USER GUIDE

invitrogen™
by life technologies™

Bac-to-Bac® TOPO® Cloning Kit

Five-minute cloning of blunt PCR products for expression in insect cells

Catalog Numbers A11098, A11099, A11100, A11101, and A11338

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Information for European customers using Mach1™-T1^R Cells

The Mach1™-T1^R *E. coli* strain is genetically modified to carry the *lac*ZΔM15 *hsd*R *lac*X74 *rec*A *end*A *ton*A genotype. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

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Contents

Blunt-End TOPO® Cloning procedure for experienced users

Introduction

This quick reference sheet is provided for experienced users of the blunt-end TOPO® Cloning procedure. If you are performing the blunt-end TOPO® Cloning procedure for the first time, follow the detailed protocols provided in the manual.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Generate PCR product</td><td>Generate PCR products using a thermostable proofreading DNA polymerase and your own protocol. End the PCR reaction with a final 7 to 30 minute extension step. Make sure that your PCR primers do not contain 5´ phosphates as they will inhibit ligation into your vector.</td></tr>
<tr><td>Perform the Blunt-end TOPO® Cloning Reaction</td><td>1. Set up one of the following blunt-end TOPO® Cloning reactions using the reagents in the order shown. For electroporation, dilute Salt Solution 4-fold to prepare a Dilute Salt Solution.

<table>
<tr><th>Reagent</th><th>Chemical Transformation</th><th>Electroporation</th></tr>
<tr><td>Fresh PCR product</td><td>0.5 to 4 µL</td><td>0.5 to 4 µL</td></tr>
<tr><td>Salt Solution</td><td>1 µL</td><td>–</td></tr>
<tr><td>Dilute Salt Solution</td><td>–</td><td>1 µL</td></tr>
<tr><td>Sterile Water</td><td>to a final volume of 5 µL</td><td>to a final volume of 5 µL</td></tr>
<tr><td>TOPO® Vector</td><td>1 µL</td><td>1 µL</td></tr>
<tr><td>Total volume</td><td>6 µL</td><td>6 µL</td></tr>
</table>
Note: The best insert:vector ratio in a TOPO® Cloning reaction is 1:1 to 2:1.

2. Mix gently and incubate for 5 minutes at room temperature.
3. Place on ice and proceed to transform One Shot® Mach1™ T1^R Chemically Competent E. coli, below.</td></tr>
<tr><td>Transform One Shot® Chemically Competent E. coli</td><td>1. For each transformation, thaw one vial of One Shot® Mach1™ T1^R Chemically Competent E. coli on ice.
2. Add 2 µL of the TOPO® Cloning reaction into a vial of One Shot® chemically competent E. coli and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Immediately transfer the vial(s) to ice, and incubate on ice for 2 minutes.
6. Add 250 µL of room temperature S.O.C. medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour (225 rpm).
8. Spread 25–100 µL from each transformation on a pre-warmed LB agar plate containing 100 µg/mL ampicillin, and incubate overnight at 37°C.</td></tr>
</table>

Control reaction

We recommend using the Control PCR Template and the Control PCR Primers included with the kit to perform the control reaction. See the protocol on pages 34–35 for instructions.

Product information

Kit contents and storage

Types of products

This manual, supplied with the products listed below, **only** provides TOPO® cloning strategy for the direct insertion of blunt-end PCR products into pFastBac™ TOPO® plasmid vectors.

- For information on expressing your protein of interest from your TOPO® expression construct, refer to the Bac-to-Bac® TOPO® Expression System manual (part no. A10606) supplied with the Bac-to-Bac® N-His TOPO® or Bac-to-Bac® C-His TOPO® Expression System kits.
- For information on secreted expression of your protein of interest from pFastBac™/HBM construct, refer to the Bac-to-Bac® HBM TOPO® Secreted Expression System manual (part no. A11341) supplied with the Bac-to-Bac® HBM TOPO® Secreted Expression System kit.

