invitrogen™

# Champion™ pET300/NT-DEST and pET301/CT-DEST Gateway® Vectors

## Destination vectors for high-level, inducible expression of N- and C-terminal 6xHis-tagged protein in *E. coli*

**Catalog no. K6300-01**

**Version D**
June 23, 2010
*25-0956*

User Manual

# Table of Contents

# Important Information

## Shipping and Storage

Champion™ pET300/NT-DEST, pET301/CT-DEST and pET300/NT-GW/Rac Kinase vectors are shipped at room temperature. Upon receipt, store lyophilized vectors at -20°C.

## Contents

The Champion™ pET300/NT-DEST and pET301/CT-DEST vector kit contains the N- and C-terminal destination vectors and an expression control plasmid as listed below:

| Item | Concentration | Amount |
|---|---|---|
| pET300/NT-DEST | lyophilized in TE, pH 8.0 | 6 µg |
| pET301/CT-DEST | lyophilized in TE, pH 8.0 | 6 µg |
| pET300/NT-GW/Rac Kinase | lyophilized in TE, pH 8.0 | 10 µg |

# Accessory Products

## Additional Products

Additional products that may be used with pET300/NT-DEST and pET301/CT-DEST are available from Invitrogen. Ordering information is provided below.

| Product | Amount | Catalog no. |
|---|---|---|
| Gateway® LR Clonase™ II Enzyme Mix | 20 reactions<br>100 reactions | 11791-020<br>11791-100 |
| One Shot® *ccd*B Survival T1$^R$ Chem. Competent Cells | 10 reactions | C7510-03 |
| BL21(DE3) Chem. Competent Cells | 20 x 50 µl | C6000-03 |
| One Shot® BL21 Star (DE3) Chem. Competent Cells | 20 x 50 µl | C6010-03 |
| One Shot® BL21(DE3) pLysS Chem. Competent Cells | 20 x 50 µl | C6060-03 |
| MagicMedia™ *E. coli* Expression Medium | 1 L SoluPouch™<br>5 x 1 L SoluPouch™<br>1 L liquid | K6801<br>K6802<br>K6803 |
| Ampicillin | 5 g | Q100-16 |
| Carbenicillin | 5 g | 10177-012 |

## Detection and Purification of Recombinant Fusion Protein

If your gene of interest is in frame with the N- or C-terminal polyhistidine (6x His) tag, you may detect your fusion protein with an antibody to the polyhistidine tag. You may also purify your recombinant fusion protein using a metal chelating system.

| Product | Quantity | Catalog no. |
|---|---|---|
| Mouse anti-His Tag monoclonal antibody | 100 µg | 37-2900 |
| ProBond™ Nickel-chelating Resin | 50 ml | R801-01 |
| | 150 ml | R801-15 |
| ProBond™ Purification System | 6 purifications | K850-01 |
| Ni-NTA Agarose | 10 ml<br>25 ml<br>100 ml | R901-15<br>R901-25<br>R901-10 |
| Purification Columns<br>(10 ml polypropylene columns) | 50 | R640-50 |

# Methods

# Overview

## Description

Champion™ pET300/NT-DEST and pET301/CT-DEST are ~7.3 kb vectors adapted for use with the Gateway® Technology. They are designed to allow high-level, inducible expression of recombinant fusion proteins in *E. coli* using the pET system. Both Champion™ pET300/NT-DEST and pET301/CT-DEST are included, allowing you to choose the best configuration and protein expression levels for your needs. A control expression plasmid, pET300/NT-GW/Rac Kinase, is included to optimize protein expression.

## The pET Expression System

The pET system was originally developed by Studier and colleagues and takes advantage of the high activity and specificity of the bacteriophage T7 RNA polymerase to allow regulated expression of heterologous genes in *E. coli* from the T7 promoter (Rosenberg *et al.*, 1987; Studier & Moffatt, 1986; Studier *et al.*, 1990). For more information about T7-regulated expression, see the next page.

