pAd/CMV/V5-DEST™ and pAd/PL-DEST™ Gateway® Vectors

Gateway®-adapted destination vectors for cloning and high-level, transient expression in mammalian cells using the ViraPower™ Adenoviral Expression System

Catalog numbers V493-20 and V494-20

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Kit Contents and Storage

Types of Kits

This manual is supplied with the following products. Catalog numbers K4930-00 and K4940-00 are also supplied with the 293A Cell Line, the 293A Cell Line manual, and the ViraPower™ Adenoviral Expression System manual.

Note: The 293A Cell Line and ViraPower™ Adenoviral Expression System manuals are available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ViraPower™ Adenoviral Gateway® Expression Kit</td>
<td>K4930-00</td>
</tr>
<tr>
<td>ViraPower™ Adenoviral Promoterless Gateway® Expression Kit</td>
<td>K4940-00</td>
</tr>
<tr>
<td>pAd/CMV/V5-DEST™ Gateway® Vector</td>
<td>V493-20</td>
</tr>
<tr>
<td>pAd/PL-DEST™ Gateway® Vector</td>
<td>V494-20</td>
</tr>
</tbody>
</table>

Shipping/Storage

The pAd/CMV/V5-DEST™ and pAd/PL-DEST™ Gateway® Vectors are shipped on dry ice. Upon receipt, store at –20°C.

Contents

The following reagents are supplied with the pAd/CMV/V5-DEST™ and pAd/PL-DEST™ Gateway® Vectors. Store at –20°C.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Composition</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAd/CMV/V5-DEST™ or pAd/PL-DEST™</td>
<td>150 ng/μl in TE Buffer, pH 8.0</td>
<td>40 μl</td>
</tr>
<tr>
<td>pAd/CMV/V5-GW//lacZ control plasmid</td>
<td>1 μg/μl in TE Buffer, pH 8.0</td>
<td>10 μl</td>
</tr>
</tbody>
</table>

*TE buffer, pH 8.0: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Product Use

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

The products below may be used with the pAd/CMV/V5-DEST™ and pAd/PL-DEST™ vectors. For more information, refer to our website (www.lifetechnologies.com) or call Technical Support (see page 25).

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ViraPower™ Adenoviral Gateway® Expression Kit</td>
<td>1 kit</td>
<td>K4930-00</td>
</tr>
<tr>
<td>ViraPower™ Adenoviral Promoterless Gateway® Expression Kit</td>
<td>1 kit</td>
<td>K4940-00</td>
</tr>
<tr>
<td>293A Cell Line</td>
<td>3 × 10⁶ cells</td>
<td>R705-07</td>
</tr>
<tr>
<td>Gateway® LR Clonase® II Enzyme Mix</td>
<td>20 reactions</td>
<td>11791-020</td>
</tr>
<tr>
<td>Library Efficiency® DB3.1™ Competent Cells</td>
<td>1 ml (5 × 0.2 ml)</td>
<td>11782-018</td>
</tr>
<tr>
<td>One Shot® TOP10 Chemically Competent E. coli</td>
<td>20 × 50 μl</td>
<td>C4040-03</td>
</tr>
<tr>
<td>Library Efficiency DH5 Chemically Competent E. coli</td>
<td>5 × 0.2 ml</td>
<td>18263-012</td>
</tr>
<tr>
<td>One Shot® coII Survival™ 2 T1® Chemically Competent E. coli</td>
<td>10 × 50 μl</td>
<td>A10460</td>
</tr>
<tr>
<td>PureLink® HiPure Plasmid Miniprep Kit</td>
<td>25 preps</td>
<td>K2100-02</td>
</tr>
<tr>
<td>PureLink® HiPure Plasmid Midiprep Kit</td>
<td>25 preps</td>
<td>K2100-04</td>
</tr>
<tr>
<td>Lipofectamine® 2000</td>
<td>0.75 ml</td>
<td>11668-027</td>
</tr>
<tr>
<td>β-Gal Antiserum</td>
<td>50 μl*</td>
<td>R901-25</td>
</tr>
<tr>
<td>β-Gal Assay Kit</td>
<td>80 ml</td>
<td>K1455-01</td>
</tr>
<tr>
<td>β-Gal Staining Kit</td>
<td>1 kit</td>
<td>K1465-01</td>
</tr>
<tr>
<td>Ampicillin Sodium Salt, irradiated</td>
<td>200 mg</td>
<td>11593-027</td>
</tr>
</tbody>
</table>

Detection of Recombinant Protein

If you express your recombinant protein from pAd/CMV/V5-DEST™, you can use an antibody to the V5 epitope for detection (see table below). Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using chemiluminescent or colorimetric detection methods. A fluorescein isothiocyanate (FITC)-conjugated antibody allows one-step detection in immunofluorescence experiments.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-V5 Antibody</td>
<td>50 μl*</td>
<td>R960-25</td>
</tr>
<tr>
<td>Anti-V5-HRP Antibody</td>
<td>50 μl*</td>
<td>R961-25</td>
</tr>
<tr>
<td>Anti-V5-AP Antibody</td>
<td>125 μl*</td>
<td>R962-25</td>
</tr>
<tr>
<td>Anti-V5-FITC Antibody</td>
<td>50 μl*</td>
<td>R963-25</td>
</tr>
</tbody>
</table>

