X)</td>
<td>4 µl</td>
<td>4 µl</td>
<td>4 µl</td>
</tr>
<tr>
<td>TE</td>
<td>10 µl</td>
<td>8 µl</td>
<td>To 16 µl</td>
</tr>
</tbody>
</table>

*For PCR products >4 kb, use at least 100 fmol of PCR product, but no more than 500 ng.

1pEXP7-tet is a ~1.4-kb linear DNA encoding the tetracycline resistance gene and its promoter for expression (Tc'), used to verify the BP reaction. The resulting Entry Clones can be used to estimate Km' transformants that contain transferred DNA (Tc').

2. Remove the BP ClONASE Enzyme Mix from -70°C and thaw on ice (~2 min).
3. Vortex BP ClONASE Enzyme Mix briefly (2 s) twice.
4. Add 4 µl of BP ClONASE Enzyme Mix. Mix well by vortexing briefly twice. Return vial to -70°C.
5. Incubate reactions at 25°C for 60 min.
6. Add 2 µl of Proteinase K Solution. Incubate for 10 min at 37°C.
7. Transform 1 µl into 50 µl of LIBRARY EFFICIENCY DH5α Competent Cells. Incubate on ice for 30 min. Heat-shock the cells at 42°C for 30 s. Place on ice for 1-2 min. Add 450 µl S.O.C. Medium and incubate at 37°C for 1 h. Alternatively, electroporation can be used to transform 1-2 µl of the BP Reaction into 25 to 40 µl electropotent E. coli. Add 450 µl S.O.C. Medium and incubate at 37°C for 1 h.
8. Spread 10 µl and 100 µl on LB plates containing 50 µg/ml kanamycin. (For E. coli cells with a transformation efficiency of 10⁸ CFU/µg, the BP Reaction gives ~3,000 colonies if the entire transformation is plated.)
9. If desired, the percent correct clones in the positive control reaction can be confirmed by streaking the kanamycin-resistant colonies onto LB plates containing 20 µg/ml tetracycline.

3.4.3 Sequencing of pENTR Clones Generated by Recombination with Donor Vectors

pENTR (attL) clones can be sequenced with dye-labeled terminator chemistries such as DYEEnamic™ energy transfer or BigDye™ reaction chemistries. The primer sequences are:

For Entry Clones derived from recombination with pDONR201:

proximal to attL1: TCGCG TTAAC GCTAG CATGG ATCTC
proximal to attL2: GTAAC ATCAG AGATT TTGAG ACAC
Use the BigDye chemistry and the following conditions:
5-min at 95°C followed by 30 cycles of PCR:
96°C for 10 s;
50°C for 5 s;
60°C for 4 min.

For small inserts (a couple of hundred bases), the following cycling conditions are recommended:
5-min at 98°C followed by 30 cycles of PCR:
98°C for 10 s;
60°C for 4 min.

3.5 Creating Expression Clones via the LR Reaction

Materials:
- Entry Clone
- Appropriate Expression System with GATEWAY Technology or your converted Destination Vector and LR CLONASE Enzyme Mix.
- S.O.C. Medium
- LB plates with 100 μg/ml ampicillin
- Appropriate host and cell growth media for expression

The reaction of an Entry Clone (attL) with a Destination Vector (attR) creates a new Expression Clone (attB).

Purify plasmid DNA with the CONCERT Rapid Plasmid Systems for best results. Alternatively, DNA can be purified using an alkaline lysis protocol, with or without RNase treatment. During alkaline lysis treatment, keep the NaOH ≤0.125 M to minimize irreversible denaturation of the supercoiled plasmid DNA.

The efficiency of the LR Reaction depends on the topology of the plasmids in the following order (most efficient to least efficient):
Either or both plasmids linear > both plasmids relaxed >> both plasmids supercoiled

All GIBCO BRL® Destination Vectors are provided linearized. If you have converted a plasmid to a Destination Vector, linearize it by cleaving at a restriction site within the region of the GATEWAY Cassette, taking care to avoid the ccdB gene. When suitable restriction sites are unknown, relax the DNA with topoisomerase I treatment (see modified LR Reaction protocol in section 5.4).

1. Add the following to 1.5-ml tubes at room temperature and mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Tube 1</th>
<th>Tube 2</th>
<th>Tube 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>4 μl</td>
<td>4 μl</td>
<td>4 μl</td>
</tr>
<tr>
<td>Positive Control</td>
<td>---</td>
<td>2 μl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>1-11 μl</td>
</tr>
<tr>
<td>LR Reaction Buffer (5X)</td>
<td>4 μl</td>
<td>4 μl</td>
<td>4 μl</td>
</tr>
<tr>
<td>pENTR-gus*, 50 ng/μl</td>
<td>---</td>
<td>2 μl</td>
<td>---</td>
</tr>
<tr>
<td>Entry Clone (100-300 ng/reaction)</td>
<td>---</td>
<td>---</td>
<td>1-11 μl</td>
</tr>
<tr>
<td>Destination Vector, linearized (~300 ng/reaction)</td>
<td>1-11 μl</td>
<td>1-11 μl</td>
<td>1-11 μl</td>
</tr>
<tr>
<td>TE</td>
<td>To 16 μl</td>
<td>To 16 μl</td>
<td>To 16 μl</td>
</tr>
</tbody>
</table>

*Note: pENTR-gus is a ~1.8 kb plasmid DNA encoding the gus gene and is used to verify the LR Reaction. The resulting Expression Clone contains both E. coli and eukaryotic translational signals upstream of the gus gene, allowing for native expression in E. coli, yeast, insect, and mammalian cells when reacted with the appropriate Destination Vector. Also, the ATG of gus is in frame with the att site for expression of N-terminal fusions.

See the calculation in the Troubleshooting section for determining the amount of Entry Clone to use in the reaction.
Methods

2. Remove LR CLONASE Enzyme Mix from -70°C and thaw on ice (~2 min).
3. Vortex LR CLONASE Enzyme Mix briefly (2 s) twice.
4. Add 4 µl of LR CLONASE Enzyme Mix. Mix well by vortexing briefly twice. Return vial to -70°C.
5. Incubate reactions at 25°C for 60 min.
6. Add 2 µl of Proteinase K Solution to all reactions. Incubate for 10 min at 37°C.
7. Transform 1 µl into 50 µl LIBRARY EFFICIENCY DH5α Competent Cells. Incubate on ice for 30 min. Heat-shock the cells at 42°C for 30 s. Add 450 µl S.O.C. Medium and incubate at 37°C for 1 h.
   Alternatively, electroporation can be used to transform 1 to 2 µl of the LR Reaction into 25-40 µl electrocompetent E. coli. Add 450 µl S.O.C. Medium and incubate at 37°C for 1 h.
8. Plate 20 µl and 100 µl on LB plates containing 100 µg/ml ampicillin. For E. coli cells with a transformation efficiency of 10^8 CFU/µg, the LR Reaction should give about 8,500 colonies if the entire transformation is plated.

Note: BsrG I cleaves within all att sites, and can be used to help characterize clones.

3.5.1 Protein Expression from GATEWAY Expression Clones

The E. coli Expression Systems with GATEWAY Technology provide the components to construct E. coli Expression Clones from Entry Clones. Use LIBRARY EFFICIENCY DH5α cells to select for Expression Clones. (Note: DH5α cells do not express T7 RNA polymerase and cannot be used for expression from T7 promoters.) Use BL21-SI cells for protein expression. In BL21-SI cells, expression of T7 RNA polymerase is under control of a salt-inducible promoter, allowing for salt induction of expression of proteins from T7 promoters (such as found in Gibco BRL E. coli Destination Vectors).

The Baculovirus Expression System with GATEWAY Technology (cat. no. 11827-011) provides the components necessary to construct the GATEWAY version of the pFASTBAC™ clone. Once constructed, this clone can be used in conjunction with the BAC-TO-BAC® Baculovirus Expression System to generate (by in vivo recombination with a bacmid) a recombinant baculovirus for expression in insect cells. In addition to the Baculovirus Expression System with GATEWAY Technology, components from the BAC-TO-BAC system are also required (including DH10BAC™ cells, CELLFECTIN® Reagent, and insect cells for expression.) Refer to the BAC-TO-BAC System manual (on the web site) for more information.

The Mammalian Expression System with GATEWAY Technology (cat. no. 11826-013) supplies the components to construct mammalian Expression Clones from Entry Clones. These Expression Clones contain the ned marker and the CMV promoter for expression. See the related products list in section 6 for cell lines and transfection reagents for mammalian expression.

3.6 Converting a Vector into a GATEWAY Destination Vector

For any vector to serve as a Destination Vector, it must have attL sites flanking the ccdB gene. Conversion of any vector to a GATEWAY Destination Vector is done by simply ligating a blunt-ended cassette, containing attR sites and ccdB (and a chloramphenicol resistance marker to select for successful ligation of the cassette) into the multiple cloning site (MCS) of the vector. The GATEWAY Vector Conversion System provides conversion cassettes in all three reading frames (see Table 4, Figures 10 and 11) for N- and C-terminal fusion vectors as well as for native expression vectors.
Table 4. Location of Cleavage Sites for a Selection of Restriction Endonucleases.

