Gateway® pDONR™ Vectors

Catalog nos. 11798-014, 12536-017, and 12535-035

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User Manual

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Kit Contents and Storage

Gateway® pDONR™ Vectors

This manual is supplied with the following vectors:

Product	Catalog no.
pDONR™201	11798-014
pDONR™221	12536-017
pDONR™/Zeo	12535-035

Shipping and Storage

pDONR™201 and pDONR™221 are shipped at room temperature. Upon receipt, store at -20°C.

pDONR™/Zeo is shipped on blue ice. Upon receipt, store the pDONR™/Zeo vector at -20ºC and the Zeocin™ at -20ºC, protected from light.

Contents

6 µg pDONR™ vector, lyophilized in TE buffer, pH 8.0.

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

Accessory Products

Additional Products

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

Product	Amount	Catalog no.
BP Clonase™ II Enzyme Mix	20 reactions	11789-020
	100 reactions	11789-100
One Shot® *ccd*B Survival™ T1^R Chemically Competent Cells	5 x 0.2 ml	C7510-03
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 x 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

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. Once you have created 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 table below for a list of the available pDONR™ vectors.

Vector	M13 Sequencing Sites	Selection Marker
pDONR™201	No	Kanamycin
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™221, 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 table above)
- pUC origin for replication and maintenance of the plasmid in *E. coli*.

For a map of pDONR™201, see page 16. For a map of pDONR™221 and pDONR™/Zeo, see page 18.

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. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 20).

*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.

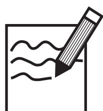
For more information on characteristics of *att* sites, refer to the Gateway® Technology with Clonase™ II manual.

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 **Performing the BP Recombination Reaction** (pages 5-10) before beginning.



Note

If you wish to go directly from an *att*B PCR product or *att*B expression clone into a destination vector without purification of 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).

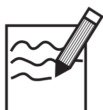
Resuspending the pDONR™ Vectors

Before you perform the BP recombination reaction, resuspend the pDONR™ vector in 40 µl of sterile water to a final concentration of 150 ng/µl.

Propagating pDONR™ Vectors

If you wish to propagate and maintain the pDONR™ vectors, we recommend using One Shot® *ccd*B Survival T1^R Chemically Competent *E. coli* (page vi) 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.

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.



Note

Although pDONR™201 contains a pUC origin, this vector replicates less efficiently resulting in lower yields of vector. pDONR™221 and pDONR™/Zeo, however, act as high-copy number plasmids.

Continued on next page

Important

For optimal efficiency, perform the BP recombination reaction using:

- **Linear** *att*B substrates (see below for 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.

Linearizing Expression Clones

If you wish 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 recommendations below).

1. Linearize 1 to 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 wish 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.

Performing the BP Reaction

Introduction

Instructions are provided in this section to perform a BP recombination reaction using an appropriate *att*B substrate and a donor vector. We recommend that you include a positive control (see below) and negative control (no BP Clonase™ II) in your experiment to help you evaluate your results.

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.

BP Clonase™ II Enzyme Mix

BP Clonase™ II enzyme mix (page vi) 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 BP Clonase™ II enzyme mix.

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

Continued on next page

Determining 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 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 below. For an example, see the next page.

- Do not use more than 250 ng of donor vector in a 10 µl BP reaction as 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 as excess DNA will inhibit the reaction.

Converting 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.

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

Continued on next page

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:

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

Materials Needed

You should have the following materials on hand before beginning:

- *att*B PCR product or linearized *att*B expression clone (see page 6 to determine the amount of DNA to use)
- pDONR™ vector (resuspend to 150 ng/µl with water)
- BP Clonase™ II enzyme mix (Invitrogen Catalog no. 11789-020; 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 BP Clonase™ II enzyme mix; thaw and keep on ice until use)
- pEXP7-tet positive control (50 ng/µl; supplied with the BP Clonase™ II enzyme mix)

Continued on next page

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

   Reminder: Return 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 **Transforming Competent Cells**, next page.

   Note: You may store the BP reaction at -20°C for up to 1 week before transformation, if desired.

Transforming Competent Cells

Introduction

Once you have performed 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 below.

***E. coli* Host Strain**

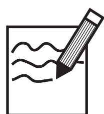
You may use any *rec*A, *end*A *E. coli* strain including TOP10, OmniMAX™ 2-T1^R or equivalent for transformation. **Do not** use *E. coli* strains that contain the F′ episome (*e.g.* TOP10F′). These strains contain the *ccd*A gene and will prevent negative selection with the *ccd*B gene.