*Amount supplied is sufficient for 25 western blots or 25 immunostaining reactions, as appropriate.
**Introduction**

Overview

pAd/CMV/V5-DEST™ (36.7 kb) and pAd/PL-DEST™ (34.9 kb) are destination vectors adapted for use with the Gateway® Technology, and are designed to allow high-level, transient expression of recombinant fusion proteins in dividing and non-dividing mammalian cells using Life Technologies’ ViraPower™ Adenoviral Expression System.

A choice of vectors allows you to generate an adenovirus expressing your recombinant protein of interest under the following conditions (see table below).

<table>
<thead>
<tr>
<th>Vector</th>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAd/CMV/V5-DEST™</td>
<td>CMV promoter</td>
<td>For high-level, constitutive expression of the gene of interest</td>
</tr>
<tr>
<td></td>
<td>C-terminal V5</td>
<td>For detection of recombinant protein using the Anti-V5 antibodies</td>
</tr>
<tr>
<td></td>
<td>epitope</td>
<td></td>
</tr>
<tr>
<td>pAd/PL-DEST™</td>
<td>No promoter</td>
<td>Allows expression of the gene or sequence of interest using your promoter of choice</td>
</tr>
<tr>
<td></td>
<td>No 3’ sequences</td>
<td>Allows addition of a C-terminal epitope tag (if desired) and a polyadenylation signal of choice</td>
</tr>
</tbody>
</table>

For more information about the Gateway® Technology and the ViraPower™ Adenoviral Expression System, see page 8.

Continued on next page
Features of the Vectors

The pAd/CMV/V5-DEST™ and pAd/PL-DEST™ vectors contain the following elements:

- Human adenovirus type 5 sequences (Ad 1-458 and 3513-35935) encoding genes and elements (e.g. Left and Right Inverted Terminal Repeats (ITRs), encapsidation signal sequence, late genes) required for proper packaging and production of adenovirus (Hitt et al., 1999; Russell, 2000)

- Human cytomegalovirus (CMV) immediate early promoter for high-level constitutive expression of the gene of interest in a wide range of mammalian cells (in pAd/CMV/V5-DEST™ only) (Andersson et al., 1989; Boshart et al., 1985; Nelson et al., 1987)

- Two recombination sites, attR1 and attR2 for recombinational cloning of the DNA sequence of interest from an entry clone

- Chloramphenicol resistance gene (Cm^R^) located between the two attR sites for counterselection

- The ccdB gene located between the attR sites for negative selection

- C-terminal V5 epitope for detection of the recombinant protein of interest (in pAd/CMV/V5-DEST™ only) (Southern et al., 1991)

- Herpes Simplex Virus thymidine kinase (TK) polyadenylation sequence for efficient transcription termination and polyadenylation of mRNA (in pAd/CMV/V5-DEST™ only) (Cole & Stacy, 1985)

- Ampicillin resistance gene for selection in E. coli

- pUC origin for high-copy replication and maintenance of the plasmid in E. coli

The control plasmid, pAd/CMV/V5-GW/ lacZ, is included for use as a positive expression control in the mammalian cell line of choice.

Continued on next page
The Gateway® Technology

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

1. Clone your gene of interest into a Gateway® entry vector of choice to create an entry clone. **Note:** If you are using pAd/PL-DEST™, your insert will need to include a promoter of choice, the gene or sequence of interest, and a polyadenylation signal.

2. Generate an expression clone by performing an LR recombination reaction between the entry clone and a Gateway® destination vector (e.g. pAd/PL-DEST™ or pAd/CMV/V5-DEST™).

3. Use your expression clone in the ViraPower™ Adenoviral Expression System (see below).

For more information about the Gateway® Technology, refer to the Gateway® Technology with Clonase® II manual. This manual is available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

The ViraPower™ Adenoviral Expression System

The ViraPower™ Adenoviral Expression System facilitates highly efficient, in vitro or in vivo delivery of a target gene to dividing and non-dividing mammalian cells using a replication-incompetent adenovirus. The System utilizes Gateway®-adapted destination vectors to allow highly efficient and rapid creation of adenoviral vectors that circumvent the need for traditional, homologous recombination and the use of recA+ bacteria or extensive DNA manipulation and ligation protocols to construct the recombinant adenovirus genome. To express your gene of interest in mammalian cells using the ViraPower™ Adenoviral Expression System, you will:

1. Create an expression clone in pAd/CMV/V5-DEST™ or pAd/PL-DEST™ using Gateway® Technology (see the previous section).

2. Digest the expression clone with Pac I to expose the viral inverted terminal repeats (ITRs).

3. Transfect your Pac I-digested expression clone into the 293A Cell Line to produce a crude adenoviral stock. Amplify the adenovirus by infecting 293A cells.