These manuals are also available at **www.lifetechnologies.com/manuals** or by contacting Technical Support (page 40).

Product	Quantity	Cat. no.
Bac-to-Bac® N-His TOPO® Cloning Kit	1 kit	A11099
Bac-to-Bac® C-His TOPO® Cloning Kit	1 kit	A11098
Bac-to-Bac® HBM TOPO® Cloning Kit	1 kit	A11338

Shipping and storage

Bac-to-Bac® TOPO® Cloning Kits are shipped on dry ice. In addition to the Bac-to-Bac® TOPO® Cloning Kit manual, each kit contains two boxes as described below. All reagents are guaranteed for six months if stored properly. Upon receipt, store boxes as detailed below.

Box	Item	Storage
1	Bac-to-Bac® N-His TOPO®, Bac-to-Bac® C-His TOPO®, or Bac-to-Bac® HBM TOPO® Cloning Reagents	–30°C to –10°C
2	One Shot® Mach1-T1^R Chemically Competent *E. coli*	–85°C to –68°C

Continued on next page

Bac-to-Bac® TOPO® Cloning Kit reagents

The cloning reagents for the Bac-to-Bac® TOPO® Cloning Kits (Box 1) are listed below. **Store the contents of Box 1 at –30°C to –10°C.**

Item	Concentration	Amount
pFastBac™/NT-TOPO® vector (only with Cat. nos. A11099 and A11101) *or* pFastBac™/CT-TOPO® vector (only with Cat. nos. A11098 and A11100) *or* pFastBac™/HBM-TOPO® vector (only with Cat. nos. A11338 and A11339)	20 mL at 10 ng/µL in 50% glycerol 50 mM Tris-HCl, pH 7.4 (at 25°C) 1 mM EDTA 2 mM DTT 0.1% Triton® X-100 100 µg/mL BSA 30 µM bromophenol blue	20 µL
10X PCR Buffer	100 mM Tris-HCl, pH 8.3 (at 42°C) 500 mM KCl 25 mM $MgCl_2$ 0.01% gelatin	100 µL
dNTP Mix	12.5 mM each dATP, dCTP, dGTP, and dTTP; neutralized at pH 8.0 in water	10 µL
Salt Solution	1.2 M NaCl 0.06 M $MgCl_2$	50 µL
Sterile Water	–	1 mL
Control PCR template	50 ng/µL in TE buffer, pH 8.0*	10 µL
Control PCR primers	100 ng/µL each in TE buffer, pH 8.0	10 µL
Polyhedrin forward sequencing primer	100 ng/µL in TE buffer, pH 8.0	20 µL
SV40 polyA reverse sequencing primer	100 ng/µL in TE buffer, pH 8.0	20 µL
pFastBac™ Gus control plasmid	0.2 ng/µL in TE buffer, pH 8.0	20 µL

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

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Mach1™ T1^R Competent Cells

The following reagents are included in the One Shot® Mach1™ T1^R Chemically Competent *E. coli* module (Box 2). Transformation efficiency of One Shot® Mach1™ T1^R *E. coli* cells is ≥ 1×10^9 cfu/µg DNA. **Store cells at –85°C to –68°C.**

Reagent	Composition	Amount
One Shot® Mach1™ Chemically Competent *E. coli*	–	21 × 50 µL
S.O.C. Medium (may be stored at room temperature or 2°C to 8°C)	2% tryptone 0.5% yeast extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 mL
pUC19 Control DNA	10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8.0	50 µL

Genotype of Mach1™ T1^R

F^- ϕ80(*lac*Z)ΔM15 Δ*lac*X74 *hsd*R(r_K^- m_K^+) Δ*rec*A1398 *end*A1 *ton*A

IMPORTANT!