<table>
<thead>
<tr>
<th>Restriction Endonuclease Cleavage Site</th>
<th>DNA</th>
<th>Not I</th>
<th>Pvu II</th>
<th>EcoR I</th>
<th>Nco I</th>
<th>Sca I</th>
<th>BssH II</th>
<th>AlwN I</th>
<th>Sma I</th>
<th>Sfc I</th>
<th>Sal I</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF A</td>
<td>129</td>
<td>348</td>
<td>450</td>
<td>751</td>
<td>865</td>
<td>944</td>
<td>1224</td>
<td>1319</td>
<td>1572</td>
<td>1578</td>
<td></td>
</tr>
<tr>
<td>RF B</td>
<td>130</td>
<td>349</td>
<td>451</td>
<td>752</td>
<td>866</td>
<td>945</td>
<td>1225</td>
<td>1320</td>
<td>1573</td>
<td>1579</td>
<td></td>
</tr>
<tr>
<td>RF C.1</td>
<td>131</td>
<td>350</td>
<td>452</td>
<td>753</td>
<td>867</td>
<td>946</td>
<td>1226</td>
<td>1321</td>
<td>1574</td>
<td>1580</td>
<td></td>
</tr>
</tbody>
</table>

3.6.1 Protocol for Constructing a GATEWAY Destination Vector

Materials:
- GATEWAY Vector Conversion System
- restriction endonucleases
- calf intestinal alkaline phosphatase
- PEG
- TE [10 mM Tris-Cl (pH 7.5), 1 mM EDTA]
- T4 DNA ligase
- LB plates with 30 µg/ml chloramphenicol

- Destination Vectors must be constructed and propagated in DB3.1 cells, a gyrA462 strain of E. coli because the ccdB gene is lethal to other strains.
- If linearizing a vector using restriction endonucleases that generate 5’ overhangs, the ends of the DNA molecules must first be made blunt (by a Klenow fill-in reaction) before the blunt-end cassette may be ligated into the vector.
- Because the reading frame cassettes are blunt-ended, they will clone in both orientations and must be screened to identify the construct with the cassette in the proper orientation.
- If you are converting a vector that encodes kanamycin resistance, use the resulting Destination Vector with Entry Clones that carry a selection marker other than Kmr. You can make this Entry Clone in a BP Reaction using a Donor Vector with a marker such as gentamicin resistance (pDONR207).
Methods

1. Determine the Gateway Reading Frame Cassette to use.
   If the Destination Vector will be used to make a fusion protein, use a Gateway Reading Frame Cassette with the correct translation reading frame. For an amino-terminal fusion protein, keep the -AAA-AAA- triplets in attR1 (see Figure 11) in phase with the translation reading frame of the fusion protein. This is the reading frame convention used in N-terminal fusion Destination Vectors from Life Technologies. For C-terminal fusion proteins, align the coding sequence in phase with -TAC-AAA- of the attR2 sequences.

   A. Write out the nucleotide sequence of your vector near the restriction site where the Gateway Cassette will be cloned. These must be written in triplets corresponding to the amino acid sequence of the fusion domain.

   B. Draw a vertical line through the sequence that corresponds to the restriction site end, after it has been digested and made blunt, (i.e., after filling in a protruding 5’-end or polishing a protruding 3’-end).

   For N-terminal fusions:
   — If the coding sequence of the blunt end terminates after a complete codon triplet, use the Reading Frame Cassette A. (See Figure 12.)
   — If the coding sequence of the blunt end encodes two bases of a complete codon triplet, use the Reading Frame Cassette B.
   — If the coding sequence of the blunt end encodes one base of a complete codon triplet, use the Reading Frame Cassette C.1.

   For C-terminal fusions:
   — If the coding sequence of the blunt end terminates after a complete codon triplet, use the Reading Frame Cassette B. (See Figure 11.)
   — If the coding sequence of the blunt end encodes two bases of a complete codon triplet, use the Reading Frame Cassette C.1.

Figure 11. Sequences at ends of Gateway Reading Frame Cassettes. The staggered cleavage sites for the Clonase enzymes are indicated in the boxed regions. Following recombination with an Entry Clone, only the outer (unshaded) sequences in attR sites contribute to the resulting attB sites in the Expression Clone. BsrGI cleaves within all att sites and can be used to help characterize clones.
—If the coding sequence of the blunt end encodes one base of a complete codon triplet, use the Reading Frame Cassette A.

For a combined N- and C-terminal fusion, the restriction endonuclease chosen must produce ends (after generating blunt ends) that are compatible with one of the three cassettes.

2. Digest your plasmid vector (1 to 5 µg) with the appropriate restriction endonucleases [where you wish your gene flanked by att sites to be after recombination]. Note: It is better to remove as many of the MCS restriction sites as possible to minimize the number of additional amino acids added to the fusion and to increase the number of unique restriction endonuclease sites in the new plasmid, which is important for linearizing the Destination Vector for the LR Reaction.

3. If necessary, convert the ends of the vector to blunt double-stranded DNA using either T4 DNA polymerase or Klenow fragment according to the manufacturer’s recommendations.

4. Remove the 5’ phosphates with alkaline phosphatase. This increases the probability of success by decreasing background associated with self-ligation of the vector.
   a. Determine the mass of DNA required for 1 pmol of the type of DNA 5’ end.
   b. To a 1.5-ml microcentrifuge tube, add 4 µl of calf intestinal alkaline phosphatase (CIAP) 10X Buffer [500 mM Tris-HCl (pH 8.5), 1 mM EDTA] and 1 pmol of DNA ends.
   c. Add autoclaved, distilled water to 39 µl.
   d. Dilute CIAP in dilution buffer such that 1 µl contains the amount of enzyme required for the appropriate 5’ end (i.e., 1 unit for 5’-recessed and blunt ends and 0.01 units for a 5’ overhang).

--- NNN NNN ATC ACA AGT TTG TAC AAA AAA GCT ---
--- NNN NNN TAG TGT TCA AAC ATG TTT TTT CGA ---

**Reading Frame Cassette A**

--- NNN NNN TCA ACA AGT TTG TAC AAA AAA GCT ---
--- NNN NNN AGT TGT TCA AAC ATG TTT TTT CGA ---

**Reading Frame Cassette B**

--- NNN NNN NAT CAA ACA AGT TTG TAC AAA AAA GCT ---
--- NNN NNN NTA GTT TGT TCA AAC ATG TTT TTT CGA ---

**Reading Frame Cassette C.1**

--- NNN NNN NNA TCA ACA AGT TTG TAC AAA AAA GCT ---
--- NNN NNN NNT AGT TGT TCA AAC ATG TTT TTT CGA ---

--- NNN NNN NNN TCA ACA AGT TTG TAC AAA AAA GCT ---
--- NNN NNN NNN TGT TGT TCA AAC ATG TTT TTT CGA ---

*cannot be TG or TA*
Methods

e. For 5’-recessed and blunt-ended DNA, incubate at 50°C for 60 min. For DNA with a 5’ overhang, incubate at 37°C for 30 min.

f. Heat-inactivate CIAP at 65°C for 15 min.

5. Adjust the DNA to a final concentration of 20 to 50 ng/μl in TE. Electrophorese 20 to 100 ng on an agarose gel to confirm digestion and recovery.

6. Combine the following at room temperature:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X T4 DNA ligase buffer</td>
<td>2 μl</td>
</tr>
<tr>
<td>vector</td>
<td>20 - 50 ng</td>
</tr>
<tr>
<td>GATEWAY Reading Frame Cassette</td>
<td>10 ng</td>
</tr>
<tr>
<td>T4 DNA ligase</td>
<td>1 unit (in 1 μl)</td>
</tr>
<tr>
<td>Final volume</td>
<td>10 μl</td>
</tr>
</tbody>
</table>

7. Incubate for 1 h at room temperature (or overnight at 16°C, whichever is most convenient).

8. Transform 1 μl into 100 μl DB3.1 Competent Cells. (Note: E. coli DB3.1 cells must be used. The ccdB gene on the GATEWAY Reading Frame Cassette will inhibit growth of other E. coli strains.)

9. After expression in S.O.C. Medium, plate 10 μl, 100 μl, and 500 μl on agar plates containing 30 μg/ml chloramphenicol. Incubate at 37°C for 16 to 20 h.

10. Isolate miniprep DNA from single colonies (16).

11. Treat the miniprep with RNase A, ethanol precipitate, and store in TE. Digest with the appropriate restriction endonuclease to determine the orientation of the cassette. Choose clones with the attR1 site next to the amino end of the protein expression function of the plasmid (see Table 4, Figure 10).

3.6.2 Analysis of Destination Vector

Besides checking for proper orientation of the cassette, it is important to check for the presence of any contaminating ampicillin-resistant plasmid and demonstrate that the ccdB gene is functioning in your Destination Vector. Even minute amounts of ampicillin-resistant plasmid result in a high background.

1. Transform equal amounts (10 - 50 pg) of Destination Vector into 100 μl of LIBRARY EFFICIENCY DH5α cells and DB3.1 Competent Cells using the protocol provided with the cells.