4. Titer the adenoviral stock and use it to transduce the mammalian cell line of choice.

5. Assay for “transient” expression of the recombinant protein.

For more information about the ViraPower™ Adenoviral Expression System, refer to the ViraPower™ Adenoviral Expression System manual. For more information about the 293A Cell Line, refer to the 293A Cell Line manual. Both manuals are available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).
Methods

Generating an Entry Clone

Introduction

To recombine your DNA sequence of interest into pAd/CMV/V5-DEST™ or pAd/PL-DEST™, you will need an entry clone containing the DNA sequence of interest. Many entry vectors are available from Life Technologies to facilitate generation of entry clones (see table below). For more information about each vector, see our website (www.lifetechnologies.com) or contact Technical Support (see page 25).

<table>
<thead>
<tr>
<th>Entry Vector</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pENTR™/D-TOPO®</td>
<td>K2400-20</td>
</tr>
<tr>
<td>pENTR™/SD/D-TOPO®</td>
<td>K2420-20</td>
</tr>
<tr>
<td>pENTR™/TEV/D-TOPO®</td>
<td>K2525-20</td>
</tr>
<tr>
<td>pENTR™/1A</td>
<td>11813-011</td>
</tr>
<tr>
<td>pENTR™/2B</td>
<td>11816-014</td>
</tr>
<tr>
<td>pENTR™/3C</td>
<td>11817-012</td>
</tr>
<tr>
<td>pENTR™/4</td>
<td>11818-010</td>
</tr>
<tr>
<td>pENTR™/11</td>
<td>11819-018</td>
</tr>
</tbody>
</table>

Once you have selected an entry vector, refer to the manual for the specific entry vector you are using for instructions to construct an entry clone. All entry vector manuals are available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

Insert Size Limitations

The size of the wild-type adenovirus type 5 genome is approximately 35.9 kb. Studies have demonstrated that recombinant adenovirus can efficiently package up to 108% of the wild-type virus size from E1 and E3-deleted vectors (Bett et al., 1994). Taking into account the size of the elements required for expression from each pAd-DEST vector, we recommend that your DNA sequence or gene of interest not exceed the size indicated for efficient packaging (see table below).

<table>
<thead>
<tr>
<th>Vector</th>
<th>Insert Size Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAd/CMV/V5-DEST™</td>
<td>6.0 kb</td>
</tr>
<tr>
<td>pAd/PL-DEST™</td>
<td>7.5 kb</td>
</tr>
</tbody>
</table>

Continued on next page
Generating an Entry Clone, continued

Points to Consider Before Recombining into pAd/CMV/V5-DEST™

pAd/CMV/V5-DEST™ is a C-terminal fusion vector; however, you may use this vector to express native proteins or C-terminal fusion proteins. Consider the following when generating your entry clone.

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

  \[(G/A)NNATG\]

- If you wish to include the V5 epitope tag, your gene in the entry clone should not contain a stop codon. In addition, the gene should be in frame with the V5 epitope tag after recombination.
- If you do not wish to include the V5 epitope tag, make sure that your gene contains a stop codon in the entry clone.

Refer to the diagram of the recombination region of pAd/CMV/V5-DEST™ on page 12 to help you design a strategy to generate your entry clone.

Points to Consider Before Recombining into pAd/PL-DEST™

pAd/PL-DEST™ allows generation of an adenovirus that contains a gene of interest whose expression is controlled by a promoter of choice. Alternatively, the vector may also be used to express small RNA molecules from their appropriate promoters. To facilitate proper expression of your gene or sequence of interest from pAd/PL-DEST™, you will need to generate an entry clone containing the following:

- A promoter of choice to control expression of the gene or sequence of interest in mammalian cells.
- The gene or sequence of interest. Note that the gene of interest should contain an ATG initiation codon in the context of a Kozak consensus sequence for proper initiation of translation in mammalian cells (Kozak, 1987; Kozak, 1990; Kozak, 1991) and a stop codon.
- A polyadenylation signal sequence of choice for proper transcription termination and polyadenylation of mRNA.

Note: You may also include an N-terminal or C-terminal fusion tag, if desired.

Refer to the diagram of the recombination region of pAd/PL-DEST™ on page 12 to help you design a strategy to generate your entry clone.
Creating Expression Clones

Introduction
After you have generated an entry clone, you will perform the LR recombination reaction to transfer the gene of interest into the pAd/CMV/V5-DEST™ or pAd/PL-DEST™ vector to create your expression clone. To ensure that you obtain the best possible results, we recommend that you read this section and the next section entitled Performing the LR Recombination Reaction (pages 13–15) before beginning.

