USER GUIDE

invitrogen™
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# Gateway® pDONR™ Vectors

**Catalog numbers** 12536-017 and 12535-035

**Revision Date** 29 March 2012

**Publication Part number** 25-0531

MAN0000291

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# Contents

# Kit Contents and Storage

## Gateway® pDONR™ Vectors

This manual is supplied with the following vectors:

| Product | Catalog no. |
|---|---|
| pDONR™221 | 12536-017 |
| pDONR™/Zeo | 12535-035 |

## Shipping and Storage

pDONR™221 is shipped at room temperature. Upon receipt, store at –30°C to –10°C.

pDONR™/Zeo is shipped on blue ice. Upon receipt, store the pDONR™/Zeo vector at –30°C to –10°C and the Zeocin™ selective antibiotic at –30°C to –10°C, protected from light.

## Contents

6 µg pDONR™ vector, supplied at 150 ng/µL in TE buffer, pH 8.0, in a total volume of 40 µL.

pDONR™/Zeo is also supplied with 1.25 mL Zeocin™ selective antibiotic. Zeocin™ selective antibiotic is provided as a 100 mg/mL solution in deionized, sterile water.

## Product Use

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

# Introduction

## Overview

### Description

pDONR™ vectors are Gateway®-adapted vectors designed to generate *att*L-flanked entry clones containing your gene of interest following recombination with an *att*B expression clone or an *att*B PCR product. After creating an entry clone, your gene of interest may then be easily shuttled into a large selection of expression vectors using the Gateway® LR recombination reaction. Refer to the following table for a list of the available pDONR™ vectors.

| Vector | M13 Sequencing Sites | Selection Marker |
|---|---|---|
| pDONR™221 | Yes | Kanamycin |
| pDONR™/Zeo | Yes | Zeocin™ |

### Features

The pDONR™ vectors contain the following elements:

- *rrn*B T1 and T2 transcription terminators for protection of the cloned gene from expression by vector-encoded promoters
- M13 Forward (–20) and M13 Reverse priming sites for sequencing of the insert (**pDONR™/Zeo only)**
- Two recombination sites, *att*P1 and *att*P2, for recombinational cloning of the gene of interest from a Gateway® expression clone or *att*B PCR product
- *ccd*B gene located between the two *att*P sites for negative selection
- Chloramphenicol resistance gene located between the two *att*P sites for counterselection
- Kanamycin or Zeocin™ resistance gene for selection in *E. coli* (see the preceding table)
- pUC origin for replication and maintenance of the plasmid in *E. coli*.

For a map of pDONR™221 and pDONR™/Zeo, see page 14.

*Continued on next page*

### The Gateway® Technology

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

1. Generate an entry clone by performing a BP recombination reaction between a pDONR™ vector (e.g. pDONR™221) and an *att*B PCR product or expression clone.
2. Generate the desired expression clone by performing an LR recombination reaction between the entry clone and a Gateway® destination vector of choice.
3. Introduce your expression clone into the system of choice for expression of your gene of interest.

For more information on the Gateway® Technology, refer to the Gateway® Technology with Clonase® II manual, which is available from **www.lifetechnologies.com/manuals** or by contacting Technical Support (page 17).

### *att*P sequence variations

The *att*P sites between the pDONR™ vectors will contain slight sequence variations which do not affect the specificity of recombination. Wild-type *att*P sites were modified to create the first-generation *att*P sites found in pDONR™201. First-generation sites were further modified to improve recombination efficiency and resulted in the second-generation *att*P sites found in pDONR™221 and pDONR™/Zeo.

For more information on characteristics of *att* sites, refer to the Gateway® Technology with Clonase® II manual.

# Methods

## General Guidelines

### Introduction

You will perform a BP recombination reaction to transfer the gene of interest in an *att*B expression clone or *att*B PCR product to a donor vector to create an entry clone. To ensure that you obtain the best possible results, we suggest that you read this section and the one entitled **Perform the BP Recombination Reaction** (pages 5–9) before beginning.

### Note

If you intend to go directly from an *att*B PCR product or *att*B expression clone into a destination vector without purifying the intermediate entry clone, refer to the Gateway® Technology with Clonase® II manual for a one-tube protocol.