2. Plate onto LB plates containing the ampicillin.

3. Transform 50 pg pUC19 into both strains. Plate onto LB plates containing 100 μg/ml ampicillin.

4. Calculate the transformation efficiency of both strains with the pUC19 control to ensure transformation reactions worked well.

Transformation efficiency (CFU/μg) = colonies/pg of DNA \times \left(1 \times 10^5 \text{ pg/μg}\right) \times \text{dilution factor(s)}

For example, if 50 pg of pUC19 yields 100 colonies when 100 μl of a 1:10 dilution of the transformation mix is plated, then:

CFU/μg = 100 \text{ CFU}/50 \text{ pg} \times \left(1 \times 10^5 \text{ pg/μg}\right) \times \left(1 \text{ ml}/0.1 \text{ ml plated}\right) \times 10 = 2 \times 10^8

5. Calculate the number of colonies obtained in both strains from transformations using the Destination Vector.

6. The Destination Vector should give >10,000 times the number of colonies in DB3.1 cells than in LIBRARY EFFICIENCY DH5α Competent Cells. Any ratio <10,000 suggests contamination of the plasmid prep with another ampicillin-resistant plasmid, or an inactive ccdB gene. DNA with ratios <10,000 will result in higher background.
3.6.3 Preparing the Destination Vector for Cloning

Linearize the Destination Vector with a restriction endonuclease or relax the DNA with topoisomerase I. About 10 times more colonies result from a Gateway reaction if the Destination Vector is linear or relaxed.

The site or sites used for linearization must be within the Gateway Reading Frame Cassette, but not within the ccdB gene. A sampling of the sites that cut within a cassette is shown in Figure 10. After restriction digestion, ethanol precipitate the DNA by adding 0.1 volume of 3 M sodium acetate, followed by 2.5 volumes of 100% ethanol. The linear Destination Vector is now ready for the LR Reaction.
## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For Both LR and BP Reactions:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Few or no colonies obtained from sample reaction, and the transformation control with pUC19 gave colonies</td>
<td>Transformation was plated with incorrect antibiotic</td>
<td>Use kanamycin for most Entry Clones. Use ampicillin for most Destination Vectors.</td>
</tr>
<tr>
<td></td>
<td>Reactions were not treated with proteinase K</td>
<td>Treat reactions with proteinase K before transformation.</td>
</tr>
<tr>
<td></td>
<td>Used incorrect <em>att</em> sites for reaction</td>
<td>Use Entry Clone (<em>attL</em>) and Destination Vector (<em>attR</em>) for the LR Reaction. Use Expression Clone (or <em>attB</em>-PCR product) and Donor Vector (<em>attP</em>) for BP Reaction.</td>
</tr>
<tr>
<td></td>
<td>DNA topology is not optimal for reaction</td>
<td>For the LR Reaction, linearize the Destination Vector within the <em>attR</em> Cassette, avoiding the <em>ccdB</em> gene.</td>
</tr>
<tr>
<td></td>
<td><em>CLONASE™ Enzyme Mix is inactive</em></td>
<td>For the BP Reaction, linearize the <em>attB</em> Expression Clone outside the <em>attB</em> sites with an appropriate restriction endonuclease or relax with topoisomerase I. Use supercoiled Donor Vector.</td>
</tr>
<tr>
<td></td>
<td>Test another aliquot of the <em>CLONASE Enzyme Mix</em>.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Check that the <em>CLONASE Enzyme Mix</em> is being stored at -70°C.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Do not freeze any aliquot more than 10 times to minimize loss of activity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pENTR-gus can be used in an LR Reaction (section 3.5) to test LR <em>CLONASE Enzyme Mix</em> activity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pEXP7-tet can be used in a BP Reaction (section 3.4) to test BP <em>CLONASE Enzyme Mix</em> activity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Used incorrect <em>CLONASE Enzyme Mix</em></td>
<td>Use the LR <em>CLONASE Enzyme Mix</em> for the LR Reaction and the BP <em>CLONASE Enzyme Mix</em> for the BP Reaction.</td>
</tr>
<tr>
<td></td>
<td>Too much PCR product was used in a BP Reaction</td>
<td>Reduce the amount of PCR product used. Remember to use ~100 fmol of Donor Vector. Therefore, to obtain an equimolar ratio of PCR product and Donor Vector, use 100 fmol of PCR product. If the PCR product is 2.5 kb, convert to nanograms using the following equation:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ng = (fmol)(N)(660 fg/fmol)(1 ng/10^6 fg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>where N is the size of the DNA in bp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100 fmol)(2500 bp)(660 fg/fmol)(1 ng/10^6 fg) = 165 ng.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Therefore, 165 ng of PCR product are required for this reaction.</td>
</tr>
<tr>
<td></td>
<td>Too much Entry Clone was used in an LR Reaction</td>
<td>Use equal fmol of Destination Vector and Entry Clone.</td>
</tr>
<tr>
<td></td>
<td>Two distinct types of colonies appear, large and small</td>
<td>For the LR Reaction, the small colonies can be unreacted Entry Clone that co-transforms with Expression Clone. When small colonies are restreaked onto LB kanamycin (50-100 µg/ml) and LB ampicillin (100 µg/ml) plates, often they only grow on the LB kanamycin.</td>
</tr>
<tr>
<td></td>
<td>Reduce the amount of Entry Clone to 100 ng per 20-µl reaction.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduce the volume of sample used for transformation to 1 µl.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase the ampicillin to 300 µg/ml.</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Possible Cause</td>
<td>Suggested Solution</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>For the BP Reaction, deletions or point mutations of the ccdB gene within the Donor (attP) Plasmid can allow E. coli to grow, although at lower rates. The negative control will give a similar number of colonies.</td>
<td>Obtain a new attP Donor Plasmid.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasmids carrying large genes may be deleted during culture, leading to two populations of colonies. Generally, larger colonies contain the deletions.</td>
<td>Incubate plates at 30°C instead of 37°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confirm whether a deletion is occurring by analyzing the DNA derived from the colonies.</td>
</tr>
<tr>
<td>For the LR Reaction, high background in absence of Entry Clone</td>
<td>Contamination of solution(s) with another plasmid carrying the same antibiotic resistance, or by bacteria carrying a resistance plasmid</td>
<td>Test for plasmid contamination by transforming with aliquots of each of the separate solutions used in the LR Reaction. Test for bacterial contamination by plating an aliquot of each solution directly onto LB ampicillin plates.</td>
</tr>
<tr>
<td></td>
<td>Reactions transformed into an F- containing E. coli which has the ccdA gene</td>
<td>Use E. coli strains without an F episome such as DH5α cells.</td>
</tr>
<tr>
<td></td>
<td>Some Destination Vectors have an inherently higher background than others, possibly due to tendency to delete some or all of the ccdB gene</td>
<td>Prepare miniprep DNA from one or more background colonies. Unstable Destination Vectors often reveal multiple bands on agarose gels. If this is the case, try using a different vector backbone in the Destination Vector.</td>
</tr>
<tr>
<td>Few or no colonies obtained from the transformation control with pUC19</td>
<td>Transformation performed incorrectly, or competent cells stored improperly</td>
<td>Verify that competent cells are stored at -70°C.</td>
</tr>
<tr>
<td></td>
<td>Dilutions were performed incorrectly</td>
<td>Repeat transformation paying special attention to dilution steps.</td>
</tr>
</tbody>
</table>

For attB-PCR Cloning: (These are in addition to general BP Reaction problems above.)

<p>| Few or no colonies obtained from BP Reaction with new attB-PCR product, and both attB-positive control and transformation control gave expected number of colonies | attB-PCR primers have a mistake in the attB1 or attB2 sequences, or are missing the four 5’ terminal Gs | Replace with correct attB-PCR primers. |
| | attB primers have high percentage of incomplete sequence | Purify long (&gt;65 nucleotides) attB-PCR primers by PAGE, to remove incomplete sequences. Alternatively, use the Adapter PCR protocol (section 5.2). |
| | For large PCR products (&gt;5 kb), too few PCR molecules added to BP Reaction | Increase the amount (ng) of PCR product to 40 to 80 fmol of PCR DNA/20-μl reaction (e.g., for an 8 kb DNA, 1 fmol ~5 ng.) Note: Do not exceed 400 ng DNA/20-μl reaction. |
| | Incubation time not sufficient | Increase incubation time to 6 to 18 h. |
| | PCR products were not purified sufficiently | Gel purify PCR product, making sure to separate product from oligonucleotides. |</p>
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry Clones migrate as 2.2-kb supercoiled plasmids</td>
<td>BP recombination reaction may have cloned primer-dimers</td>
<td>Purify PCR products &gt;500 bp by precipitating with PEG/MgCl₂ solution. Alternatively, excise the correct size DNA product from an agarose gel, and use the eluted, purified DNA in the BP Reaction. Use a PLATINUM™ DNA polymerase for automatic hot-start PCR giving higher specificity. Redesign primers to minimize potential mutual priming sites leading to primer-dimers.</td>
</tr>
<tr>
<td>Low yield of PCR product from PEG precipitation</td>
<td>PCR product not diluted with TE</td>
<td>Dilute with 150 μl TE before adding the PEG MgCl₂ solution.</td>
</tr>
<tr>
<td></td>
<td>Centrifugation step too short or centrifugation speed too low</td>
<td>Increase time and speed of the centrifugation step to 30 min and 15,000 x g.</td>
</tr>
<tr>
<td></td>
<td>Loss of PEG pellet</td>
<td>Take care when removing the tube from the microcentrifuge and keep track of the orientation of the outer edge of the tube where the pellet is located.</td>
</tr>
</tbody>
</table>