Experimental Outline
To generate an expression clone, you will:
1. Perform an LR recombination reaction using the attL-containing entry clone and the attR-containing pAd/CMV/V5-DEST™ or pAd/PL-DEST™ vector. Note: Both the entry clone and the destination vector should be supercoiled (see Important Note below).
2. Transform the reaction mixture into a suitable *E. coli* host (see page 13).
3. Select for expression clones (see the next page for illustrations of the recombination region of expression clones in pAd/CMV/V5-DEST™ or pAd/PL-DEST™).

Important
The pAd/CMV/V5-DEST™ and pAd/PL-DEST™ vectors are supplied as supercoiled plasmids. Although the Gateway® Technology manual has previously recommended using a linearized destination vector for more efficient recombination, further testing at Life Technologies has found that linearization of pAd/CMV/V5-DEST™ and pAd/PL-DEST™ is not required to obtain optimal results for any downstream application.

Destination Vectors
Each destination vector is supplied in solution at a concentration of 150 ng/μl in TE Buffer, pH 8.0, and is ready-to-use in the LR recombination reaction.

Propagating the Destination Vectors
If you wish to propagate and maintain the pAd/CMV/V5-DEST™ and pAd/PL-DEST™ vectors, we recommend using One Shot™ ccdB Survival™ 2 T1R Chemically Competent *E. coli* Cells from Life Technologies (see page 5) for transformation. The ccdB Survival™ 2 T1R *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the ccdB gene. To maintain integrity of the vector, select for transformants in media containing 50–100 μg/ml ampicillin or carbenicillin and 15–30 μg/ml chloramphenicol.

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

Continued on next page
Creating Expression Clones, continued

Recombination Region of pAd/CMV/V5-DEST™

The recombination region of the expression clone resulting from pAd/CMV/V5-DEST™ x entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pAd/CMV/V5-DEST™ vector by recombination. Non-shaded regions are derived from the pAd/CMV/V5-DEST™ vector.
- Bases 1414 and 3657 of the pAd/CMV/V5-DEST™ sequence are marked.

Recombination Region of pAd/PL-DEST™

The recombination region of the expression clone resulting from pAd/PL-DEST™ x entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pAd/PL-DEST™ vector by recombination. Non-shaded regions are derived from the pAd/PL-DEST™ vector.
- Bases 519 and 2202 of the pAd/PL-DEST™ sequence are marked.
Performing the LR Recombination Reaction

Introduction

Once you have obtained an entry clone containing your gene of interest, you may perform an LR recombination reaction between the entry clone and pAd/CMV/V5-DEST™ or pAd/PL-DEST™, and transform the reaction mixture into a suitable E. coli host (see E. coli Host) to select for an expression clone. We recommend including a negative control (no LR Clonase® II) in your experiment to help you evaluate your results.

E. coli Host

You may use any recA, endA E. coli strain including TOP10, DH5α™, or equivalent for transformation (see page 5 for ordering information). Do not transform the LR reaction mixture into E. coli strains that contain the F′ episome (e.g. TOP10F′). These strains contain the ccdA gene and will prevent negative selection with the ccdB gene.

LR Clonase® II Enzyme Mix

To catalyze the LR recombination reaction, you will use LR Clonase® II enzyme mix, which is available separately from Life Technologies (see page 5). The LR Clonase® II enzyme mix combines the proprietary enzyme formulation and 5X LR Clonase® Reaction Buffer previously supplied as separate components in LR Clonase® enzyme mix into an optimized single-tube format for easier set-up of the LR recombination reaction. Use the protocol provided on page 14 to perform the LR recombination reaction using LR Clonase® II enzyme mix.

Note: You may perform the LR recombination reaction using LR Clonase® enzyme mix, if desired. To use LR Clonase® enzyme mix, follow the protocol provided with the product. Do not use the protocol for LR Clonase® II enzyme mix provided in this manual.

Materials Needed

You should have the following materials on hand before beginning:

- Purified plasmid DNA of your entry clone (50–150 ng/μl in TE Buffer, pH 8.0)
- pAd/CMV/V5-DEST™ or pAd/PL-DEST™ vector (150 ng/μl in TE Buffer, pH 8.0)
- LR Clonase® II enzyme mix (see page 5); keep at –20°C until immediately before use
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- 2 μg/μl Proteinase K solution (supplied with the LR Clonase® II enzyme mix; thaw and keep on ice until use)
- pENTR™-gus positive control (supplied with the LR Clonase® II enzyme mix)
- Appropriate competent E. coli host and growth media for expression
- S.O.C. Medium
- LB agar plates containing 100 μg/ml ampicillin or carbenicillin to select for expression clones

Continued on next page
Performing the LR Recombination Reaction, continued

**Important**  
Use care when handling the pAd/CMV/V5-DEST™ or pAd/PL-DEST™ plasmid DNA. The pAd-DEST plasmids are large (> 34 kb in size) and excessive manipulations can shear the DNA, resulting in reduced LR recombination efficiency. When working with the pAd-DEST plasmids, do not vortex or pipet the solution vigorously.