## Features

Champion™ pET300/NT-DEST and pET301/CT-DEST vectors contain the following elements:

- T7*lac* promoter for high-level expression of the gene of interest in *E. coli* (see next page for more information)
- Two recombination sites, *att*R1 and *att*R2, downstream of the T7 promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene located between the two *att*R sites for counterscreening
- The *ccd*B gene located between the two *att*R sites for negative selection
- N- or C-terminal 6x His tag for detection and purification
- Ampicillin resistance gene for selection in *E. coli*
- pBR322 origin for low-copy replication and maintenance of the plasmid in *E. coli*
- *lac*I gene encoding the lac repressor to reduce basal transcription from the T7*lac* promoter

For maps of pET300/NT-DEST and pET301/CT-DEST, see pages 16-17.

*Continued on next page*

# Overview, continued

## 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 gene of interest into multiple vector systems. To express your gene of interest using Gateway® cloning technology, simply:

1. Clone your gene of interest into a Gateway® entry vector to create an entry clone.
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and pET300/NT-DEST or pET301/CT-DEST
3. Transform your expression clone into a BL21 strain of choice, and induce expression of your protein with your method of choice.

For more information on the Gateway® System, refer to the Gateway® Technology Manual. This manual is available for downloading from www.invitrogen.com or by contacting Technical Support (page 20).

## T7-Regulated Expression

pET300/NT-DEST and pET301/CT-DEST contain elements from bacteriophage T7 to control expression of heterologous genes in *E. coli*. In the vector, expression of the gene of interest is controlled by a strong bacteriophage T7 promoter that has been modified to contain a *lac* operator sequence (see next page). In bacteriophage T7, the T7 promoter drives expression of gene 10. T7 RNA polymerase specifically recognizes this promoter. To express the gene of interest, it is necessary to deliver T7 RNA polymerase to the cells by inducing expression of the polymerase.

*Continued on next page*

# Overview, continued

### T7*lac* Promoter

pET300/NT-DEST and pET301/CT-DEST contain the T7*lac* promoter to drive expression of the gene of interest. The T7*lac* promoter consists of a *lac* operator sequence placed downstream of the T7 promoter. The *lac* operator serves as a binding site for the lac repressor (encoded by the *lac*I gene) and functions to further repress T7 RNA polymerase-induced basal transcription of the gene of interest in BL21 strains.

### BL21 Strains

The BL21(DE3) *E. coli* strain is specifically designed for expression of genes regulated by the T7 promoter. Basal level expression of T7 polymerase, particularly in BL21(DE3) cells, may lead to plasmid instability if your gene of interest is toxic to *E. coli*. You may also use BL21 Star™(DE3) and BL21 Star™(DE3)pLysS strains if your protein is toxic to *E. coli*. See page v for ordering information.

# Methods

## Generating an Entry Clone

### Introduction

To recombine your gene of interest into pET300/NT-DEST or pET301/CT-DEST, you will need one or more entry clones containing the gene of interest. Refer to the manual for the specific entry vector you are using for detailed instructions to construct an entry clone.

### Choosing an Entry Vector

You may generate entry clones in a number of ways:

- Use pENTR™/D-TOPO®, pCR8®/GW/TOPO® or pENTR™/SD/D-TOPO (for pET301/CT-DEST) to rapidly clone of your promoter and gene of interest using TOPO® Cloning technology.
- Perform a BP recombination reaction using a PCR product containing *att*B sites and an *att*P-containing pDONR™ vector to create your entry clone. A large selection of pDONR™ vectors is available from Invitrogen.

For more information about these products, go to www.invitrogen.com or contact Technical Support (page 20).

### Recombining into pET300/NT-DEST

pET300/NT-DEST is an N-terminal fusion vector and contains an ATG initiation codon and a Shine-Dalgarno ribosome binding site (RBS) with optimal spacing for proper translation. Your gene of interest in the entry clone must:

- Be in frame with the N-terminal tag after recombination.
- Contain a stop codon.

Refer to the diagram of the recombination region of pET300/NT-DEST on page 7 for more information.

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# Generating an Entry Clone, continued

### Recombining into pET301/CT-DEST

To recombine your gene of interest into pET301/CT-DEST your entry clone must include:

- A Shine-Delgarno ribosome binding sequence
- An ATG initiation sequence
- If you wish to include the 6x His tag, your gene in the entry clone **should not** contain a stop codon.
- If you DO NOT wish to fuse your gene of interest to the 6xHis tag, your gene should contain a stop codon in the entry clone.