Although this protocol allows you to generate expression clones more rapidly than the standard BP reaction followed by the LR reaction, fewer expression clones will be obtained (generally 10–20% of the total number of entry clones).

### Propagate pDONR™ Vectors

If you intend to propagate and maintain the pDONR™ vectors, we recommend using One Shot® *ccd*B Survival 2 T1$^R$ Chemically Competent *E. coli* (page 16) for transformation. The *ccd*B Survival 2 T1$^R$ *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene.

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

*Continued on next page*

**Important**

For optimal efficiency, perform the BP recombination reaction using:

- **Linear** *att*B substrates (see the following guidelines to linearize *att*B expression clones)
- **Supercoiled** *att*P-containing pDONR™ vector

**Note:** Supercoiled or relaxed *att*B substrates may be used, but will react less efficiently than linear *att*B substrates.

### Linearize Expression Clones

If you intend to perform a BP recombination reaction using an *att*B expression clone, we recommend that you linearize the expression clone using a suitable restriction enzyme (see the following recommendations).

1. Linearize 1–2 µg of the expression clone with a unique restriction enzyme that does not digest within the gene of interest and is located outside the *att*B 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.
3. Pellet the DNA by centrifugation. Wash the pellet twice with 70% ethanol.
4. Dissolve the DNA in 1X TE Buffer, pH 8.0 to a final concentration of 50–150 ng/µL.


If you intend to perform a BP recombination reaction using an *att*B PCR product, we recommend purifying the PCR product to remove *att*B primers and any *att*B primer-dimers. These primers and primer-dimers can recombine efficiently with the pDONR™ vector in the BP reaction and may increase background after transformation into *E. coli*. Refer to the Gateway® Technology with Clonase® II manual for a purification protocol using PEG/$MgCl_2$ precipitation.

**Note:** Standard PCR product purification protocols using phenol/chloroform extraction followed by sodium acetate and ethanol or isopropanol precipitation are not recommended for use in purifying *att*B PCR products. These protocols generally have exclusion limits less than 100 bp and do not efficiently remove large primer-dimer products.

# Perform the BP Reaction

## Positive Control

pEXP7-tet is provided as a positive control for the BP reaction. pEXP7-tet is an approximately 1.4-kb linear fragment and contains *att*B sites flanking the tetracycline resistance gene and its promoter ($Tc^r$). Using the pEXP7-tet fragment in a BP reaction with a donor vector results in entry clones that express the tetracycline resistance gene. The efficiency of the BP recombination reaction can easily be determined by streaking entry clones onto LB plates containing 20 µg/mL tetracycline.

## Gateway® BP Clonase® II Enzyme Mix

Gateway® BP Clonase® II enzyme mix (page 16) combines the proprietary enzyme formulation and 5X BP Reaction Buffer previously supplied as separate components in Gateway® BP Clonase® enzyme mix into an optimized single tube format to allow easier set-up of the BP recombination reaction. Use the protocol provided in this section to perform the BP recombination reaction using Gateway® BP Clonase® II enzyme mix.

**Note:** You may perform the BP recombination reaction using Gateway® BP Clonase® enzyme mix, if desired. To use Gateway® BP Clonase® enzyme mix, follow the protocol provided with the product. **Do not** use the protocol for Gateway® BP Clonase® II enzyme mix provided on page 7.

## Determine how much *att*B DNA and donor vector to use in the reaction

For optimal efficiency, we recommend using the following amounts of *att*B PCR product (or linearized *att*B expression clone) and donor vector in a 10 µL BP recombination reaction with Gateway® BP Clonase® II enzyme mix:

- An equimolar amount of *att*B PCR product (or linearized *att*B expression clone) and the donor vector
- 50 femtomoles (fmol) **each** of *att*B PCR product (or linearized *att*B expression clone) and donor vector is preferred, but the amount of *att*B PCR product used may range from 20–50 fmol

  **Note:** 50 fmol of donor vector) is approximately 150 ng
- For large PCR products (>4 kb), use at least 50 fmol of *att*B PCR product, but no more than 250 ng

For a formula to convert fmol of DNA to nanograms (ng), see **Convert femtomoles (fmol) to nanograms (ng)**. For an example, see page 6.