Preparation Entry Clones with Restriction Endonucleases and Ligase:

<table>
<thead>
<tr>
<th>Few or no colonies obtained</th>
<th>ccb Cassette still present within Entry Vector</th>
<th>Excise with appropriate restriction endonuclease(s).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligation did not work</td>
<td>Include ligation positive control linearized plasmid, with and without ligase.</td>
<td></td>
</tr>
<tr>
<td>Transformation was plated with incorrect antibiotic</td>
<td>Use kanamycin for most Entry Clones. Use ampicillin for most Destination Vectors.</td>
<td></td>
</tr>
</tbody>
</table>

Protein Synthesis using attB Expression Clones:

<table>
<thead>
<tr>
<th>No protein of expected molecular weight seen on SDS-PAGE</th>
<th>Protein is being degraded by endogenous proteases, especially for proteins &gt;100 kDa</th>
<th>Use lon⁺ and ompT⁺ strains for E. coli expression (such as BL21-SI cells). Incubate plates at 30°C instead of at 37°C. Compare expression using different N-terminal and/or C-terminal fusion tags, and in other types of host cells, such as yeast, insect, or mammalian cells. For expression in E. coli from T7 promoters (such as pDEST™14, pDEST15, and pDEST17), use a strain such as BL21-SI cells that express T7 RNA polymerase. DH5α cells cannot be used for expression from T7 promoters. Expression Clones made from pDEST8, pDEST10, and pDEST20 must first be recombined with bacmid DNA (BAC-TO-BAC® system). The resultant baculovirus can then be used for expression in insect cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein contains secondary modifications that increase apparent molecular weight</td>
<td>Compare expression in other types of host cells, such as yeast, insect, or mammalian cells.</td>
<td></td>
</tr>
<tr>
<td>No fusion protein of expected molecular weight seen on SDS-PAGE</td>
<td>Incorrect reading frame of Entry Clone</td>
<td>Verify that attB-PCR primers were designed with gene in correct reading frame. Verify that Entry Clone was constructed with gene in correct reading frame. Verify that Destination Vector was constructed with correct reading frame.</td>
</tr>
</tbody>
</table>
Additional Information

5.1 “One-Tube” Protocol: A Protocol for Cloning attB-PCR Products Directly into Destination Vectors

This one-tube protocol moves attB-PCR products into a Destination Vector in 2 steps - a BP Reaction followed by an LR Reaction without purification of the intermediate Entry Clone. This protocol is more rapid than the protocol in section 3.3. However, here the Expression Clone is obtained from an Entry Clone that was not unique, so this protocol requires sequence validation of the Expression Clone.

Also, this protocol can transfer a gene from one Expression Clone into another Destination Vector. Linearize the Expression Clone within the plasmid backbone for an optimal BP Reaction and to eliminate false-positive colonies due to co-transformation.

1. In a 1.5-ml tube, prepare a 25-μl BP Reaction as follows:

   **Component**  | **Volume (μl)**
   --- | ---
   attB DNA (100-200 ng)  | 5
   attP DNA (pDONR™201, 150 ng/μl)  | 2.5
   BP Reaction Buffer (5X)  | 5
   TE  | sufficient to bring the volume to 20 μl
   BP CLONASE™ Enzyme Mix  | 5
   **Final volume**  | 25

   2. Mix and incubate for 4 h at 25°C.
   3a. Remove 5 μl of the reaction to a separate tube. Add 0.5 μl of proteinase K solution. Incubate for 10 min at 37°C.
   3b. Transform 100 μl of competent cells with 1 μl of the mixture. Plate on LB plates containing 50 μg/ml kanamycin. These colonies can be used to isolate Entry Clones and assess the BP Reaction efficiency.

4. To the remaining 20-μl reaction, add:

   **Component**  | **Volume (μl)**
   --- | ---
   NaCl (0.75 M)  | 1
   Destination Vector linearized (150 ng/ml)  | 3
   LR CLONASE™ Enzyme Mix  | 6
   **Final volume**  | 30

   5. Mix and incubate for 2 h at 25°C.
   6. Add 3 μl of proteinase K solution. Incubate for 10 min at 37°C.
   7. Transform 100 μl of competent cells with 1 μl of the reaction. Plate on LB plates containing 100 μg/ml ampicillin (for Ap′ Destination Vectors).

   The total number of Expression Clone colonies is usually 10% to 20% of the total number of Entry Clone colonies.

5.2 attB Adapter PCR for Preparation of attB-flanked PCR Products

Use this protocol to replace the standard protocol (section 3.3.1) to prepare attB-flanked PCR products when primers are >70 bases. This protocol requires 2 sets of primers, one for the gene-specific amplification and the second set to install complete attB sequences (adapter-primers attB1 and attB2).
Design template-specific primers with 12 bases of attB1 and attB2 at their 5'-ends as shown below:

12 attB1: AA AAA GCA GGC TNN - forward template-specific primer
12 attB2: A GAA AGC TGG GTN - reverse template-specific primer

In addition, the following adapter-primers will be needed to install the full 29-b attB sequences:

attB1 adapter primer:  G GGG ACA AGT TTG TAC AAA AAA GCA GGC T
attB2 adapter primer:  GGG GAC CAC TTT GTA CAA GAA AGC TGG GT

1. Prepare a 50-μl PCR containing 10 pmol of each template-specific primer (with 12 attB) and the appropriate amount of template DNA.
2. Incubate at 95°C for 2 min. Perform 10 cycles of PCR:
   - 94°C for 15 s;
   - 50-60°C for 30 s;
   - 68°C for 1 min/kb of target.
3. Transfer 10 μl to a 40-μl PCR mixture containing 40 pmol each of the attB1 and attB2 adapter-primers.
4. Incubate at 95°C for 1 min. Perform 5 cycles of PCR:
   - 94°C for 15 s;
   - 45°C for 30 s;
   - 68°C for 1 min/kb of target.
5. Perform 15-20 cycles of PCR:
   - 94°C for 15 s;
   - 55°C for 30 s;
   - 68°C for 1 min/kb of target.
6. Check quality and recovery on a gel.
7. Refer to section 3.3.2 to purify the attB-flanked PCR product.

5.3 Blunt Cloning of PCR Products

Generally PCR products do not have 5’ phosphates (because the primers are usually 5’-OH), and they are not necessarily blunt (23). The following protocol simultaneously creates blunt, 5’- phosphorylated ends.

Materials:
- PCR product
- T4 polynucleotide kinase and buffer
- T4 DNA polymerase
- 30% PEG/30 mM MgCl₂
- T4 DNA ligase and buffer
- Dephosphorylated Entry Vector
- LIBRARY EFFICIENCY® competent cells
- S.O.C. medium
- LB plates containing 50 μg/ml kanamycin

1. In a 0.5-ml tube, precipitate ~40 ng of PCR product (as judged from an agarose gel) by adding 0.1 volume of 3 M sodium acetate followed by 2.5 volumes of 100% ethanol.
2. Add the following to the DNA:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled H₂O</td>
<td>...........................................</td>
</tr>
<tr>
<td>10 mM ATP</td>
<td>...........................................</td>
</tr>
<tr>
<td>2 mM dNTPs (i.e., 2 mM each dATP, dCTP, dTTP, and dGTP)</td>
<td>.................</td>
</tr>
<tr>
<td>5X T4 Forward Reaction Buffer [350 mM Tris·HCl (pH 7.6), 50 mM MgCl₂, 500 mM KCl, 5 mM 2-mercaptoethanol]</td>
<td>...........................................</td>
</tr>
<tr>
<td>T4 polynucleotide kinase (10 units/μl)</td>
<td>...........................................</td>
</tr>
<tr>
<td>T4 DNA polymerase</td>
<td>...........................................</td>
</tr>
</tbody>
</table>
3. Incubate at 37°C for 10 min, then at 65°C for 15 min. Cool on ice for 5 min. Centrifuge briefly to bring any condensate to the bottom of the tube.
4. Add 5 µl of 30% PEG 8000/30 mM MgCl₂. Mix and centrifuge immediately at room temperature for 10 min.
5. Carefully remove and discard supernatant.
6. Dissolve the invisible pellet in 10 µl containing 2 µl 5X T4 DNA ligase buffer, 0.5 units T4 DNA ligase, and about 50 ng of blunt, dephosphorylated Entry Vector.
7. Incubate at 25°C for 1 h, then at 65°C for 10 min.
8. Add 40 µl TE, transform 2 µl into 100 µl of of LIBRARY EFFICIENCY DH5α Competent Cells.
9. Plate on LB plates containing 50 µg/ml kanamycin.
10. Isolate miniprep DNA from single colonies (16). Treat the miniprep with RNase A and store in TE. Cut with the appropriate restriction endonuclease to determine the orientation of the PCR fragment. Choose clones with the attL1 site next to the amino end of the open reading frame.