Refer to the diagram of the recombination region of pET301/CT-DEST on page 8 for more information.

# Generating an Expression Clone

## Introduction

After you have generated an entry clone, you will perform the LR recombination reaction to transfer the gene of interest into the pET300/NT-DEST or pET301/CT-DEST vector to create your expression clone. To ensure that you obtain the best results, we recommend that you read this section and the next section entitled **Performing the LR Recombination Reaction** (pages 9-12) before beginning.

## Experimental Outline

To generate an expression clone, you will:

1. Perform an LR recombination reaction using the *att*L-containing entry clone(s) and the *att*R-containing pET300/NT-DEST or pET301/CT-DEST.
2. Transform the reaction mixture into suitable *E. coli*.
3. Select for expression clones (refer to pages 7-8 for a diagram of the recombination regions of the resulting expression clones).

## Resuspending Vectors

Resuspend pET300/NT-DEST and pET301/CT-DEST to 150 ng/µl each in sterile water.

## Propagating the Vectors

If you wish to propagate and maintain pET300/NT-DEST and pET301/CT-DEST, we recommend using One Shot® *ccd*B Survival T1$^R$ Chemically Competent *E. coli* from Invitrogen for transformation. The *ccd*B Survival T1$^R$ *E. coli* strain is resistant to ccdB effects and can support the propagation of plasmids containing the *ccd*B gene. To maintain the integrity of the vector, select for transformants in media containing 50-100 µg/ml ampicillin and 15-30 µg/ml chloramphenicol.

**Note: Do not** use general *E. coli* cloning strains including TOP10 or DH5 for propagation and maintenance as these strains are sensitive to ccdB effects.

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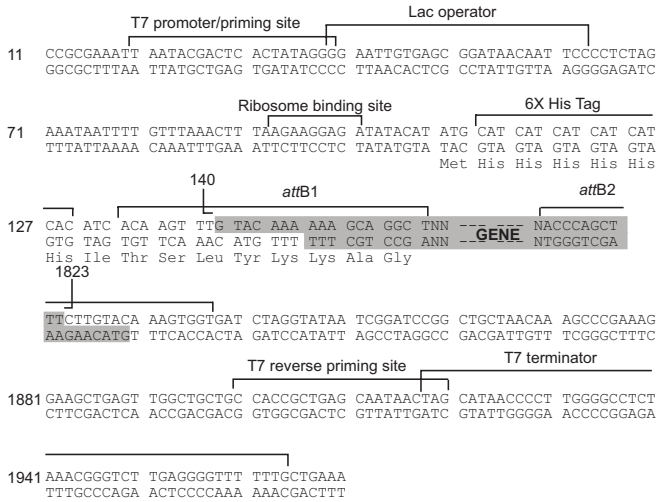
# Generating an Expression Clone, continued

## Recombination Region of pET300/NT-DEST

The recombination region of the expression clone resulting from pET300/NT-DEST × entry clone is shown below.

**Features of the Recombination Region:**

- Shaded regions correspond to DNA sequences transferred from the entry clone into pET300/NT-DEST by recombination. Non-shaded regions are derived from the pET300/NT-DEST vector.
- The underlined nucleotides flanking the shaded region correspond to bases 140 and 1823, respectively, of the pET300/NT-DEST vector sequence.

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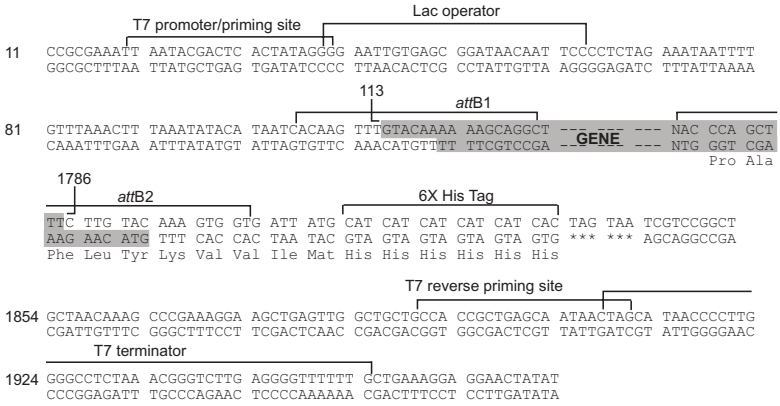
# Generating an Entry Clone, continued

## Recombination Region of pET301/CT-DEST

The recombination region of the expression clone resulting from pET301/CT-DEST × entry clone is shown below.