**Setting Up the LR Recombination Reaction**  
Follow this procedure to perform the LR reaction between the pAd/CMV/V5-DEST™ or pAd/PL-DEST™ vector and your entry clone. To include a negative control, set up a separate reaction but omit the LR Clonase® II enzyme mix.

1. Add the following components to 0.5 ml microcentrifuge tubes at room temperature and mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry clone (50–150 ng/reaction)</td>
<td>1-7 μl</td>
</tr>
<tr>
<td>pENTR™-gus (50 ng/μl)</td>
<td>--</td>
</tr>
<tr>
<td>Destination vector (300 ng/reaction)</td>
<td>2 μl</td>
</tr>
<tr>
<td>TE Buffer, pH 8.0</td>
<td>4 μl</td>
</tr>
</tbody>
</table>

2. Remove the LR Clonase® II enzyme mix from –20°C and thaw on ice (~2 minutes).
3. Vortex the LR Clonase® II enzyme mix briefly twice (2 seconds each time).
4. To each sample above, add 2 μl of LR Clonase® II enzyme mix. Mix well by pipetting up and down.
   
   **Reminder:** Return LR Clonase® II enzyme mix to –20°C immediately after use.
5. Incubate reactions at 25°C for 1 hour.
   
   **Note:** Extending the incubation time to 18 hours typically yields more colonies.
6. Add 1 μl of the Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Transform 2–3 μl of the LR recombination reaction into a suitable *E. coli* host (follow the manufacturer’s instructions) and select for expression clones.
   
   **Note:** You may store the LR reaction at –20°C for up to 1 week before transformation, if desired.

**What You Should See**  
If you use *E. coli* cells with a transformation efficiency of $1 \times 10^8$ cfu/μg, you should see >5000 colonies if the entire LR reaction is transformed and plated.

*Continued on next page*
Performing the LR Recombination Reaction, continued

Confirming the Expression Clone

The ccdB gene mutates at a low frequency, resulting in a low number of false positives. True expression clones will be ampicillin/carbenicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated ccdB gene will be ampicillin/carbenicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 μg/ml chloramphenicol. A true expression clone should not grow in the presence of chloramphenicol.

Sequencing

To confirm that your gene of interest is in the correct orientation and in frame with a fusion tag (if present), you may sequence your expression construct. We recommend using the following primer binding sites to help you sequence your expression construct. Refer to the diagrams on page 12 for the location of the primer binding sites in each vector.

**Note:** For your convenience, Life Technologies has a custom primer synthesis service. For more information, see our website ([www.lifetechnologies.com](http://www.lifetechnologies.com)) or call Technical Support (see page 25).

<table>
<thead>
<tr>
<th>Vector</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAd/CMV/V5-DEST™</td>
<td>T7 promoter/priming site</td>
<td>5’-TAATACGACTCACTATAGGG-3’</td>
</tr>
<tr>
<td></td>
<td>V5(C-term) reverse priming site</td>
<td>5’-ACCGAGGAGAGGTACTAGGGAT-3’</td>
</tr>
<tr>
<td>pAd/PL-DEST™</td>
<td>pAd forward priming site</td>
<td>5’-GACTTTGACCGTTTACGTGGAGAC-3’</td>
</tr>
<tr>
<td></td>
<td>pAd reverse priming site</td>
<td>5’-CCTTAAGCCACGCACACATTT-3’</td>
</tr>
</tbody>
</table>
Expression and Analysis

Introduction

Once you have obtained purified plasmid DNA of your pAd/CMV/V5-DEST™ or pAd/PL-DEST™ expression construct, you will prepare the vector for use in Life Technologies’ ViraPower™ Adenoviral Expression System by digesting with Pac I. The Pac I-digested vector is used to transfect 293A cells to produce an adenoviral stock. After amplification, this adenoviral stock may be used to transduce your mammalian cell line of choice to express your recombinant protein (see experimental outline below).

1. Generate the adenoviral expression clone containing your DNA of interest. Digest the purified plasmid with Pac I to expose the ITRs.

2. Transfect the 293A producer cell line with your adenoviral expression clone. Harvest cells and prepare a crude viral lysate.

3. Amplify the adenovirus by infecting 293A producer cells with the crude viral lysate. Determine the titer of your adenoviral stock.

4. Add the viral supernatant to your mammalian cell line of interest.

5. Assay for recombinant protein of interest.

Continued on next page
Expression and Analysis, continued

Plasmid Preparation

Once you have generated your pAd/CMV/V5-DEST™ or pAd/PL-DEST™ expression clone, prepare purified plasmid DNA. You may use any method of choice to prepare purified plasmid DNA. We recommend isolating plasmid DNA using the PureLink® HiPure Plasmid Midiprep Kit (see page 5) or CsCl gradient centrifugation.

**Note:** We recommend performing restriction analysis to verify the integrity of your expression construct after plasmid preparation.