5.4 Modified LR Reaction with Topoisomerase I

Use this protocol to relax Destination Vectors when suitable restriction sites are unavailable. The expected colony output for this modified protocol is ~50% less than when using linear Destination Vectors, and 5-10 times greater than reactions using supercoiled Destination Vectors.

1. Prepare the LR Reaction (described in section 3.5) by adding the following to a 1.5-ml tube at room temperature.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR Reaction Buffer (5X)</td>
<td>4</td>
</tr>
<tr>
<td>Supercoiled Entry Clone (100-300 ng)</td>
<td>1-9</td>
</tr>
<tr>
<td>Supercoiled Destination Vector (300 ng)</td>
<td>1-9</td>
</tr>
<tr>
<td>Topoisomerase I (15 units/µg total DNA)</td>
<td>0.6-2</td>
</tr>
<tr>
<td>TE</td>
<td>to 16</td>
</tr>
</tbody>
</table>

2. Remove LR CLONASE Enzyme Mix from -70°C and thaw on ice (~2 min).
3. Vortex LR CLONASE Enzyme Mix briefly (2 s) twice.
4. Add 4 µl of LR CLONASE Enzyme Mix. Mix well by vortexing briefly twice. Return vial to -70°C.
5. Incubate reactions at 25°C for 60 min.
6. Add 2 µl of proteinase K solution. Incubate for 10 min at 37°C.
7. Proceed with transformation of E. coli.

5.5 Transferring Clones from cDNA Libraries Made in GATEWAY™ Vectors

There are several things to consider when working with a clone isolated from a cDNA library constructed in a GATEWAY vector, such as SUPERSCRIPT™ cDNA libraries supplied in pCMV•SPORT6 (which contains attB sites). These include whether the clone is full-length and whether the protein will be expressed as a native protein or as a fusion protein.

While libraries contain many full-length open reading frames, some clones may be a partial reading frame, or may contain the entire ORF plus 5' untranslated (5' UTR) sequence as well. Contained within the 5' UTR of a cDNA is the ribosome recognition sequence for the organism from which the cDNA was derived. Therefore, a full-length cDNA derived from mammalian cells can be used for native expression in mammalian cells without prior characterization but cannot be used for native expression in E. coli, as no Shine-Dalgarno sequence is present. A Shine-Dalgarno sequence can be supplied either by cloning the cDNA into an Entry Vector that contains a Shine-Dalgarno sequence, or by introducing a Shine-Dalgarno
sequence by PCR when amplifying the cDNA with primers containing attB sequences and cloning the PCR product by recombination. (See Section 3.3 for cloning of PCR products).

The length and content of the clone is important in expressing fusion proteins. For full-length cDNA, the 5' UTR will be translated as a part of the fusion protein. This may present problems as the additional codons may interfere with the expression or function of the protein, or the 5' UTR may contain stop codons. If the ORF is not full-length, a truncated portion of the protein of interest will be expressed within the fusion. To express any cDNA isolated from a library as an N-terminal fusion protein, the reading frame of the gene must be in frame with the reading frame of the attB1 site (see Figure 9). There is one chance in three that the cDNA will be in frame with the attB1 site and allow for fusion protein expression. A researcher can construct three Destination Vectors representing the three reading frames through the attB1 sites so that any given cDNA clone can be expressed in one of the three vectors. Alternatively, to assure that the ORF encoded by the cDNA will be in frame with an N-terminal fusion protein sequence, use PCR to install attB sites, so that the AAA-AAA sequence within attB1 is in phase with the ORF.

The major consideration in generating C-terminal fusion proteins from cDNAs is that cDNAs contain one or more stop codons, which must be removed before C-terminal fusion expression is possible. This may be done by subcloning the gene into an Entry Vector by classic methods, so that no stop codon is present. Alternatively, it may be done by amplifying the gene by PCR using attB primers where the stop codon has been eliminated from the gene-specific sequence.
5.6 GATEWAY™ Vector Restriction Maps

5.6.1 Entry Vectors

Entry Vectors contain a pUC origin of replication and the kanamycin resistance gene (Km<sup>r</sup>) for maintenance in *E. coli*.

All Entry Vectors consist of the same vector backbone (outside of the attL sites) but differ in the sequences and cloning sites provided between the attL sites. Details of the regions between the attL<sub>1</sub> and attL<sub>2</sub> sites for each Entry Vector follow the circle map as well as endonucleases that do not cleave the vectors.

Restriction endonucleases that cleave pENTR<sub>1A</sub> once are shown on the outer circle. The positions refer to the 5<sup>´</sup>-base of the recognition site.

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine™ section of Life Technologies’ web page, http://www.lifetech.com.
### Sequences within the attL sites of Entry Vectors:

The amino acids shown before the ccdB gene are added to the N-terminus of your protein only if a translation start site is provided in the Destination Vector (such as with an N-terminal fusion). Clone your sequence in frame with the AAA AAA for N-terminal fusion proteins. Clone your sequence in frame with TTT GTA for C-terminal fusion proteins.

If a blunt-ended fragment containing a 5'-ATG is cloned into the Xmn I site of pENTR1A, 2B, 3C, or 4, the adenine at position -3 of the underlined ACC sites provides a Kozak eukaryotic ribosome recognition sequence for initiation of translation.

**pENTR1A sequence: 145-674 nucleotides**

<table>
<thead>
<tr>
<th>Restriction Endonucleases that do not cleave pENTR1A:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asc I</td>
</tr>
<tr>
<td>Ava II</td>
</tr>
<tr>
<td>Avr II</td>
</tr>
<tr>
<td>Bcl I</td>
</tr>
<tr>
<td>Bgl I</td>
</tr>
<tr>
<td>Bgl II</td>
</tr>
<tr>
<td>Bpu1102 I</td>
</tr>
<tr>
<td>BsaA I</td>
</tr>
<tr>
<td>BseB I</td>
</tr>
<tr>
<td>Bsg I</td>
</tr>
<tr>
<td>BspM I</td>
</tr>
</tbody>
</table>

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine™ section of Life Technologies' web page, http://www.lifetech.com.
pENTR2B sequence: 145-675 nucleotides

> ATT Lys Lys Ala Gly Val Pro Leu Gly Ser Gly Thr Glu Pro Val Pro Asn

Restriction Endonucleases that do not cleave pENTR2B:

- Asc I
- BstE II
- Gsu I
- PinA I
- Spe I
- Ava I
- Clu I
- Hind III
- Pme I
- SpI
- Atr II
- Cvn I
- Hpa I
- PshA I
- Spe II
- Bcl I
- Dra I
- Kpn II
- PsI II
- Sst I
- Bgl II
- Dra III
- Mlu I
- Rsr II
- Sst II
- Bpu1102 I
- Eam I
- Nco I
- Sca I
- Sty I
- BsaA I
- EcoR I
- Nde I
- Sex A I
- Sun I
- BsoR I
- Eco72 I
- Ngo A IV
- Sfi I
- Swa I
- BspG I
- Fse I
- Nsp V
- Sgr A I
- Tth I
- BspM I
- Fsp I
- Pac I
- Sna B I
- Xcm I

pENTR3C sequence: 145-680 nucleotides

> ATT Lys Lys Ala Gly Val Pro Leu Gly Ser Gly Thr Glu Pro Val Pro Asn

Restriction Endonucleases that do not cleave pENTR3C:

- Asc I
- BstE II
- Hind III
- Pme I
- Spe I
- Ava I
- Clu I
- Hpa I
- PshA I
- Spe II
- Atr II
- Cvn I
- Kpn II
- PsI II
- Sst I
- Bcl I
- Dra I
- Mlu I
- Rsr II
- Sst II
- Bpu1102 I
- Eam I
- Nco I
- Sca I
- Sty I
- BsaA I
- EcoR I
- Nde I
- Sex A I
- Sun I
- BsoR I
- Eco72 I
- Ngo A IV
- Sfi I
- Swa I
- BspG I
- Fse I
- Nsp V
- Sgr A I
- Tth I
- BspM I
- Fsp I
- Pac I
- Sna B I
- Xcm I

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the 'Tech-Online™' section of Life Technologies' web page, http://www.lifetech.com.
Additional Information

pENTR4 sequence: 145-677 nucleotides

ACT TTG TAC AAA AAA GCA GGC TCC ACC ATG GGA ACC AAT TCA AGC GAG TGC AAC TGT GAC TGG ATC CGG TAG CTA ATT C TGA AAC ATG TTT TTT CCT CGG AGG TGG TAC TCC TCT TGT TTA AGT CAG CTG ACC TAG GCG ATG GCT TAK G thr leu tyr lys ala gly ser thr met gly thr asn ser val asp trp ile arg tyr arg ile

Restriction Endonucleases that do not cleave pENTR4:

- Asc I
- BstE II
- Hind III
- Pme I
- Sph I
- Ava II
- Cia I
- Hpa I
- PshA I
- Ssa8387 I
- Avr II
- Cvn I
- Kpn2 I
- Psp5 II
- Sat I
- Bcl I
- Dra I
- Mlu I
- Rsr II
- Sal I
- Bgl II
- Dra III
- Mun I
- Sap I
- Stu I
- Bgl II
- Eam105 I
- Nar I
- Sca I
- Sun I
- Bpu1102 I
- Eco47 III
- Nde I
- SexA I
- Swa I
- Bsa A I
- Eco72 I
- NgoA IV
- Sfl I
- Thh111 I
- Bse R I
- Fse I
- Nsp V
- SgrA I
- Xcm I
- Bsg I
- Fsp I
- Pac I
- SnaB I
- Bsp M I
- Gsu I
- PinA I
- Spe I

pENTR11 sequence: 145-701 nucleotides

Restriction Endonucleases that do not cleave pENTR11:

- Asc I
- BstE II
- Hind III
- Pme I
- Sph I
- Ava II
- Cia I
- Hpa I
- PshA I
- Ssa8387 I
- Avr II
- Cvn I
- Kpn2 I
- Psp5 II
- Sat I
- Bcl I
- Dra I
- Mlu I
- Rsr II
- Sal I
- Bgl I
- Dra III
- Mun I
- Sap I
- Stu I
- Bgl II
- Eam105 I
- Nar I
- Sca I
- Sun I
- Bpu1102 I
- Eco47 III
- Nde I
- SexA I
- Swa I
- Bsa A I
- Eco72 I
- NgoA IV
- Sfl I
- Thh111 I
- Bse R I
- Fse I
- Nsp V
- SgrA I
- Xcm I
- Bsg I
- Fsp I
- Pac I
- SnaB I
- Bsp M I
- Gsu I
- PinA I
- Spe I

*The AAGGAG/A and ACC sites correspond to the Shine-Dalgarno (prokaryotes) and Kozak eukaryotic ribosome recognition sequences preceding the initiating ATG.

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine™ section of Life Technologies' web page, http://www.lifetech.com.
5.6.2 E. coli Destination Vectors

pDEST14 Vector for Native Protein Expression from a T7 Promoter

Recombination Region of the Expression Clone resulting from pDEST14 × Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 75 and 1897. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST14 by recombination. Non-shaded regions are derived from pDEST14.

Restriction endonucleases that do not cleave pDEST14 DNA:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Accession</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aff II</td>
<td>1101</td>
<td>4313</td>
</tr>
<tr>
<td>Apa I</td>
<td>1428</td>
<td>389</td>
</tr>
<tr>
<td>Asc I</td>
<td>654</td>
<td>2427</td>
</tr>
<tr>
<td>Avr II</td>
<td>1523</td>
<td>5363</td>
</tr>
<tr>
<td>Bcl I</td>
<td>345</td>
<td>4563</td>
</tr>
<tr>
<td>BseR I</td>
<td>2581</td>
<td>4382</td>
</tr>
</tbody>
</table>

Restriction endonucleases that cleave pDEST14 DNA twice:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Accession</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aff III</td>
<td>1101</td>
<td>4313</td>
</tr>
<tr>
<td>AlwN I</td>
<td>1428</td>
<td>389</td>
</tr>
<tr>
<td>Apo I</td>
<td>654</td>
<td>2427</td>
</tr>
<tr>
<td>Ava I</td>
<td>1523</td>
<td>5363</td>
</tr>
<tr>
<td>Bar I</td>
<td>6307</td>
<td>6321</td>
</tr>
<tr>
<td>BsaAI</td>
<td>345</td>
<td>4563</td>
</tr>
<tr>
<td>BspM I</td>
<td>1776</td>
<td>5725</td>
</tr>
<tr>
<td>BsrI</td>
<td>2581</td>
<td>4382</td>
</tr>
</tbody>
</table>

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine™ section of Life Technologies’ web page, http://www.lifetech.com.
Additional Information

pDEST17 Vector for N-terminal Histidine Fusion Protein Expression from a T7 Promoter

Recombination Region of the Expression Clone resulting from pDEST17 x Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 148 and 1829. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST17 by recombination. Non-shaded regions are derived from pDEST17.

Restriction endonucleases that do not cleave pDEST17 DNA:

<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>pDEST17 DNA</th>
<th>pDEST17 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afl II</td>
<td>BstE II</td>
<td>PinA I</td>
</tr>
<tr>
<td>Apa I</td>
<td>Cvn I</td>
<td>Mlu I</td>
</tr>
<tr>
<td>Asc I</td>
<td>Dra III</td>
<td>Mun I</td>
</tr>
<tr>
<td>Avr II</td>
<td>Eco72 I</td>
<td>Nsi I</td>
</tr>
<tr>
<td>Bcl I</td>
<td>Fse I</td>
<td>Nsp V</td>
</tr>
<tr>
<td>BseRI</td>
<td>Hpa I</td>
<td>Pac I</td>
</tr>
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</table>

Restriction endonucleases that cleave pDEST17 DNA twice:

<table>
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<tr>
<th>Restriction Enzyme</th>
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<th>pDEST17 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlwNI</td>
<td>1360</td>
<td>3831</td>
</tr>
<tr>
<td>Apo I</td>
<td>586</td>
<td>2359</td>
</tr>
<tr>
<td>Ava I</td>
<td>1455</td>
<td>5295</td>
</tr>
<tr>
<td>BamHI</td>
<td>336</td>
<td>1039</td>
</tr>
<tr>
<td>BanII</td>
<td>6239</td>
<td>6253</td>
</tr>
<tr>
<td>Bgl II</td>
<td>1</td>
<td>1033</td>
</tr>
<tr>
<td>BspMI</td>
<td>1710</td>
<td>5657</td>
</tr>
<tr>
<td>BstBI</td>
<td>2513</td>
<td>4314</td>
</tr>
</tbody>
</table>

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine™ section of Life Technologies' web page, http://www.lifetech.com.
The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the TECH-ONLINE℠ section of Life Technologies’ web page, http://www.lifetech.com.

pDEST15 Vector for N-terminal GST Fusion Expression from a T7 Promoter

Recombination Region of the Expression Clone resulting from pDEST15 × Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 800 and 2481. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST15 by recombination. Non-shaded regions are derived from pDEST15.

Restriction endonucleases that do not cleave pDEST15 DNA:

- Afl I
- Cvn I
- Mlu I
- Rsr II
- Sse837 I
- Xho I
- Apa I
- Dra III
- Mun I
- SexA I
- Sst I
- Asc I
- Eco72 I
- Nsi I
- Sfi I
- Sst II
- Avr II
- Fse I
- Pac I
- Sgf I
- Stu I
- BseR I
- Hpa I
- Pin A
- SnaB I
- Sun I
- BstE II
- Kpn I
- Pme I
- Spe I
- Xcm I

Restriction endonucleases that cleave pDEST15 DNA twice:

- Afl III: 343, 4908
- Eco57 I: 3333, 4381
- Pvu II: 1116, 5319
- AlwN I: 2012, 4494
- EcoN I: 111, 6760
- Sap I: 189, 5030
- Apo I: 1238, 3022
- EcoR I: 1238, 3022
- Sma I: 2107, 2506
- Ban II: 6902, 6916
- EcoV: 2644, 2835
- Ssp I: 1548, 3213
- Bsg I: 370, 5733
- Esp3 I: 1456, 5261
- Vsp I: 23, 3844
- BspLU11 I: 343, 4908
- Nco I: 777, 1539
- Xba I: 63, 1685
- BspM I: 2362, 6320
- Psp6 II: 5902, 5944
- Xma III: 918, 6444
- BstI107 I: 1771, 5139
- Pst I: 2360, 3774
- Dre I: 4800, 5215
- Pvu I: 3648, 6734

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the TECH-ONLINE℠ section of Life Technologies’ web page, http://www.lifetech.com.
5.6.3 Baculovirus Destination Vectors

pDEST8 Vector for Native Protein Expression from a Polyhedrin Promoter

Recombination Region of the Expression Clone resulting from pDEST8 x Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 168 and 1990. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST8 by recombination. Non-shaded regions are derived from pDEST8.