The parental strain of Mach1™-T1^R *E. coli* is the non-K-12, wild-type W strain (ATCC #9637, S. A. Waksman). Although the parental strain is generally classified as Biosafety Level 1 (BL-1), we recommend that you consult the safety department of your institution to verify the Biosafety Level.

Primers

Bac-to-Bac® TOPO® Cloning Kits contain the following primers to sequence your insert.

Primer	Sequence
Polyhedrin forward primer	5'-AAATGATAACCATCTCGC-3'
SV40 polyA reverse primer	5'-GGTATGGCTGATTATGATC-3'

Gus control plasmid

Bac-to-Bac® TOPO® Cloning and Bac-to-Bac® TOPO® Expression System Kits include the control expression plasmid pFastBac™ Gus, which contains the Gus gene. When the recombinant baculovirus produced from the control plasmid is used to infect insect cells, it allows the expression of β-glucuronidase, which can be used in a rapid, qualitative assay for expression. See page 36 for details.

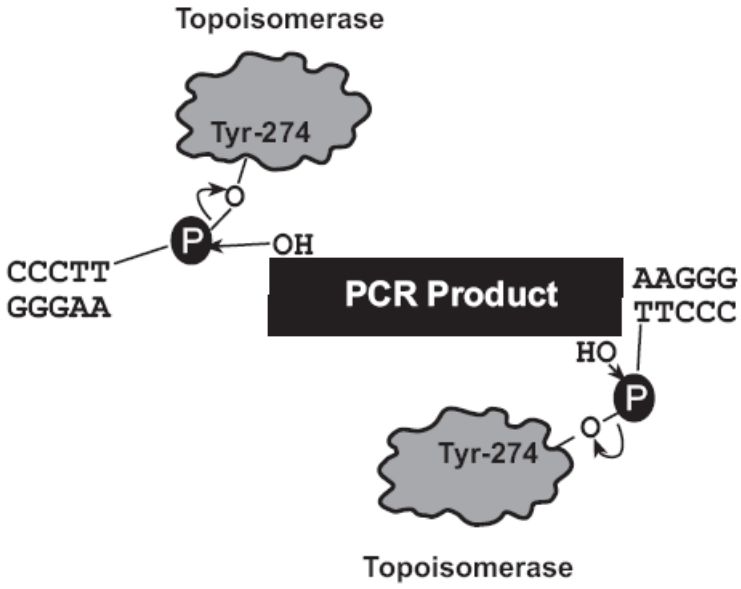
Description of the system

Product features

The Bac-to-Bac® N-His TOPO®, Bac-to-Bac® C-His TOPO®, and Bac-to-Bac® HBM TOPO® Cloning Kits include the pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, and pFastBac™/HBM-TOPO® cloning vectors, respectively. These vectors provide the means for the direct insertion of a **blunt-end PCR product** into a plasmid vector in a highly efficient, 5-minute, one-step cloning reaction (TOPO® Cloning). The ability to clone blunt-end PCR products allows the use of proofreading polymerases to amplify the gene of interest. No ligase, post-PCR procedures, or PCR primers containing specific sequences are required.

How Topoisomerase I works

Topoisomerase I from *Vaccinia* virus binds to duplex DNA at specific sites and cleaves the phosphodiester backbone after 5′-CCCTT in one strand (Shuman, 1991). The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3′ phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5′ hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase (Shuman, 1994). TOPO® Cloning exploits this reaction to efficiently clone PCR products (see diagram below).



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TOPO® cloning

The pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, and pFastBac™/HBM-TOPO® cloning vectors are supplied linearized with *Vaccinia* virus DNA topoisomerase I covalently bound to the 3´ end of each DNA strand (referred to as "TOPO®-activated" vector). DNA topoisomerase I facilitates the cloning of blunt-end PCR products encoding the gene of interest into the cloning vectors, which can then be transformed into chemically competent cells or electroporated directly into electrocompetent cells for analysis.