Selection Media

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

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

Donor Vector	Selection Media
pDONR™201	LB + 50 µg/ml kanamycin
pDONR™221	LB + 50 µg/ml kanamycin
pDONR™/Zeo	Low Salt LB + 50 µg/ml Zeocin™ (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 14 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 15.

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Transforming Competent Cells, Continued

Transforming 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 1×10^8 cfu/µg, the BP reaction should give you >1500 colonies if the entire BP reaction is transformed and plated.

Verifying 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.

Analyzing Entry Clones

Analyzing 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 vi).
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.

Analyzing Transformants by PCR

You may also analyze positive transformants using PCR. Use a primer that hybridizes within the vector (see next page 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 vi)
- Appropriate forward and reverse PCR primers, 20 µM each (see next page 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 reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at +4°C.
6. Visualize by agarose gel electrophoresis.

Continued on next page

Recommended Primers

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

pDONR™201	
Forward primer	5′-TCGCG TTAAC GCTAG CATGG ATCTC-3′
Reverse primer	5′-GTAAC ATCAG AGATT TTGAG ACAC-3′

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

Recombination Region of pDONR™201

The recombination region of the expression clone resulting from pDONR™201 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to DNA sequences transferred from the *att*B substrate into pDONR™201 by recombination. Non-shaded regions are derived from the pDONR™201 vector.
- Bases 413 and 2656 of the pDONR™201 vector sequence are marked.

```
                          Forward priming site
293   CCTACTCTCG CGTTAACGCT AGCATGGATC TCGGGCCCCA AATAATGATT TTATTTTGAC
                                       AGCCCGGGGT TTATTACTAA AATAAAACTG

353   TGATAGTGAC CTGTTCGTTG CAACAAATTG ATGAGCAATG CTTTTTTATA ATG CCA AGT
      ACTATCACTG GACAAGCAAC GTTGTTTAAC TACTCGTTAC GAAAAAATAT TAC GGT TCA
                                        attL1
       413                                                     2656
412   TTG TAC AAA AAA GCA GGC TNN --- --- --- NAC CCA GCT TTC TTG TAC AAA
      AAC ATG TTT TTT CGT CCG ANN ---Gene--- NTG GGT CGA AAG AAC ATG TTT

2666  GTG GGC ATT ATAAGAAAGC ATTGCTTATC AATTTGTTGC AACGAACAGG TCACTATCAG
      CAC CCG TAA TATTCTTTCG TAACGAATAG TTAAACAACG TTGCTTGTCC AGTGATAGTC
                                        attL2
                                                       Reverse priming site
2725  TCAAAATAAA ATCATTATTT GCCATCCAGC TGCAGCTCTG GCCCGTGTCT CAAAATCTCT
      AGTTTTATTT TAGTAATAAA CGGTAGGTCG

2785  GATGTTACAT TGCACAAGAT AAAAATATAT CATCATGAAC AATAAAACTG TCTGCTTACA
```

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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 below.

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.

```
                    M13 Forward (-20) priming site
531   GACGTTGTAA AACGACGGCC AGTCTTAAGC TCGGGCCCCA AATAATGATT TTATTTTGAC
                                       AGCCCGGGGT TTATTACTAA AATAAAACTG

591   TGATAGTGAC CTGTTCGTTG CAACACATTG ATGAGCAATG CTTTTTTATA ATG CCA ACT
      ACTATCACTG GACAAGCAAC GTTGTGTAAC TACTCGTTAC GAAAAAATAT TAC GGT TGA
                                     attL1
       651                                                        2897
650   TTG TAC AAA AAA GCA GGC TNN --- --- --- NAC CCA GCT TTC TTG TAC AAA
      AAC ATG TTT TTT CGT CCG ANN --- Gene --- NTG GGT CGA AAG AAC ATG TTT

2907  GTT GGC ATT ATAAGAAAGC ATTGCTTATC AATTTGTTGC AACGAACAGG TCACTATCAG
      CAA CCG TAA TATTCTTTCG TAACGAATAG TTAAACAACG TTGCTTGTCC AGTGATAGTC
                                     attL2

2966  TCAAAATAAA ATCATTATTT GCCATCCAGC TGATATCCCC TATAGTGAGT CGTATTACAT
      AGTTTTATTT TAGTAATAAA CGGTAGGTCG

                 M13 Reverse priming site
3026  GGTCATAGCT GTTTCCTGGC AGCTCTGGCC CGTGTCTCAA AATCTCTGAT GTTACATTGC
```