*Continued on next page*


- Do not use more than 250 ng of donor vector in a 10 µL BP reaction because this will affect the efficiency of the reaction.
- Do not exceed more than 0.5 µg of total DNA (donor vector plus *att*B PCR product) in a 10 µL BP reaction because excess DNA will inhibit the reaction.

### Convert femtomoles (fmol) to nanograms (ng)

Use the following formula to convert femtomoles (fmol) of DNA to nanograms (ng) of DNA where N is the size of the DNA in bp.

$$ng = (fmol)(N)(\frac{660\,fg}{fmol})(\frac{1\,ng}{10^6\,fg})$$

### Example of fmol to ng conversion

In this example, you need to use 50 fmol of an *att*B PCR product in the BP reaction. The *att*B PCR product is 2.5 kb in size. Calculate the amount of *att*B PCR product required for the reaction (in ng) by using the equation above:

$$(50\,fmol)(2500\,bp)(\frac{660\,fg}{fmol})(\frac{1\,ng}{10^6\,fg}) = 82.5\,ng\ of\ PCR\ product$$

### Required materials

- *att*B PCR product or linearized *att*B expression clone (see page 5 to determine the amount of DNA to use)
- Gateway® BP Clonase® II enzyme mix (see page 16 for ordering information; 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 Gateway® BP Clonase® II enzyme mix; thaw and keep on ice until use)
- pEXP7-tet positive control (50 ng/µL; supplied with the Gateway® BP Clonase® II enzyme mix)

*Components supplied with the kit:*

- pDONR™ vector (150 ng/ µL)

*Continued on next page*

### Perform the BP Reaction

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

| Components | Sample | Positive Control | Negative Control |
|---|---|---|---|
| *att*B PCR product or linearized *att*B expression clone (20–50 fmol) | 1–7 µL | — | 1–7 µL |
| pDONR™ vector (150 ng/µL) | 1 µL | 1 µL | 1 µL |
| pEXP7-tet positive control (50 ng/µL) | — | 2 µL | — |
| TE Buffer, pH 8.0 | to 8 µL | 5 µL | to 10 µL |

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

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

   **Note:** For most applications, a 1-hour incubation will yield a sufficient number of entry clones. Depending on your needs, the length of the recombination reaction can be extended up to 18 hours. An overnight incubation typically yields 5–10 times more colonies than a 1-hour incubation. For large PCR products (≥5 kb), longer incubations (i.e. overnight incubation) will increase the yield of colonies and are recommended.
6. Add 1 µL of the Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Proceed to **Transform Competent Cells**, page 8.

   **Note:** You may store the BP reaction at –20°C for up to 1 week before transformation.

# Transform Competent Cells

## Introduction

After performing the BP recombination reaction, you will transform competent *E. coli* and select for entry clones using the appropriate antibiotic. General guidelines for transforming competent cells are provided in this section.

## *E. coli* host strain

You may use any *rec*A, *end*A *E. coli* strain including TOP10, OmniMAX™ 2-T1$^R$ or equivalent for transformation. **Do not** use *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.

## Selection media

Refer to the following table for the appropriate selection medium to use to select for entry clones. You will need 2 LB plates containing the appropriate antibiotic for each transformation. Pre-warm plates at 37ºC for 30 minutes.

If you are using pDONR™/Zeo, you will need to use Low Salt LB agar for selection (see the following **Note**).

| Donor Vector | Selection Media |
|---|---|
| pDONR™221 | LB + 50 µg/mL Kanamycin |
| pDONR™/Zeo | Low Salt LB + 50 µg/mL Zeocin™ selective antibiotic (see Note below) |

**Note**

The Zeocin™ resistance gene in pDONR™/Zeo allows selection of *E. coli* transformants using Zeocin™ antibiotic. For selection, use Low Salt LB agar plates containing 50 µg/mL Zeocin™ (see page 12 for a recipe). Note that for Zeocin™ to be active, the salt concentration of the bacterial medium must remain low (<90 mM) and the pH must be 7.5. For more information on storing and handling Zeocin™, refer to page 13.