Once the transformants are analyzed for correct orientation and reading frame, the recombinant pFastBac™ TOPO® vector can be used for generating recombinant bacmid DNA to be transfected into insect cells to generate baculovirus for protein expression and further downstream applications.

For more information on generating bacmid DNA and protein expression in insect cells, refer to the Bac-to-Bac® TOPO® Expression System manual (part no. A10606) or the Bac-to-Bac® HBM TOPO® Secreted Expression System manual (part no. A11341) available at **www.lifetechnologies.com**, or contact Technical Support (page 40).

Features of the pFastBac™/ NT-TOPO® and CT-TOPO® vectors

The pFastBac™/NT-TOPO® and pFastBac™/CT-TOPO® vectors contain the following elements. These features have been functionally tested and the vectors have been fully sequenced.

- Strong polyhedrin (P_H) promoter for high-level baculovirus-based protein expression in insect cells
- TOPO® Cloning site for rapid and efficient cloning of **blunt-end** PCR products amplified with proofreading polymerases
- N- or C-terminal polyhistidine tag (pFastBac™/NT-TOPO® and pFastBac™/CT-TOPO®, respectively) for simple purification of recombinant proteins
- TEV protease cleavage site for removal of the polyhistidine tag following protein purification using AcTEV™ protease
- SV40 polyadenylation signal for efficient transcription termination and polyadenylation of the recombinant transcript
- Mini-Tn7 elements for site-specific transposition of your gene into the baculovirus shuttle vector (bacmid DNA) propagated in *E. coli*
- Ampicillin (*bla*) resistance gene (β-lactamase) for selection of transformants in *E. coli*
- pUC origin for high copy replication and maintenance of the plasmid in *E. coli*
- Gentamicin resistance gene for selection of transformants containing recombinant bacmid DNA

For vector maps of pFastBac™/NT-TOPO® and pFastBac™/CT-TOPO®, see pages 27 and 28, respectively.

Continued on next page

Features of the pFastBac™/ HBM-TOPO® vector

The pFastBac™/HBM-TOPO® vector contains the following elements. These features have been functionally tested and the vector has been fully sequenced.

- Strong polyhedrin (P_H) promoter for high-level baculovirus-based protein expression in insect cells
- TOPO® Cloning site for rapid and efficient cloning of **blunt-end** PCR products amplified with proofreading polymerases
- N-terminal Honey Bee Melittin (HBM) secretion signal coding sequence for secretion of the cloned gene product into the extracellular medium
- C-terminal polyhistidine tag for simple purification of recombinant proteins
- TEV protease cleavage site for removal of the polyhistidine tag following protein purification using AcTEV™ protease
- SV40 polyadenylation signal for efficient transcription termination and polyadenylation of the recombinant transcript
- Mini-Tn7 elements for site-specific transposition of your gene into the baculovirus shuttle vector (bacmid DNA) propagated in *E. coli*
- Ampicillin (*bla*) resistance gene (β-lactamase) for selection of transformants in *E. coli*
- pUC origin for high copy replication and maintenance of the plasmid in *E. coli*
- Gentamicin resistance gene for selection of transformants containing recombinant bacmid DNA

For the vector map of pFastBac™/HBM-TOPO®, see page 29.