**Features of the Recombination Region:**

- Shaded regions correspond to DNA sequences transferred from the entry clone into pET301/CT-DEST by recombination. Non-shaded regions are derived from the pET301/CT-DEST vector.
- The underlined nucleotides flanking the shaded region correspond to bases 113 and 1786, respectively, of the pET301/CT-DEST vector sequence.



# 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 pET300/NT-DEST or pET301/CT-DEST and transform the reaction mixture into a suitable *E. coli* host (see below) to select for an expression clone. We recommend including a negative control (no LR Clonase™ II) to help you evaluate your results.

## *E. coli* Host

You may use any *rec*A, *end*A *E. coli* strain including TOP10, DH5α™, or equivalent for transformation (see page v for ordering information).

**Do not** transform the LR reaction mixture into *ccd*B Survival T1$^R$ Chemically Competent *E. coli* or *E. coli* strains that contain the F′ episome (*e.g.* TOP10F′). These strains contain the *ccd*A gene and will prevent negative selection with the *ccd*B gene.

## LR Clonase™ II Enzyme Mix

LR Clonase™ II enzyme mix is available separately from Invitrogen. The LR Clonase™ II enzyme mix combines the proprietary enzyme formulation and 5X LR Clonase™ Reaction Buffer in an optimized single-tube format for easy set-up of the LR recombination reaction. Use the protocol on page 11 to perform the LR recombination reaction using LR Clonase™ II enzyme mix.

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# Performing the LR Recombination Reaction, continued

## Materials Needed

You should have the following materials:

- Purified plasmid DNA of your entry clone (150 ng/µl in TE, pH 8.0)
- pET300/NT-DEST or pET301/CT-DEST (150 ng/µl in TE, pH 8.0)
- LR Clonase™ II enzyme mix (keep at -20°C until immediately before use)
- 1X TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- 2 µg/µl Proteinase K solution (supplied with the enzyme mix; thaw and keep on ice until use)
- Appropriate competent *E. coli* host and growth media
- S.O.C. Medium
- LB agar plates containing 100 µg/ml ampicillin

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# Performing the LR Recombination Reaction, continued

## Setting Up the LR Reaction

Follow this procedure to perform the LR reaction between your entry clone and a destination vector. To include a negative control, set up a second sample reaction, but omit the LR Clonase™ II enzyme mix.

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

| Component | Sample |
|---|---|
| Entry clone (50-150 ng/rxn) | 1-7 µl |
| Destination vector (150 ng/µl) | 1 µl |
| 1X TE Buffer, pH 8.0 | to 8 µl |

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 1 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.

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# Performing the LR Recombination Reaction, continued

## What You Should See

If you use *E. coli* cells with a transformation efficiency of $\geq 1 \times 10^8$ cfu/µg, a typical LR reaction should give > 5,000 colonies if the entire reaction is transformed and plated.

## Confirming the Expression Clone

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

## Sequencing

To confirm that your gene of interest is in frame with the N- or C-terminal 6X His tag, you may sequence your expression construct, if desired. Refer to the diagrams on pages 7-8 for the location of the primer binding sites.

For your convenience, Invitrogen offers a custom primer synthesis service. For more information, go to www.invitrogen.com or contact Technical Support (page 20).

| Primer | Sequence |
|---|---|
| T7 Promoter | 5′-TAATACGACTCACTATAGGG-3′ |
| T7 Reverse | 5′-TAGTTATTGCTCAGCGGTGG-3′ |

## The Next Step

Once you have generated your expression clone, you will need to transform it into a BL21 *E. coli* strain for expression studies. See page v for recommended BL21 host strains and media, and proceed to **Expression and Analysis**, next section.

# Expression and Analysis

## Introduction

This section provides general guidelines for expressing and analyzing your protein of interest. For detailed information on transforming your BL21 strain, inducing expression, and analyzing samples, refer to your specific BL21 *E. coli* strain manual.