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## Transform Competent Cells, Continued

| | |
|---|---|
| **Transform competent cells** | Transform 1 µL of the BP recombination reaction into a suitable *E. coli* host (follow the manufacturer's instructions) and select for entry clones using the appropriate antibiotic. We recommend plating 2 different volumes to ensure that at least 1 plate has well-spaced colonies. |
| **What you should see** | If you use *E. coli* cells with a transformation efficiency of $1 \times 10^8$ cfu/µg, the BP reaction should give you >1500 colonies if the entire BP reaction is transformed and plated. |
| **Verify pEXP7-tet entry clones** | If you included the pEXP7-tet control in your BP reaction, the efficiency of the BP reaction may be assessed by streaking the kanamycin-resistant colonies onto LB agar plates containing 20 µg/mL tetracycline. True entry clones should be tetracycline-resistant. |

# Analyze Entry Clones

## Analyze positive clones

1. Pick 5 colonies and culture them overnight in LB medium containing the appropriate antibiotic.
2. Isolate plasmid DNA using your method of choice. We recommend using the PureLink® HQ Mini Plasmid Purification Kit (page 16).
3. Analyze the entry clones by restriction analysis to confirm the presence and correct orientation of the insert. Use a restriction enzyme or a combination of enzymes that cut once in the vector and once in the insert.

## Analyze transformants by PCR

You may also analyze positive transformants using PCR. Use a primer that hybridizes within the vector (see page 11 for suggested primer sequences) and one that hybridizes within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, you may want to perform restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template. The protocol below is provided for your convenience. Other protocols are suitable.

**Materials Needed:**

- PCR SuperMix High Fidelity (page 16)
- Appropriate forward and reverse PCR primers, 20 µM each (see page 11 for suggested primer sequences)

**Protocol:**

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

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## Recommended Primers

We recommend using the following primers to analyze entry clones. Refer to the following diagram or on page 11 for the location of the primer binding sites.

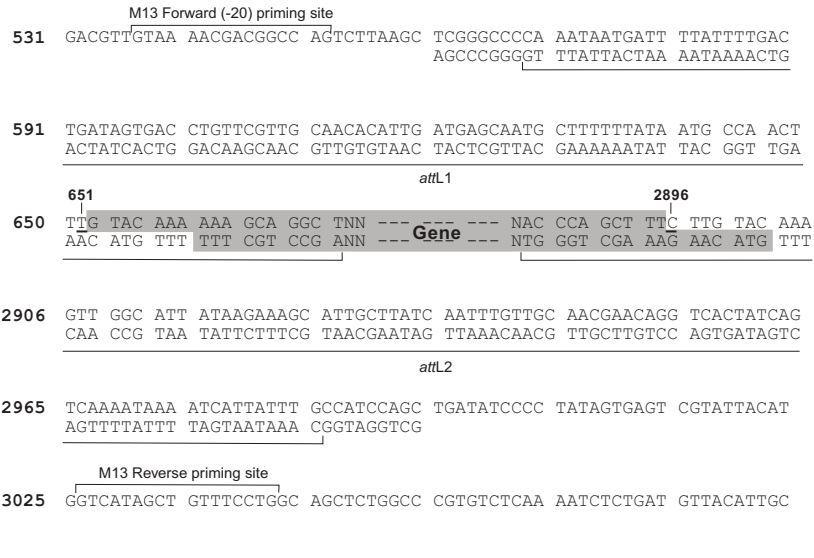
| pDONR™221 and pDONR™/Zeo | |
|---|---|
| M13 Forward (–20) primer | 5′-GTAAAACGACGGCCAG-3′ |
| M13 Reverse primer | 5′-CAGGAAACAGCTATGAC-3′ |

## Recombination region of pDONR™221 and pDONR™/Zeo

The recombination region of the expression clone resulting from pDONR™221 × entry clone or pDONR™/Zeo × entry clone is shown in the following figure.