Pac I Digestion

Before you can transfect your expression clone into 293A cells, you must expose the left and right viral ITRs on the vector to allow proper viral replication and packaging. Both pAd/CMV/V5-DEST™ and pAd/PL-DEST™ vectors contain Pac I restriction sites (see maps on pages 20 and 22, respectively for the location of the Pac I sites). Digestion of the vector with Pac I allows exposure of the left and right viral ITRs and removal of the bacterial sequences (i.e. pUC origin and ampicillin resistance gene). **Note:** Make sure that your DNA sequence of interest does not contain any Pac I restriction sites.

1. Digest at least 5 μg of purified plasmid DNA of your pAd/CMV/V5-DEST™ or pAd/PL-DEST™ expression construct with Pac I (New England Biolabs, Cat. no. R0547S). Follow the manufacturer’s instructions.
2. Purify the digested plasmid DNA using phenol/chloroform extraction followed by ethanol precipitation or a DNA purification kit (e.g. Life Technologies’ PureLink® HiPure Plasmid Miniprep Kit; see page 5). **Note:** Gel purification is not required.
3. Resuspend or elute the purified plasmid, as appropriate in sterile water or TE Buffer, pH 8.0 to a final concentration of 0.1–3.0 μg/μl.

Positive Control

pAd/CMV/V5-GW/β-galactosidase is included with the each kit for use as a positive control for expression in the ViraPower™ Adenoviral Expression System. In pAd/CMV/V5-GW/β-galactosidase is expressed as a C-terminally tagged fusion protein that may be easily detected by western blot or functional assay. To use pAd/CMV/V5-GW/β-galactosidase as a positive control, you will need to digest the vector with Pac I using the protocol above. The Pac I-digested plasmid may then be used in your transfection experiment to generate an adenoviral stock.

For details about the vector, see page 24. To propagate and maintain the plasmid:

1. Use the 1 μg/μl stock solution provided to transform a recA, endA E. coli strain like TOP10, DH5α™-T18, or equivalent. Use 10 ng of plasmid for transformation.
2. Select transformants on LB agar plates containing 50–100 μg/ml ampicillin or carbenicillin.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

*Continued on next page*
Expression and Analysis, continued

**Important**

**Reminder:** Use care when handling your pAd-DEST expression clone and pAd/CMV/V5-GW/lacZ plasmid DNA. The adenoviral plasmids are large (>34 kb in size) and excessive manipulations can shear the DNA, resulting in reduced transfection efficiency and lower viral titers. When working with the plasmids, **do not vortex or pipet the solution vigorously.**

**Materials to Have on Hand**

To express your gene of interest from pAd/CMV/V5-DEST™ or pAd/PL-DEST™ using Life Technologies’ ViraPower™ Adenoviral Expression System, you will need to have the following reagents:

- A cell line that stably expresses the E1 proteins (E1a and E1b) for producing viral stocks. We recommend using the 293A Cell Line. This cell line, a subclone of the 293 cell line, supplies the E1 proteins required for production of replication-competent adenovirus and exhibits a flattened morphology to enhance visualization of plaques.

- Transfection reagent for efficient delivery of the pAd/CMV/V5-DEST™ or pAd/PL-DEST™ expression construct to 293A cells. We recommend using Lipofectamine® 2000 Reagent for optimal transfection efficiency.

For more information about the 293A Cell Line and Lipofectamine® 2000 Reagent, see the 293A Cell Line manual and the Lipofectamine® 2000 Reagent manual, respectively. Both manuals are available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

**Obtaining Reagents**

The 293A Cell Line and Lipofectamine® 2000 Reagent are available separately from Life Technologies (see page 5 for ordering information). The 293A Cell Line is also supplied with each ViraPower™ Adenoviral Expression System (Cat. nos. K4930-00 and K4940-00).

*Continued on next page*
Expression and Analysis, continued

Producing Viral Stocks and Transducing Mammalian Cells

Refer to the ViraPower™ Adenoviral Expression System manual for detailed guidelines and protocols to:

- Transfect your Pac I-digested pAd/CMV/V5-DEST™ or pAd/PL-DEST™ expression construct into the 293A Cell Line to generate an adenoviral stock. Remember to generate an adenoviral stock of the pAd/CMV/V5-GW/lacZ positive control.
- Amplify the adenovirus by infecting 293A cells.
- Determine the titer of your adenoviral stock.
- Transduce your Ad/CMV/V5-DEST™ or Ad/PL-DEST™ adenoviral construct into the mammalian cell line of interest at the appropriate multiplicity of infection (MOI).

Detecting Recombinant Protein

To detect expression of your recombinant fusion protein from pAd/CMV/V5-DEST™ or pAd/PL-DEST™, you may perform:

- Western blot analysis using an antibody to your protein or the Anti-V5, Anti-V5-HRP, or Anti-V5-AP antibodies available from Life Technologies (pAd/CMV/V5-DEST™ only).
- Immunofluorescence using an antibody to your protein or the Anti-V5-FITC antibody available from Life Technologies (pAd/CMV/V5-DEST™ only).
- Functional analysis

For more information about the Anti-V5 antibodies, refer to our website (www.lifetechnologies.com) or call Technical Support (see page 25). Ordering information is provided on page 5.