## Basic Strategy

The basic steps needed to induce expression of your gene in a BL21 *E. coli* strain are outlined below.

1. Isolate plasmid DNA using standard procedures and transform your construct into BL21 cells (see page 3). Use pET303/NT-GW/Rac Kinase included with the kit as a positive control (see next page)
2. Grow the transformants and induce expression over several hours. Take several time points to determine the optimal time of expression. Alternatively, you can grow *E. coli* using MagicMedia™ (see Recommendation, below).


MagicMedia *E. coli* Expression Medium allows high yield of T7-regulated heterologous protein expression without time-consuming steps such as monitoring O.D. or adding inducing agents such as IPTG. MagicMedia™ is available separately from Invitrogen, see page v for ordering information or go to www.invitrogen.com for more details.

## Plasmid Preparation

You may prepare plasmid DNA using your method of choice. We recommend using the PureLink™ HiPure Plasmid Midiprep Kit for isolation of pure plasmid DNA. Note that since you are purifying a low-copy number plasmid, you may need to increase the amount of bacterial culture that you use to prepare your plasmid construct.

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# Expression and Analysis, continued

## Choosing a Selection Agent

For most purposes, ampicillin works well for selection of transformants and expression experiments. However, if you find that your expression level is low, you may want to use carbenicillin instead. The resistance gene for ampicillin encodes a protein called β-lactamase. This protein is secreted into the medium where it hydrolyzes ampicillin, inactivating the antibiotic. Since β-lactamase is catalytic, ampicillin is rapidly removed from the medium, resulting in non-selective conditions. If your plasmid is unstable, this may result in the loss of plasmid and low expression levels.

## Using Carbenicillin

Carbenicillin is generally more stable than ampicillin, and studies have shown that using carbenicillin in place of ampicillin may help to increase expression levels by preventing loss of the expression plasmid. If you wish to use carbenicillin, perform your transformation and expression experiments in LB containing 50 µg/ml carbenicillin.

**Note:** If your gene of interest is highly toxic, increasing the concentration of carbenicillin used from 50 µg/ml to 200 µg/ml may help to increase expression levels.

## Expression Control Vector pET303/NT-GW/Rac Kinase

pET303/NT-GW/Rac Kinase is provided for use as a positive control vector for protein synthesis in a suitable *E. coli* host. This vector allows expression of a 6x His N-terminally tagged fusion protein of 57.7 kDa (Jones *et al.*, 1991). For details about the vector, see page 19. To propagate and maintain the plasmid:

1. Resuspend the vector in 10 µl of sterile water to prepare a 1 µg/µl stock solution.
2. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1$^R$, or equivalent. Use 10 ng of plasmid for transformation.
3. Select transformants on LB agar plates containing 50-100 µg/ml ampicillin.
4. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

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# Expression and Analysis, continued

### Detection of Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by western blot analysis, you may use antibodies against the polyhistidine tag (such as mouse anti-His monoclonal antibody, available separately from Invitrogen) or an antibody to your protein of interest. For more information see page v, go to www.invitrogen.com, or contact Technical Support (page 20).

**Note**

The N-terminal peptide containing the 6x His tag will add approximately 2 kDa to your protein. The C-terminal peptide containing the 6x His tag will add approximately 2.5 kDa to your protein.

### Purification of Recombinant Fusion Proteins

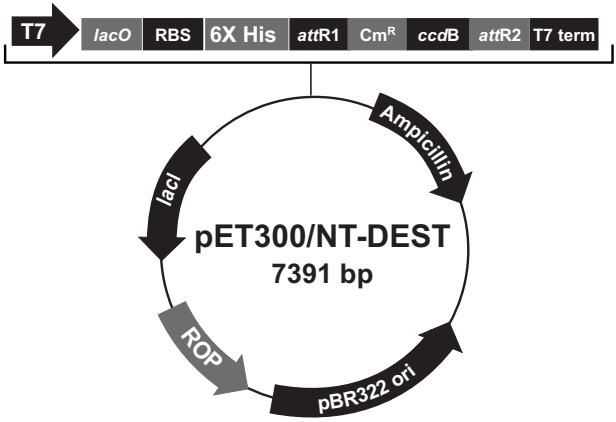
The presence of the N- or C-terminal 6x His tag in your recombinant fusion protein allows use of a metal-chelating resin such as ProBond™ to purify your fusion protein. The ProBond™ Purification System and bulk ProBond™ resin are available from Invitrogen (see page v for ordering information). Invitrogen also offers Ni-NTA Agarose for purification of proteins containing a 6x His tag.
**Note:** Other metal-chelating resins and purification methods are suitable.