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™

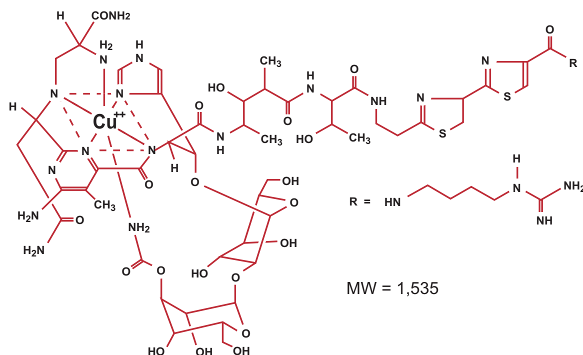
Introduction

Zeocin™ 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™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

Molecular Weight, Formula, and Structure

The formula for Zeocin™ is $C_{60}H_{89}N_{21}O_{21}S_3$ and the molecular weight is 1,535. The structure of Zeocin™ is shown below.



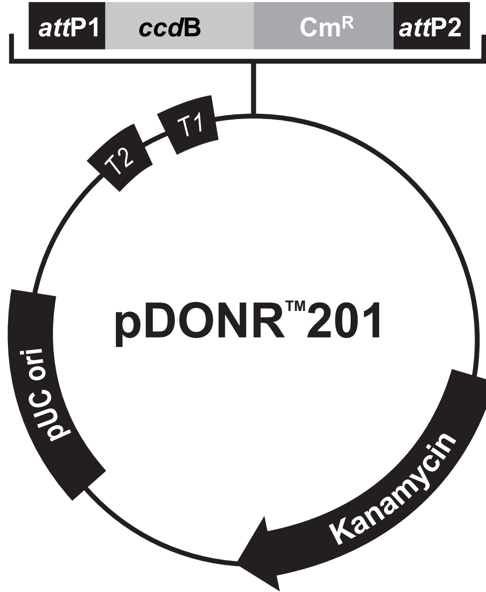
Handling Zeocin™

- High ionic strength and acidity or basicity inhibit the activity of Zeocin™. 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 14 for a recipe).
- Store Zeocin™ at -20°C and thaw on ice before use.
- Zeocin™ 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™201

Map of pDONR™201

The map below shows the elements of pDONR™201. The complete sequence of pDONR™201 is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 20).



Comments for:	**pDONR™201** **4470 nucleotides**
*rrn*B T2 transcription termination sequence (c):	73-100
rrnB T1 transcription termination sequence (c):	232-275
Recommended forward priming site:	300-324
*att*P1:	332-563
*ccd*B gene (c):	959-1264
Chloramphenicol resistance gene (c):	1606-2265
*att*P2 (c):	2513-2744
Recommended reverse priming site:	2769-2792
Kanamycin resistance gene:	2868-3677
pUC origin:	3794-4467

(c) = complementary strand

Continued on next page

Features of pDONR™201

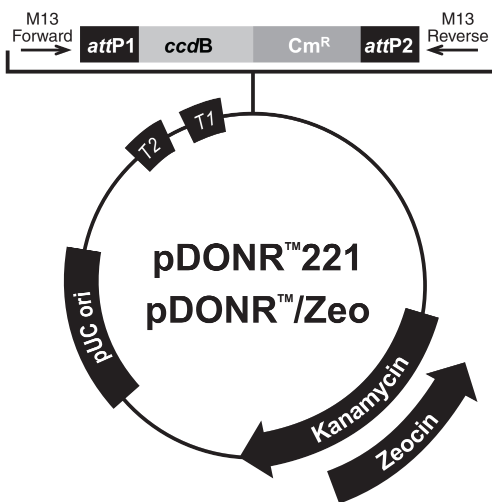
pDONR™201 (4470 bp) contains the following elements. Features have been functionally tested.

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)
*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
Kanamycin resistance gene	Allows selection of the plasmid in *E. coli*
pUC origin (see Note on page 3)	Allows replication and maintenance in *E. coli*

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

Map of pDONR™221 and pDONR™/Zeo

The map below shows the elements of pDONR™221 and pDONR™/Zeo. The complete sequences of pDONR™221 and pDONR™/Zeo are available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 20).



Comments for:	pDONR™221 4762 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):	1847-2506	1847-2506
*att*P2 (c):	2754-2985	2754-2985
T7 Promoter/priming site (c):	3000-3019	3003-3022
M13 Reverse priming site:	3027-3043	3027-3043
Kanamycin resistance gene:	3156-3965	---
EM7 promoter (c):	---	3486-3552
Zeocin resistance gene (c):	---	3111-3485
pUC origin:	4086-4759	3615-4288

Continued on next page

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

Features of pDONR™221 and pDONR™/Zeo

pDONR™221 (4762 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 promote **(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*

Technical Support

Web Resources

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.
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MSDS

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Certificate of Analysis

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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 24.

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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.

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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.

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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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