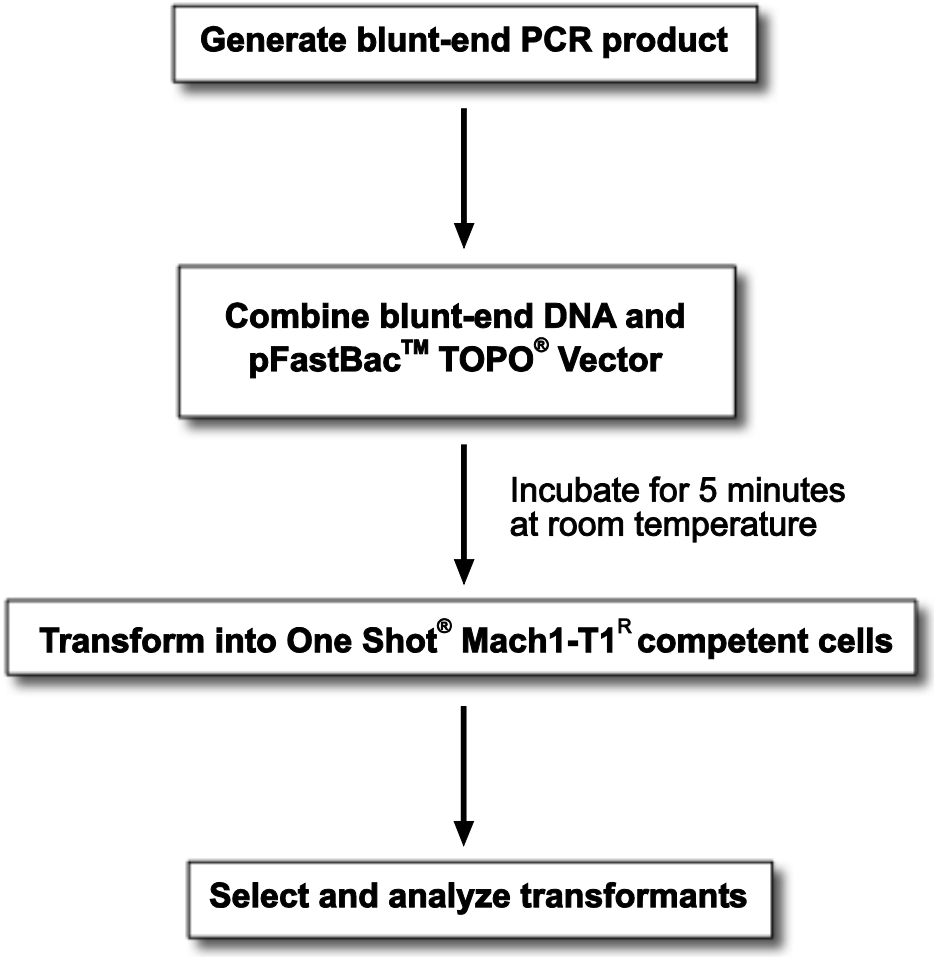
Continued on next page

Experiment outline

Experiment outline

To TOPO® Clone your gene of interest into pFastBac™ TOPO® vectors, perform the following steps:

1. Generate a PCR product containing your gene of interest with a thermostable proofreading DNA polymerase (e.g., AccuPrime™ *Pfx* DNA Polymerase, Platinum® *Pfx* DNA Polymerase).
2. TOPO® Clone your blunt-end PCR product into the pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, or pFastBac™/HBM-TOPO® vector, and use the reaction to transform One Shot® Mach1™ T1^R Chemically Competent *E. coli*.
3. Pick colonies, isolate plasmid DNA, and screen for insert directionality by sequencing expression clones with primers provided in the kit.



Methods

Generating blunt-end PCR products

Introduction

This kit is specifically designed to clone **blunt-end** PCR products generated by thermostable proofreading polymerases such as AccuPrime™ *Pfx* DNA Polymerase and Platinum® *Pfx* DNA Polymerase. Follow the guidelines below to design your PCR primers and to produce your blunt-end PCR product.

Note

Do not add 5´ phosphates to your primers for PCR. The PCR product synthesized will not ligate into pFastBac™ TOPO® vectors.