**Features of the Recombination Region:**

- Shaded regions correspond to DNA sequences transferred from the *att*B substrate into pDONR™221 or pDONR™/Zeo by recombination. Non-shaded regions are derived from the pDONR™221 or pDONR™/Zeo vector.
- Bases 651 and 2897 of the pDONR™221 or pDONR™/Zeo vector sequence are marked.



# Appendix

## Recipes

### Low Salt LB Medium with Zeocin™

10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 mL. Adjust the pH to 7.5 with 5 M NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 lbs/sq. in. and 121°C for 20 minutes.
3. Thaw Zeocin™ on ice and vortex before removing an aliquot.
4. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 50 µg/mL final concentration.
5. Store plates at 4°C in the dark. Plates containing Zeocin™ are stable for 1–2 weeks.

# Zeocin™ Selective Antibiotic

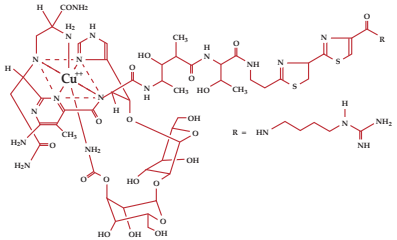
## Introduction

Zeocin™ selective antibiotic is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. It shows strong toxicity against bacteria, fungi, plants and mammalian cell lines (Calmels *et al.*, 1991; Drocourt *et al.*, 1990; Gatignol *et al.*, 1987; Mulsant *et al.*, 1988; Perez *et al.*, 1989).

A Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This 13,665 Da protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), binds stoichiometrically to Zeocin™ selective antibiotic and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™ selective antibiotic.

## Molecular weight, Formula, and Structure

The formula for Zeocin™ is $C_{55}H_{86}O_{21}N_{20}S_2Cu$-HCl and the molecular weight is 1527.5. The structure of Zeocin™ is:



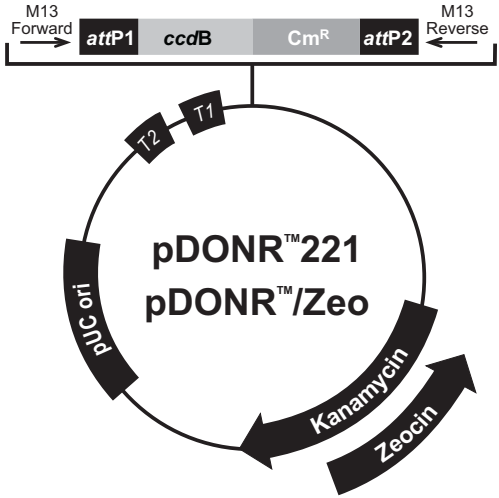
## Handling Zeocin™ selective antibiotic

- High ionic strength and acidity or basicity inhibit the activity of Zeocin™ selective antibiotic. Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active (see page 12 for a recipe).
- Store Zeocin™ selective antibiotic at –20°C and thaw on ice before use.
- Zeocin™ selective antibiotic is light sensitive. Store the drug and plates or medium containing the drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses when handling Zeocin™-containing solutions.
- Do not ingest or inhale solutions containing the drug.

# Map and Features of pDONR™221 and pDONR™/Zeo

## Map of pDONR™221 and pDONR™/Zeo

The following map shows the elements of pDONR™221 and pDONR™/Zeo. The complete sequences of pDONR™221 and pDONR™/Zeo are available from **www.lifetechnologies.com** or by contacting Technical Support (page 17).




| Comments for: | pDONR™221 4761 nucleotides | pDONR™/Zeo 4291 nucleotides |
|---|---|---|
| *rrn*B T2 transcription termination sequence (c): | 268-295 | 268-295 |
| rrnB T1 transcription termination sequence (c): | 427-470 | 427-470 |
| M13 Forward (-20) priming site: | 537-552 | 537-552 |
| *att*P1: | 570-801 | 570-801 |
| *ccd*B gene (c): | 1197-1502 | 1197-1502 |
| Chloramphenicol resistance gene (c): | 1825-2505 | 1847-2506 |
| *att*P2 (c): | 2753-2984 | 2754-2985 |
| M13 Reverse priming site: | 3026-3042 | 3027-3043 |
| Kanamycin resistance gene: | 3155-3964 | --- |
| EM7 promoter (c): | --- | 3486-3552 |
| Zeocin resistance gene (c): | --- | 3111-3485 |
| pUC origin: | 4085-4758 | 3615-4288 |