Assay for β-galactosidase Activity

The β-galactosidase protein expressed from the pAd/CMV/V5-GW/lacZ control, adenoviral construct is approximately 120 kDa in size. You may assay for β-galactosidase expression by western blot analysis, activity assay using cell-free lysates (Miller, 1972), or by staining the cells for activity. Life Technologies offers the β-Gal Antiserum, β-Gal Assay Kit, and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page 5 for ordering information).

Note: You may also detect β-galactosidase expression using the Anti-V5 antibodies.

Note

The C-terminal peptide containing the V5 epitope and the attB2 site will add approximately 4.3 kDa to the size of your protein.
Appendix

Map and Features of pAd/CMV/V5-DEST™

The map below shows the elements of pAd/CMV/V5-DEST™. DNA from the entry clone replaces the region between bases 1414 and 3657. The vector sequence of pAd/CMV/V5-DEST™ is available from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

Comments for pAd/CMV/V5-DEST™
36686 nucleotides

Human Ad5 sequences (wt 1-458; includes 5’ L-ITR and packaging signal): 1-458
pAd forward priming site: bases 361-384
CMV promoter: bases 728-1315
T7 promoter/priming site: bases 1359-1378
attR1 site: bases 1407-1531
ccdB gene: bases 1960-2265 (C)
Chloramphenicol resistance gene (CmR): bases 2607-3266 (C)
attR2 site: bases 3547-3671
V5 epitope: bases 3697-3738
TK polyadenylation signal: bases 3765-4036
Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3’ R-ITR): bases 4056-34604
pAd reverse priming site: bases 4059-4082
pUC origin: bases 34781-35442 (C)
Ampicillin (bla) resistance gene: bases 35568-36428 (C)
bla promoter: bases 36429-36527 (C)
Pac I restriction sites: bases 34610 and 36684
(C) = complementary strand

Continued on next page
## Features of the Vector

The pAd/CMV/V5-DEST™ vector (36686 bp) contains the following elements. All features have been functionally tested.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human adenovirus type 5 sequences</td>
<td>Encodes all elements (except E1 and E3 proteins) required to produce replication-incompetent adenovirus (Russell, 2000) including:</td>
</tr>
</tbody>
</table>
| (corresponds to wild-type 1–458 and 3513–35935 sequence) | • Left and right ITRs  
• Encapsidation signal for packaging  
• E2 and E4 regions  
• Late genes                                      |
| **Note:** The E1 and E3 regions are deleted.            |                                                                                                                                       |
| pAd forward priming site                             | Allows sequencing of the insert.                                                                                                     |
| CMV promoter                                         | Allows high-level expression of the gene of interest (Andersson et al., 1989; Boshart et al., 1985; Nelson et al., 1987).                 |
| T7 promoter/priming site                             | Allows *in vitro* transcription in the sense orientation and sequencing through the insert.                                           |
| *att*R1 and *att*R2 sites                            | Bacteriophage λ-derived DNA recombination sequences that permit recombinational cloning of the gene of interest from a Gateway® entry clone (Landy, 1989). |
| *cddB* gene                                          | Allows negative selection of the plasmid.                                                                                             |
| Chloramphenicol resistance gene (Cm<sup>4</sup>)      | Allows counterselection of the plasmid.                                                                                               |
| V5 epitope                                           | Allows detection of the recombinant fusion protein by the Anti-V5 Antibodies (Southern et al., 1991).                                   |
| Herpes Simplex Virus thymidine kinase (TK) polyadenylation signal | Allows efficient transcription termination and polyadenylation of mRNA (Cole & Stacy, 1985).                                         |
| pAd reverse priming site                             | Allows sequencing of the insert in the anti-sense orientation.                                                                      |
| pUC origin                                           | Allows high-copy replication and maintenance in *E. coli*.                                                                            |
| *bla* promoter                                        | Allows expression of the ampicillin resistance gene.                                                                                 |
| Ampicillin resistance gene (β-lactamase)              | Allows selection of the plasmid in *E. coli*.                                                                                         |
| *Pac* I restriction sites (positions 34610 and 36684)  | Allows exposure of the left and right ITRs required for viral replication and packaging.                                              |
Map and Features of pAd/PL-DEST™

The map below shows the elements of pAd/PL-DEST™. DNA from the entry clone replaces the region between bases 519 and 2202. The vector sequence of pAd/PL-DEST™ is available from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