Restriction endonucleases that do not cleave pDEST8 DNA:

<table>
<thead>
<tr>
<th>Aat II</th>
<th>Cla I</th>
<th>Hin d III</th>
<th>Nsp V</th>
<th>SexA I</th>
<th>Sst I</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlwN I</td>
<td>Cva I</td>
<td>Kpn I</td>
<td>Pac I</td>
<td>Sfl I</td>
<td>Stu I</td>
</tr>
<tr>
<td>Afl II</td>
<td>Cvn I</td>
<td>Kpn I</td>
<td>Pac I</td>
<td>Sfl I</td>
<td>Stu I</td>
</tr>
<tr>
<td>Apa I</td>
<td>Eco47 III</td>
<td>Nde I</td>
<td>Pme I</td>
<td>SgrA I</td>
<td>Swa I</td>
</tr>
<tr>
<td>Asc I</td>
<td>Eco72 I</td>
<td>Nde I</td>
<td>Pme I</td>
<td>SgrA I</td>
<td>Swa I</td>
</tr>
<tr>
<td>Bpu1102 I</td>
<td>EcoN I</td>
<td>Nhe I</td>
<td>PshA I</td>
<td>Spe I</td>
<td>Xba I</td>
</tr>
<tr>
<td>Bsg I</td>
<td>EcoO109 I</td>
<td>Nru I</td>
<td>Psp5 II</td>
<td>Sph I</td>
<td>Xcm I</td>
</tr>
<tr>
<td>BseE I</td>
<td>Fse I</td>
<td>Nsi I</td>
<td>Rsr II</td>
<td>Sse8387 I</td>
<td>Xho I</td>
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Restriction endonucleases that cleave pDEST8 DNA twice:

<table>
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<th>AlwN I</th>
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<th>4496</th>
<th>BstII 107 I</th>
<th>2</th>
<th>1280</th>
<th>Nsp I</th>
<th>4910</th>
<th>5892</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ban I</td>
<td>2837</td>
<td>4069</td>
<td>BstXI</td>
<td>1728</td>
<td>5356</td>
<td>PstI</td>
<td>406</td>
<td>973</td>
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<tr>
<td>Bgl II</td>
<td>5193</td>
<td>5663</td>
<td>DraIII</td>
<td>2876</td>
<td>6224</td>
<td>Rca I</td>
<td>3182</td>
<td>4190</td>
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<td>BspL1 I</td>
<td>4910</td>
<td>5892</td>
<td>Eam1105 I</td>
<td>2522</td>
<td>4017</td>
<td>Tfi I</td>
<td>1097</td>
<td>4936</td>
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<tr>
<td>BssS I</td>
<td>3353</td>
<td>4737</td>
<td>Gsu I</td>
<td>848</td>
<td>3932</td>
<td>Xmr I</td>
<td>3418</td>
<td>6443</td>
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The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine™ section of Life Technologies’ web page, http://www.lifetech.com.
The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the TECH-ONLINE™ section of Life Technologies' web page, http://www.lifetech.com.

Recombination Region of the Expression Clone resulting from pDEST10 x Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 345 and 2167. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST10 by recombination. Non-shaded regions are derived from pDEST10.

Restriction endonucleases that do not cleave pDEST10 DNA:

<table>
<thead>
<tr>
<th>Aaf I</th>
<th>BspE I</th>
<th>EcoO109 I</th>
<th>Nsi I</th>
<th>SexA I</th>
<th>Swa I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aff I</td>
<td>Cla I</td>
<td>Fse I</td>
<td>Pac I</td>
<td>Sfi I</td>
<td>Xcm I</td>
</tr>
<tr>
<td>Apa I</td>
<td>Cva I</td>
<td>Nar I</td>
<td>PinA I</td>
<td>Sgr I</td>
<td></td>
</tr>
<tr>
<td>Asc I</td>
<td>Eco47 III</td>
<td>Nde I</td>
<td>Pme I</td>
<td>SgrA I</td>
<td></td>
</tr>
<tr>
<td>Bpu102 I</td>
<td>Eco72 I</td>
<td>Nhe I</td>
<td>PshA I</td>
<td>Sse8387 I</td>
<td></td>
</tr>
<tr>
<td>Bsg I</td>
<td>EcoN I</td>
<td>Nru I</td>
<td>Psp5 I</td>
<td>Sun I</td>
<td></td>
</tr>
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</table>

Restriction endonucleases that cleave pDEST10 DNA twice:

<table>
<thead>
<tr>
<th>AluN I</th>
<th>1698</th>
<th>4770</th>
<th>BspX I</th>
<th>1905</th>
<th>5630</th>
<th>Not I</th>
<th>462</th>
<th>2233</th>
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</thead>
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<tr>
<td>BamHI I</td>
<td>1377</td>
<td>2191</td>
<td>Dra III</td>
<td>3150</td>
<td>6498</td>
<td>PflM I</td>
<td>583</td>
<td>1150</td>
</tr>
<tr>
<td>Ban I</td>
<td>2220</td>
<td>3077</td>
<td>Eam1105 I</td>
<td>2795</td>
<td>4291</td>
<td>Pst I</td>
<td>2046</td>
<td>2256</td>
</tr>
<tr>
<td>Bgl II</td>
<td>5467</td>
<td>5937</td>
<td>EcoR I</td>
<td>924</td>
<td>2198</td>
<td>Rca I</td>
<td>3456</td>
<td>4464</td>
</tr>
<tr>
<td>BspLU11 I</td>
<td>5184</td>
<td>6166</td>
<td>EcoRV</td>
<td>298</td>
<td>5743</td>
<td>Sal I</td>
<td>2052</td>
<td>2214</td>
</tr>
<tr>
<td>BssSI I</td>
<td>3627</td>
<td>5011</td>
<td>Gsu I</td>
<td>1025</td>
<td>4206</td>
<td>Xmn I</td>
<td>9</td>
<td>3692</td>
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<tr>
<td>BstI107 I</td>
<td>94</td>
<td>1457</td>
<td>Nco I</td>
<td>1225</td>
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The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the TECH-ONLINE™ section of Life Technologies' web page, http://www.lifetech.com.
The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the TECH-ONLINE™ section of Life Technologies' web page, http://www.lifetech.com.
5.6.4 Mammalian Destination Vectors

pDEST12.2 Vector for Native Protein Expression from a CMV Promoter

Recombination Region of the Expression Clone resulting from pDEST12.2 x Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 738 and 2419. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST12.2 by recombination. Non-shaded regions are derived from pDEST12.2. Transcription starts at nucleotide 537.

Restriction endonucleases that do not cleave pDEST12.2 DNA:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>ApaI</th>
<th>BsrEII</th>
<th>EcoRV</th>
<th>PshAI</th>
<th>SnaI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AscI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SwaI</td>
</tr>
<tr>
<td>BglII</td>
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<td></td>
<td>XbaI</td>
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<td>Bpu102I</td>
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Restriction endonucleases that cleave pDEST12.2 DNA twice:

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<th>BsaHI</th>
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<tr>
<td>ApaI</td>
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<td>2304</td>
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<td>4092</td>
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<td>ClaI</td>
<td>3048</td>
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<td>CfoI</td>
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<td>2781</td>
<td>605</td>
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<td>3301</td>
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<td>5281</td>
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<td>3742</td>
<td>4786</td>
<td>5890</td>
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The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tecor-OnLine™ section of Life Technologies’ web page, http://www.lifetech.com.
**Additional Information**

**pDEST26 Vector for N-terminal Histidine Fusion Protein Expression from a CMV Promoter**

Recombination Region of the Expression Clone resulting from pDEST26 x Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 679 and 2360. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST26 by recombination. Non-shaded regions are derived from pDEST26. Transcription starts at nucleotide 537.

Restriction endonucleases that do not cleave pDEST26 DNA:

- **Apa I**
- **Cvn I**
- **Fse I**
- **Pme I**
- **Spe I**
- **Xho I**
- **Asc I**
- **Eco47 III**
- **Mlu I**
- **PshI I**
- **Sse8387 I**
- **Bpu102 I**
- **Eco72 I**
- **Nru I**
- **Psp6 II**
- **Sun I**
- **Bsg I**
- **EcoN I**
- **Pag I**
- **Sgf I**
- **Swa I**
- **BscE II**
- **EcoR V**
- **PinA I**
- **SgrA I**
- **Xcm I**

Restriction endonucleases that cleave pDEST26 DNA twice:

- **Acc I**
- **1650**
- **2245**
- **BstX I**
- **2098**
- **4942**
- **Pst I**
- **2239**
- **4277**
- **AlwN I**
- **1891**
- **6678**
- **Cla I**
- **2990**
- **5008**
- **Pvu I**
- **3074**
- **5832**
- **Ave II**
- **617**
- **4034**
- **KpnI I**
- **605**
- **1113**
- **Sac I**
- **1532**
- **5721**
- **Bsa I**
- **2120**
- **6132**
- **Nde I**
- **187**
- **2604**
- **Xma III**
- **797**
- **4134**
- **BssH II**
- **1611**
- **4625**
- **NgoAI IV**
- **3243**
- **4728**

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLineSM section of Life Technologies' web page, http://www.lifetech.com.
pDEST27 Vector for N-terminal GST Fusion Protein Expression from a CMV Promoter

Recombination Region of the Expression Clone resulting from pDEST27 × Entry Clone. DNA from the Entry Clone replaces the region between nucleotides 1321 and 3002. Shaded regions correspond to those DNA sequences transferred from the Entry Clone into pDEST27 by recombination. Non-shaded regions are derived from pDEST27. Transcription starts at nucleotide 537.