(c) = complementary strand

*Continued on next page*

# Map and Features of pDONR™221 and pDONR™/Zeo, Continued

## Features of pDONR™221 and pDONR™/Zeo

pDONR™221 (4761 bp) and pDONR™/Zeo (4291 bp) contain the following elements. Features have been functionally tested.

| Feature | Benefit |
|---|---|
| *rrn*B T1 and T2 transcription terminators | Protects the cloned gene from expression by vector-encoded promoters, thereby reducing possible toxicity (Orosz *et al.*, 1991) |
| M13 Forward (–20) priming site | Allows sequencing in the sense orientation |
| *att*P1 and *att*P2 sites | Bacteriophage λ-derived DNA recombination sequences that allow recombinational cloning of the gene of interest from a Gateway® expression clone or *att*B PCR product (Landy, 1989) |
| *ccd*B gene | Allows negative selection of the plasmid |
| Chloramphenicol resistance gene ($Cm^R$) | Allows counterselection of the plasmid |
| T7 promoter/priming site | Allows *in vitro* transcription and sequencing in the anti-sense orientation |
| M13 Reverse priming site | Allows sequencing in the anti-sense orientation |
| Kanamycin resistance gene **(pDONR™221 only)** | Allows selection of the plasmid in *E. coli* |
| EM7 promoter **(pDONR™/Zeo only)** | Allows expression of the Zeocin™ resistance gene in *E. coli*. |
| Zeocin™ resistance gene **(pDONR™/Zeo only)** | Allows selection of the plasmid in *E. coli* |
| pUC origin | Allows high-copy replication and maintenance in *E. coli* |

# Accessory Products

## Additional products

Additional products that may be used with the Gateway® pDONR™ vectors are available. Ordering information is provided below.

| Product | Amount | Catalog no. |
|---|---|---|
| Gateway® BP Clonase® II Enzyme Mix | 20 reactions | 11789-020 |
| | 100 reactions | 11789-100 |
| One Shot® *ccd*B Survival™ 2 T1$^R$ Chemically Competent Cells | 5 × 0.2 mL | A10460 |
| One Shot® TOP10 Chemically Competent Cells | 10 reactions | C4040-10 |
| | 20 reactions | C4040-03 |
| One Shot® TOP10 Electrocompetent Cells | 10 reactions | C4040-50 |
| | 20 reactions | C4040-52 |
| One Shot® OmniMAX™ 2 T1$^R$ Chemically Competent Cells | 20 reactions | C8540-03 |
| Library Efficiency® DH5α™ Competent Cells | 5 × 0.2 mL | 18263-012 |
| Kanamycin Sulfate | 5 g | 11815-024 |
| Zeocin™ Selection Reagent | 1 g | R250-01 |
| | 5 g | R250-05 |
| PureLink® HQ Mini Plasmid DNA Purification Kit | 100 preps | K2100-01 |
| PCR SuperMix High Fidelity | 100 reactions | 10790-020 |

# Technical Support

## Obtaining support

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

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, Safety Data Sheets (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 Invitrogen's policy for the use and distribution of Gateway® clones, see the section entitled **Gateway® Clone Distribution Policy**, page 21.

# Gateway® Clone Distribution Policy

## Introduction

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

## Gateway® Entry Clones

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

## Gateway® Expression Clones

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

## Additional Terms and Conditions

We would ask that such distributors of Gateway® entry and expression clones indicate that such clones may be used only for research purposes, that such clones incorporate the Gateway® Technology, and that the purchase of Gateway® Clonase® from Life Technologies is required for carrying out the Gateway® recombinational cloning reaction. This should allow researchers to readily identify Gateway® containing clones and facilitate their use of this powerful technology in their research. Use of Life Technologies' Gateway® Technology, including Gateway® clones, for purposes other than scientific research may require a license and questions concerning such commercial use should be directed to **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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