# Appendix

# Map of pET300/NT-DEST

## Map of pET300/NT-DEST

The map below shows the elements of pET300/NT-DEST. DNA from the entry clone replaces the region between bases 140 and 1823. **The complete sequence of pET300/NT-DEST is available from www.invitrogen.com or by contacting Technical Support (page 20.)**




**Comments for pET300/NT-DEST**
**7391 nucleotides**

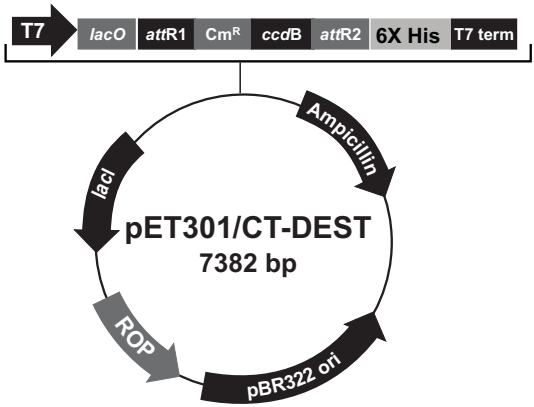
T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
lac operator (*lac*O): bases 39-63
Ribosome binding site (RBS): bases 93-101
6X His Tag: bases 112-129
*att*R1: bases 133-257
Chloramphenicol resistance gene: bases 366-1025
*ccd*B gene: bases 1367-1672
*att*R2: bases 1713-1837
T7 reverse priming site: bases 1900-1920
T7 transcription termination region: bases 1918-1964
*bla* promoter: bases 2294-2398
Ampicillin (*bla*) resistance gene: bases 2393-3253
pBR322 origin: bases 3464-4195 (c)
*ROP* ORF: bases 4439-4630 (c)
*lacI* ORF: bases 5942-7054 (c)

(c) = complementary strand

# Map of pET301/CT-DEST

## Map of pET301/CT-DEST

The map below shows the elements of pET301/CT-DEST. DNA from the entry clone replaces the region between bases 113 and 1786. **The complete sequence of pET301/CT-DEST is available from www.invitrogen.com or by contacting Technical Support (page 20).**




**Comments for pET301/CT-DEST**
**7382 nucleotides**

T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
lac operator (*lac*O): bases 39-63
*att*R1: bases 106-230
Chloramphenicol resistance gene: bases 339-998
*ccd*B gene: bases 1340-1645
*att*R2: bases 1686-1810
6X His Tag: bases 1818-1835
T7 reverse priming site: bases 1891-1911
T7 transcription termination region: bases 1909-1955
*bla* promoter: bases 2285-2389
Ampicillin (*bla*) resistance gene: bases 2326-3244
pBR322 origin: bases 3455-4186 (c)
*ROP* ORF: bases 4430-4621 (c)
*lacI* ORF: bases 5933-7045 (c)

(c) = complementary strand

# Features of pET300/NT-DEST and pET301/CT-DEST

## Features of the Vectors

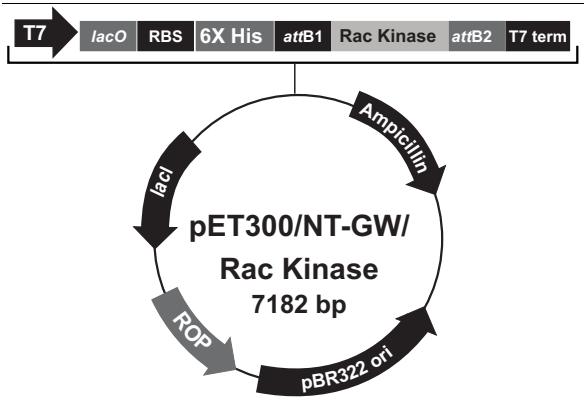
The pET300/NT-DEST and pET301/CT-DEST vectors contain the following elements. Features have been functionally tested.