Restriction endonucleases that do not cleave pDEST27 DNA:

- Apa I
- Eco47 III
- Nru I
- Psp5 II
- Sun I

Restriction endonucleases that cleave pDEST27 DNA twice:

- Acc I
- 2292
- 2887
- BsoLu11 I
- 870
- 7734
- NgoA IV
- 3885
- 5370

- Afl III
- 870
- 7734
- BssH II
- 2253
- 5267
- Nsp V
- 1028
- 5551

- AlwNI
- 2533
- 7320
- BstXI I
- 2740
- 5584
- Pst I
- 2881
- 4919

- Avr II
- 617
- 4676
- Cla I
- 3632
- 5650
- Pvu II
- 3716
- 6474

- Bcl I
- 1066
- 3393
- KpnI II
- 605
- 1755
- Xma III
- 1439
- 4776

- Bsa I
- 2762
- 6774
- Nde I
- 187
- 3246
- Xmn I
- 1021
- 6242

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tccl-OnLine™ section of Life Technologies’ web page, http://www.lifetech.com.
5.6.5 Donor Vector for BP Reactions

**pDONRTM201 Vector for Production of KmR Entry Clones**

**pDONRTM201 Vector.** DNA from the PCR product or Expression Clone replaces the region between nucleotides 111 and 2352. The vector contains T1-T2 transcription terminators to minimize possible toxic effects of cloned genes expressing from vector-encoded promoters. pDONR201 Vector must be propagated in DB3.1™ cells because of the ccdB gene.

Restriction Endonucleases that do not cleave pDONRT201:

- **Aaf II**
- **Bgl II**
- **Cfi I**
- **Avr II**
- **Bam HI**
- **Bgl II**
- **BseRI**

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the TECH-OnLINE™ section of Life Technologies’ web page, http://www.lifetech.com.
References

## Related Products

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<th>Product</th>
<th>Size</th>
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<td><strong>GATEWAY™ Products</strong></td>
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<td><strong>Systems (see Section 3.1 for components)</strong></td>
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<td>(with LIBRARY EFFICIENCY® DH5α™ Competent Cells)</td>
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<td>E. coli Expression System (with GATEWAY Technology)</td>
<td>20 reactions</td>
<td>11823-010</td>
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<tr>
<td>(with BL21-SI™ Competent Cells)</td>
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<tr>
<td>Baculovirus Expression System (with GATEWAY Technology)</td>
<td>20 reactions</td>
<td>11827-011</td>
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<td>Mammalian Expression System (with GATEWAY Technology)</td>
<td>20 reactions</td>
<td>11826-013</td>
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<tr>
<td>GATEWAY Vector Conversion System</td>
<td>20 reactions</td>
<td>11828-019</td>
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<tr>
<td><strong>Enzymes</strong></td>
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<tr>
<td>GATEWAY BP CLONASE Enzyme Mix</td>
<td>20 reactions</td>
<td>11789-013</td>
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<tr>
<td>GATEWAY LR CLONASE Enzyme Mix</td>
<td>20 reactions</td>
<td>11791-019</td>
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<tr>
<td><strong>Entry Vectors (see Table 2)</strong></td>
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<tr>
<td>GATEWAY pENTR™1A Vector (500 ng/μl)</td>
<td>20 μl</td>
<td>11813-011</td>
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<tr>
<td>GATEWAY pENTR2B Vector (500 ng/μl)</td>
<td>20 μl</td>
<td>11816-014</td>
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<tr>
<td>GATEWAY pENTR3C Vector (500 ng/μl)</td>
<td>20 μl</td>
<td>11817-012</td>
</tr>
<tr>
<td>GATEWAY pENTR4 Vector (500 ng/μl)</td>
<td>20 μl</td>
<td>11818-010</td>
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<td>GATEWAY pENTR11 Vector (500 ng/μl)</td>
<td>20 μl</td>
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<td><strong>Destination Vectors (see Table 3)</strong></td>
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<tr>
<td>GATEWAY pDEST™14 Vector (150 ng/μl)</td>
<td>40 μl</td>
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<td>GATEWAY pDEST15 Vector (150 ng/μl)</td>
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<td>11802-014</td>
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<tr>
<td>GATEWAY pDEST17 Vector (150 ng/μl)</td>
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<td>GATEWAY pDEST8 Vector (150 ng/μl)</td>
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<tr>
<td>GATEWAY pDEST10 Vector (150 ng/μl)</td>
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<td>11806-015</td>
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<td>GATEWAY pDEST20 Vector (150 ng/μl)</td>
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<tr>
<td>GATEWAY pDEST12.2 Vector (150 ng/μl)</td>
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<tr>
<td>GATEWAY pDEST26 Vector (150 ng/μl)</td>
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<td>GATEWAY pDEST27 Vector (150 ng/μl)</td>
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<td><strong>Donor Vectors</strong></td>
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<tr>
<td>GATEWAY pDONR™201 Vector (150 ng/μl)</td>
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<td><strong>Competent Cells</strong></td>
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<td>LIBRARY EFFICIENCY® DB3.1™ Competent Cells</td>
<td>5 × 0.2 ml</td>
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<tr>
<td>LIBRARY EFFICIENCY® DH5α™ Competent Cells</td>
<td>5 × 0.2 ml</td>
<td>18263-012</td>
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<tr>
<td>BL21-SI Competent Cells</td>
<td>5 × 0.2 ml</td>
<td>11665-015</td>
</tr>
<tr>
<td>MAX EFFICIENCY® DH10Bac™ Competent Cells</td>
<td>5 × 0.1 ml</td>
<td>10361-012</td>
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<tr>
<td><strong>Other Related Products:</strong></td>
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<tr>
<td><strong>Bacterial Expression:</strong></td>
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<tr>
<td>Bluo-gal</td>
<td>100 mg</td>
<td>15519-010</td>
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<tr>
<td>X-gal</td>
<td>100 mg</td>
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<tr>
<td>IPTG</td>
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<td>S.O.C. Medium</td>
<td>10 × 10 ml</td>
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<tr>
<td>Ampicillin Sodium salt, lyophilized</td>
<td>5 ml</td>
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<tr>
<td>Kanamycin Sulfate</td>
<td>1 g</td>
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<tr>
<td>LB Broth (1X), liquid</td>
<td>500 ml</td>
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<td>LB Agar, powder (Lennox L Agar)</td>
<td>500 g</td>
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<td>Product</td>
<td>Size</td>
<td>Cat. No</td>
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<td><strong>Mammalian and Insect Expression:</strong></td>
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<tr>
<td>BAC-TO-BAC® Baculovirus Expression System</td>
<td>5 reactions</td>
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<tr>
<td>LIPOFECTAMINE™ 2000 Reagent</td>
<td>1.5 ml</td>
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<tr>
<td>CELLFECTIN® Reagent</td>
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<tr>
<td>SF-900 II SFM (1X), liquid</td>
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<td>10902-096</td>
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<tr>
<td>Sf9 Cells, SFM Adapted</td>
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<tr>
<td>Sf21 Cells, SFM Adapted</td>
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<tr>
<td>CD-CHO Medium</td>
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<td>CHO-S Cells</td>
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<td>293 SFM II</td>
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<td>293-F Cells</td>
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<td>VP SFM</td>
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<tr>
<td>COS-7L Cells</td>
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<tr>
<td>GENETICIN® Selective Antibiotic, liquid</td>
<td>20 ml</td>
<td>10131-035</td>
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<td><strong>PCR/RT-PCR Products:</strong></td>
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<tr>
<td>Custom Primers-GATEWAY attB modifications*</td>
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<tr>
<td>PLATINUM® Pfx DNA Polymerase</td>
<td>50 units</td>
<td>11708-047</td>
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<tr>
<td>PLATINUM® Taq DNA Polymerase High Fidelity</td>
<td>500 units</td>
<td>11304-029</td>
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<tr>
<td>THERMOSCRIP™ PCR Cloning Enhancer</td>
<td>100 units</td>
<td>11255-014</td>
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<td>THERMOSCRIP™ RT-PCR System plus PLATINUM Taq DNA Polymerase High Fidelity</td>
<td>100 reactions</td>
<td>11146-040</td>
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<td><strong>DNA Purification:</strong></td>
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<tr>
<td>CONCERT High Purity Plasmid Miniprep System</td>
<td>25 reactions</td>
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<td>CONCERT High Purity Plasmid Midiprep System</td>
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<tr>
<td>CONCERT High Purity Plasmid Maxiprep System</td>
<td>10 reactions</td>
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<tr>
<td>CONCERT Rapid Plasmid Miniprep System</td>
<td>50 reactions</td>
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<td>CONCERT Rapid Plasmid Midiprep System</td>
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<td>CONCERT Rapid Plasmid Maxiprep System</td>
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<tr>
<td>CONCERT Matrix Gel Extraction System</td>
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<td>DNA Analysis Products:</td>
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<tr>
<td>CLONECHECKER™ System</td>
<td>100 reactions</td>
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<td>1 Kb PLUS DNA Ladder</td>
<td>250 μg</td>
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<td>Low DNA Mass Ladder</td>
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<tr>
<td>High DNA Mass Ladder</td>
<td>200 μl</td>
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<tr>
<td>Low Melting Point Agarose</td>
<td>50 g</td>
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<tr>
<td>Kodak Digital Science™ EDAS 120 System, Windows version</td>
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<td>10947-042</td>
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<td>Macronintosh version</td>
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<td>10947-059</td>
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</table>
Related Products

Protein Analysis Products:
BENCHMARK Protein Ladder 2 × 250 μl 10747-012
BENCHMARK Prestained Protein Ladder 2 × 250 μl 10748-010

*See our website (lifetech.com) for information about Custom Primers.
**See our website for an updated list of GATEWAY-compatible libraries.

Additional sizes of these products are also available.