Comments for pAd/PL-DEST™
34864 nucleotides

Human Ad5 sequences (wt 1-458; includes 5′ L-ITR and packaging signal): 1-458
pAd forward priming site: bases 361-384
attR1 site: bases 512-636
Chloramphenicol resistance gene (CmR): bases 745-1404
ccdB gene: bases 1746-2051
attR2 site: bases 2092-2216
Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3′ R-ITR): bases 2234-32782
pAd reverse priming site: bases 2237-2260
pUC origin: bases 32959-33620 (C)
Ampicillin (bla) resistance gene: bases 33746-34606 (C)
bla promoter: bases 34607-34705 (C)
Pac I restriction sites: bases 32788 and 34862
(C) = complementary strand

Continued on next page
Map and Features of pAd/PL-DEST™, continued

The pAd/PL-DEST™ vector (34864 bp) contains the following elements. All features have been functionally tested.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human adenovirus type 5 sequences (corresponds to wild-type 1–458 and 3513–35935 sequence) &lt;br&gt;<strong>Note:</strong> The E1 and E3 regions are deleted.</td>
<td>Encodes all elements (except E1 and E3 proteins) required to produce replication-incompetent adenovirus (Russell, 2000) including: &lt;br&gt;- Left and right ITRs &lt;br&gt;- Encapsidation signal for packaging &lt;br&gt;- E2 and E4 regions &lt;br&gt;- Late genes</td>
</tr>
<tr>
<td>pAd forward priming site</td>
<td>Allows sequencing of the insert.</td>
</tr>
<tr>
<td><em>att</em>&lt;sub&gt;R1&lt;/sub&gt; and <em>att</em>&lt;sub&gt;R2&lt;/sub&gt; sites</td>
<td>Bacteriophage λ-derived DNA recombination sequences that permit recombinational cloning of the DNA sequence of interest from a Gateway&lt;sup&gt;®&lt;/sup&gt; entry clone (Landy, 1989).</td>
</tr>
<tr>
<td>Chloramphenicol resistance gene (Cm&lt;sup&gt;R&lt;/sup&gt;)</td>
<td>Allows counterselection of the plasmid.</td>
</tr>
<tr>
<td><em>ccdB</em> gene</td>
<td>Allows negative selection of the plasmid.</td>
</tr>
<tr>
<td>pAd reverse priming site</td>
<td>Allows sequencing of the insert in the anti-sense orientation.</td>
</tr>
<tr>
<td>pUC origin</td>
<td>Allows high-copy replication and maintenance in <em>E. coli</em>.</td>
</tr>
<tr>
<td><em>bla</em> promoter</td>
<td>Allows expression of the ampicillin resistance gene.</td>
</tr>
<tr>
<td>Ampicillin resistance gene (&lt;span&gt;β&lt;/span&gt;-lactamase)</td>
<td>Allows selection of the plasmid in <em>E. coli</em>.</td>
</tr>
<tr>
<td><em>Pac</em> I restriction sites (positions 32788 and 34862)</td>
<td>Allows exposure of the left and right ITRs required for viral replication and packaging.</td>
</tr>
</tbody>
</table>
**Map of pAd/CMV/V5-GW/lacZ**

**Description**

pAd/CMV/V5-GW/lacZ is a 37567 bp control vector expressing β-galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the lacZ gene and pAd/CMV/V5-DEST™. β-galactosidase is expressed as a C-terminal V5 fusion protein with a molecular weight of approximately 120 kDa.

**Map of pAd/CMV/V5-GW/lacZ**

The map below shows the elements of pAd/CMV/V5-GW/lacZ. The vector sequence of the vector is available from our website (www.lifetechnologies.com) or by contacting Technical Support (see page 25).

**Comments for pAd/CMV/V5-GW/lacZ**

37567 nucleotides

Human Ad5 sequences (wt 1-458; includes 5’ L-ITR and packaging signal): 1-458
- pAd forward priming site: bases 361-384
- CMV promoter: bases 728-1315
- T7 promoter/priming site: bases 1359-1378
- attB1 site: bases 1407-1431
- lacZ ORF: bases 1452-4508
- attB2 site: bases 4528-4552
- V5 epitope: bases 4578-4619
- TK polyadenylation signal: bases 4646-4917
- Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3’ R-ITR): bases 4937-35485
- pAd reverse priming site: bases 4940-4963
- pUC origin: bases 35662-36323 (C)
- Ampicillin (bla) resistance gene: bases 36449-37309 (C)
- bla promoter: bases 37310-37408 (C)
- Pac I restriction sites: bases 35491 and 37565
(C) = complementary strand
Technical Support

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

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)

Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

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**Gateway® Clone Distribution Policy**

For additional information about Life Technologies’ policy for the use and distribution of Gateway® clones, see the section entitled Gateway® Clone Distribution Policy, page 27.

*Continued on next page*
Gateway® Clone Distribution Policy

Introduction

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

Gateway® Entry Clones

Life Technologies understands that Gateway® entry clones, containing attL1 and attL2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Life Technologies.

Gateway® Expression Clones

Life Technologies also understands that Gateway® expression clones, containing attB1 and attB2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Life Technologies. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee ($10 per clone) payable to Life Technologies.

Additional Terms and Conditions

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


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Notes