| Feature | Benefit |
|---|---|
| T7 promoter | Allows high-level, inducible expression of your recombinant protein in *E. coli* strains expressing the T7 RNA polymerase |
| T7 primer binding site | Allows sequencing of the insert |
| *lac* operator (*lac*O) | Binding site for lac repressor that serves to reduce basal expression of the recombinant protein |
| Ribosome binding site (pET300/NT-DEST only) | Optimally spaced from the initiation ATG for efficient translation of insert |
| *att*R1 and *att*R2 sites | Allows recombinational cloning of the gene of interest from an entry clone |
| Chloramphenicol resistance gene | Allows counterscreening of expression clones |
| *ccd*B gene | Allows negative selection of expression clones |
| N-terminal or C-terminal 6x His tag | Allows purification of the recombinant protein on metal-chelating resin such as ProBond™ |
| T7 reverse primer binding site | Allows sequencing of the insert |
| T7 transcription termination region | Sequence from bacteriophage T7 which allows efficient transcription termination. |
| *bla* promoter | Allows expression of the ampicillin resistance gene |
| Ampicillin resistance gene | Allows selection of transformants in *E. coli* |
| pBR322 origin | Allows replication and maintenance in *E. coli* |
| *ROP* ORF | Interacts with the pBR322 origin to facilitate low-copy replication in *E. coli.* |
| *lac*I ORF | Encodes lac repressor which binds to the T7*lac* promoter to block basal transcription of the gene of interest. Also binds the *lac*UV5 promoter in BL21 strains containing the λDE3 lysogen to repress transcription of T7 RNA polymerase |

# Map of pET300/NT-GW/Rac Kinase

## Map of pET300/NT-GW/Rac Kinase

The map below shows the elements of pET300/NT-GW/Rac Kinase. **The complete sequence of pET300/NT-GW/Rac Kinase is available from www.invitrogen.com or by contacting Technical Support (page 20).**




**Comments for pET300/NT-GW/RacKinase 7182 nucleotides**

T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
lac operator (*lac*O): bases 39-63
Ribosome binding site (RBS): bases 93-101
6X His Tag: bases 112-129
*att*B1: bases 134-154
Rac Kinase gene: bases 160-1599
*att*B2: bases 1607-1627
T7 reverse priming site: bases 1691-1711
T7 transcription termination region: bases 1709-1755
*bla* promoter: bases 2085-2189
Ampicillin (*bla*) resistance gene: bases 2184-3041
pBR322 origin: bases 3255-3986 (c)
*ROP* ORF: bases 4230-4421 (c)
*lacI* ORF: bases 5733-6845 (c)

(c) = complementary strand

# Technical Support

## World Wide Web

Visit the Invitrogen web site at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

## Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web site (www.invitrogen.com).

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## MSDS Requests

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

## Certificate of Analysis

Product qualification is described in the Certificate of Analysis (CofA), available on our web site by product lot number at www.invitrogen.com/cofa.

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# Technical Support, continued

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# Purchaser Notification

## Introduction

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This product and its use is the subject of one or more of U.S. Patent Nos. 5,888,732, 6,143,557, 6,171,861, 6,270,969, and 6,277,608 and/or other pending U.S. and foreign patent applications owned by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for profit entity). The purchase of this product does not convey a license under any method claims in the foregoing patents or patent applications, or to use this product with any recombination sites other than those purchased from Invitrogen Corporation or its authorized distributor. The right to use methods claimed in the foregoing patents or patent applications with this product for research purposes only can only be acquired by the use of Clonase™ purchased from Invitrogen Corporation or its authorized distributors. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the employment of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Notwithstanding the preceding, any buyer who is employed in an academic or government institution may transfer materials made with this product to a third party who has a license from Invitrogen under the patents identified above to distribute such materials. Transfer of such materials and/or information to collaborators does not convey rights to practice any methods claimed in the foregoing patents or patent applications. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

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# Purchaser Notification, continued

## Limited Use Label License No. 30: T7 Expression System

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# Gateway® Clone Distribution Policy

## Introduction

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

## Gateway® Entry Clones

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

## Gateway® Expression Clones

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

## Additional Terms and Conditions

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

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