Considerations for pFastBac™/ NT-TOPO®

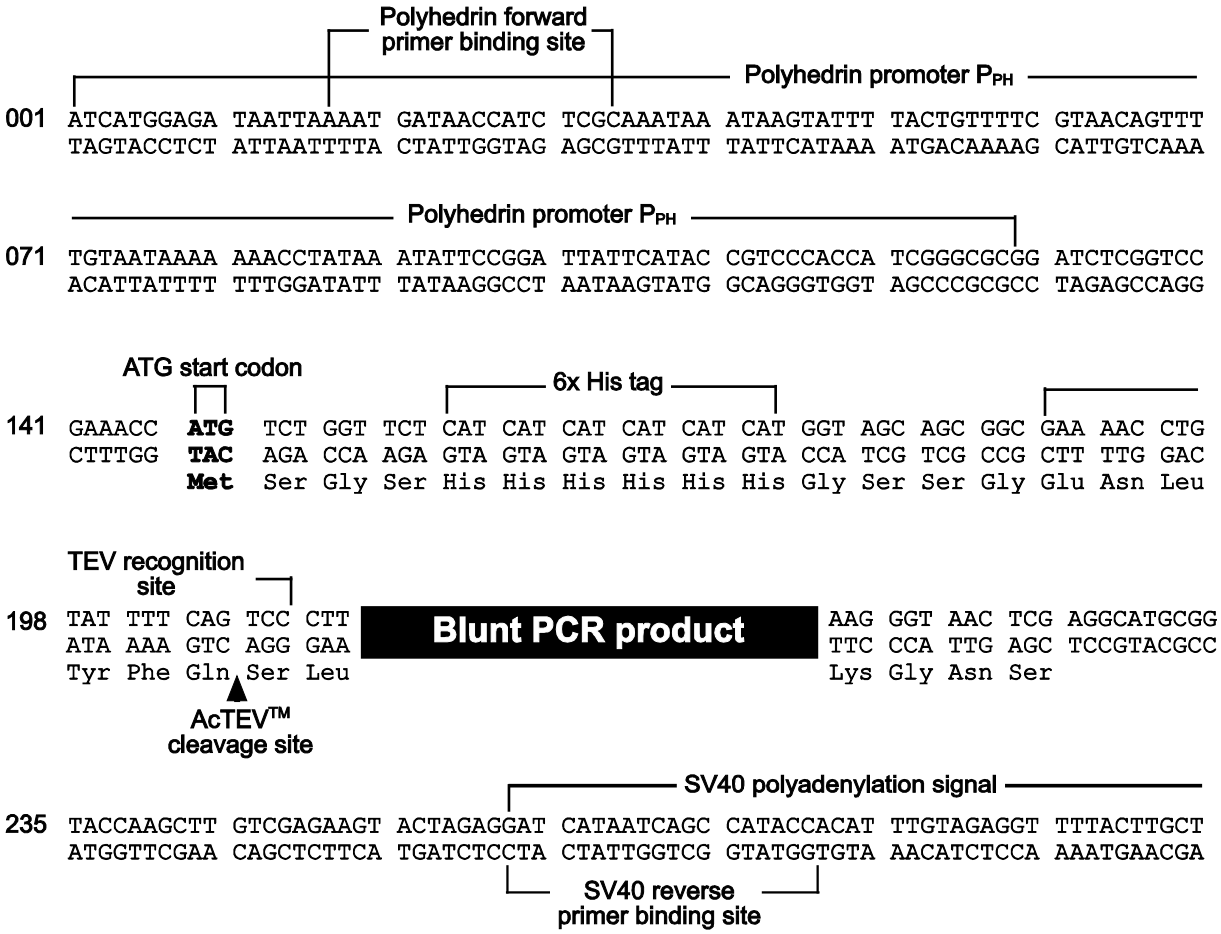
The cloning of a blunt-end PCR product into a pFastBac™/NT-TOPO® vector is a rapid and efficient process. However, to ensure proper expression of your recombinant protein, pay attention to the general considerations outlined below:

- The pFastBac™/NT-TOPO® vector contains the ATG start codon immediately upstream of the N-terminal polyhistidine tag; therefore, it is not necessary to include the initiation codon when designing your insert. However, your insert **may** include an ATG. Since the ribosome generally recognizes the first ATG, unless the internal ATG is in a particularly good context, the ATG in front of the polyhistidine will be used for initiation.
- It is best to eliminate the untranslated leader sequence from your insert and have the ATG start codon as close to the polyhedrin promoter as possible.
- Your insert must contain a stop codon.
- Do not include the Kozak sequence in the insert cloned into the pFastBac™/NT-TOPO® vector, because this sequence is not required for translation initiation in insect cells.
- If the gene of interest contains a polyadenylation signal, the first signal from the gene is recognized and the second signal in the vector is ignored. This does not affect expression.
- The cloning step presents the only limitation to the size of the insert. While the baculovirus genome can accommodate inserts of considerable size, large plasmids are more difficult to transform into *E. coli*.

Continued on next page

Cloning site of pFastBac™/ NT-TOPO® vector

Below is the TOPO® Cloning site of the pFastBac™/NT-TOPO® vector. **The vector sequence is available at www.lifetechnologies.com or by contacting Technical Support (page 40).**



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Considerations for pFastBac™/ CT-TOPO®

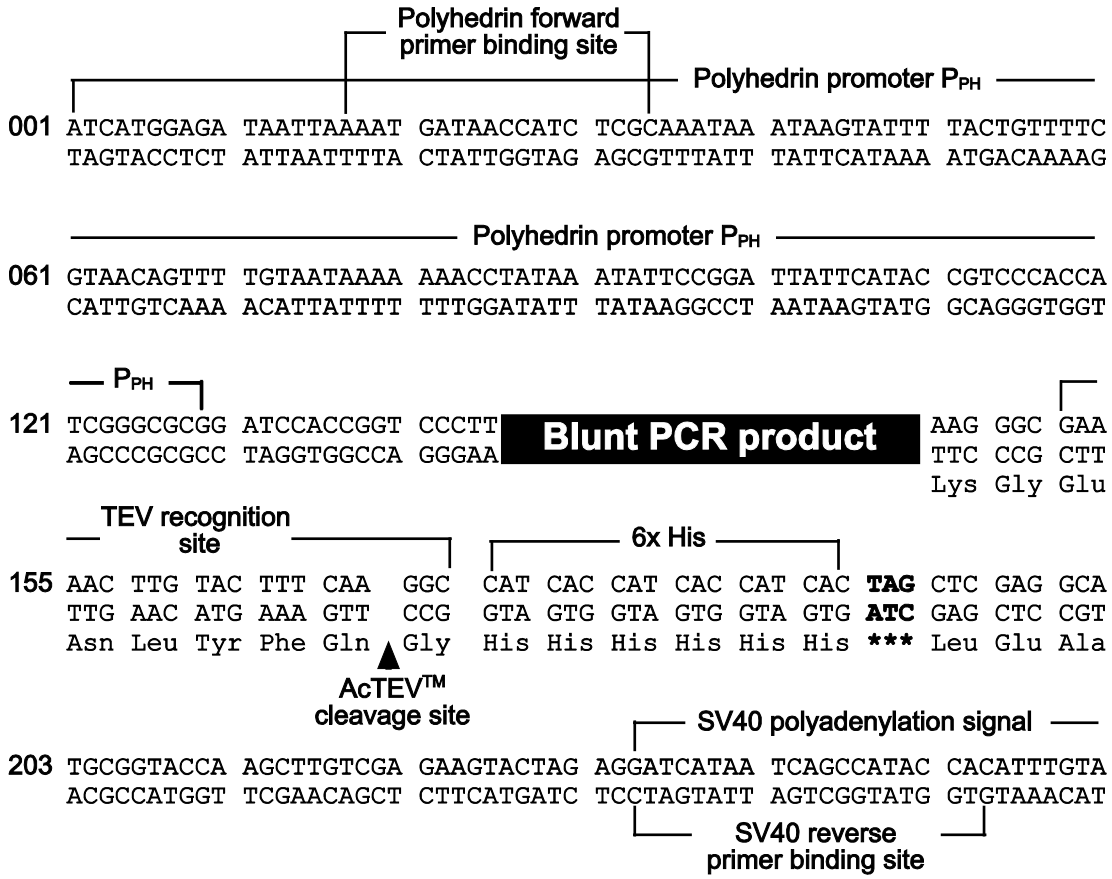
The cloning of a blunt-end PCR product into a pFastBac™/CT-TOPO® vector is a rapid and efficient process. However, to ensure proper expression of your recombinant protein, it is important to pay attention to the general considerations outlined below:

- When using the pFastBac™/CT-TOPO® vector, your insert must contain the ATG initiation codon.
- It is best to eliminate the untranslated leader sequence from your insert, and have the ATG start codon as close to the polyhedrin promoter as possible.
- It is not necessary to include the Kozak sequence in the insert cloned into the pFastBac™/CT-TOPO® vector; this sequence is not required for translation initiation in insect cells. However, in some cases, the Kozak sequence acts as an enhancer.
- Do not include a stop codon in the reverse primer for PCR if you want to use the C-terminal polyhistidine tag, because the pFastBac™/CT-TOPO® vector contains a stop codon immediately downstream of the C-terminal polyhistidine tag.
- If the gene of interest contains a polyadenylation signal, the first signal from the gene is recognized and the second signal in the vector is ignored. This does not affect expression.
- You can design your reverse primer to include a stop codon to omit the C-terminal tag encoded by the pFastBac™/CT-TOPO® vector, and express your protein in its native state.
- Similarly, you can design your PCR product to encode a different C-terminal tag followed by a stop codon to eliminate the pFastBac™/CT-TOPO® polyhistidine tag.
- The cloning step presents the only limitation to the size of the insert. While the baculovirus genome can accommodate inserts of considerable size, large plasmids are more difficult to transform into *E. coli*.

Continued on next page

Cloning site of pFastBac™/ CT-TOPO® vector

Below is the TOPO® Cloning site of the pFastBac™/CT-TOPO® vector. **The vector sequence is available at www.lifetechnologies.com or by contacting Technical Support (page 40).**



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Considerations for pFastBac™/ HBM-TOPO®

The cloning of a blunt-end PCR product into a pFastBac™/HBM-TOPO® vector is a rapid and efficient process. However, to ensure proper expression of your recombinant protein, it is important to pay attention to the general considerations outlined below:

- The pFastBac™/HBM-TOPO® vector contains the ATG start codon immediately upstream of the N-terminal HBM secretion signal sequence; therefore it is not necessary to include the initiation codon when designing your insert. However, your insert **may** include an ATG. Since the ribosome generally recognizes the first ATG, unless the internal ATG is in a particularly good context, the ATG in front of the polyhistidine will be used for initiation.
- Do not include the Kozak sequence in the insert cloned into the pFastBac™/HBM-TOPO® vector; this sequence is not required for translation initiation in insect cells.
- Do **not** include a stop codon in the reverse primer for PCR if you want to use the C-terminal polyhistidine tag, because the pFastBac™/HBM-TOPO® vector contains a stop codon immediately downstream of the C-terminal polyhistidine tag.
- If the gene of interest contains a polyadenylation signal, the first signal from the gene is recognized and the second signal in the vector is ignored. This does not affect expression.
- You can design your reverse primer to include a stop codon to omit the C-terminal tag encoded by the pFastBac™/HBM-TOPO® vector, and express your protein in its native state.
- Similarly, you can design your PCR product to encode a different C-terminal tag followed by a stop codon to eliminate the pFastBac™/HBM-TOPO® polyhistidine tag.
- The cloning step presents the only limitation to the size of the insert. While the baculovirus genome can accommodate inserts of considerable size, large plasmids are more difficult to transform into *E. coli*.

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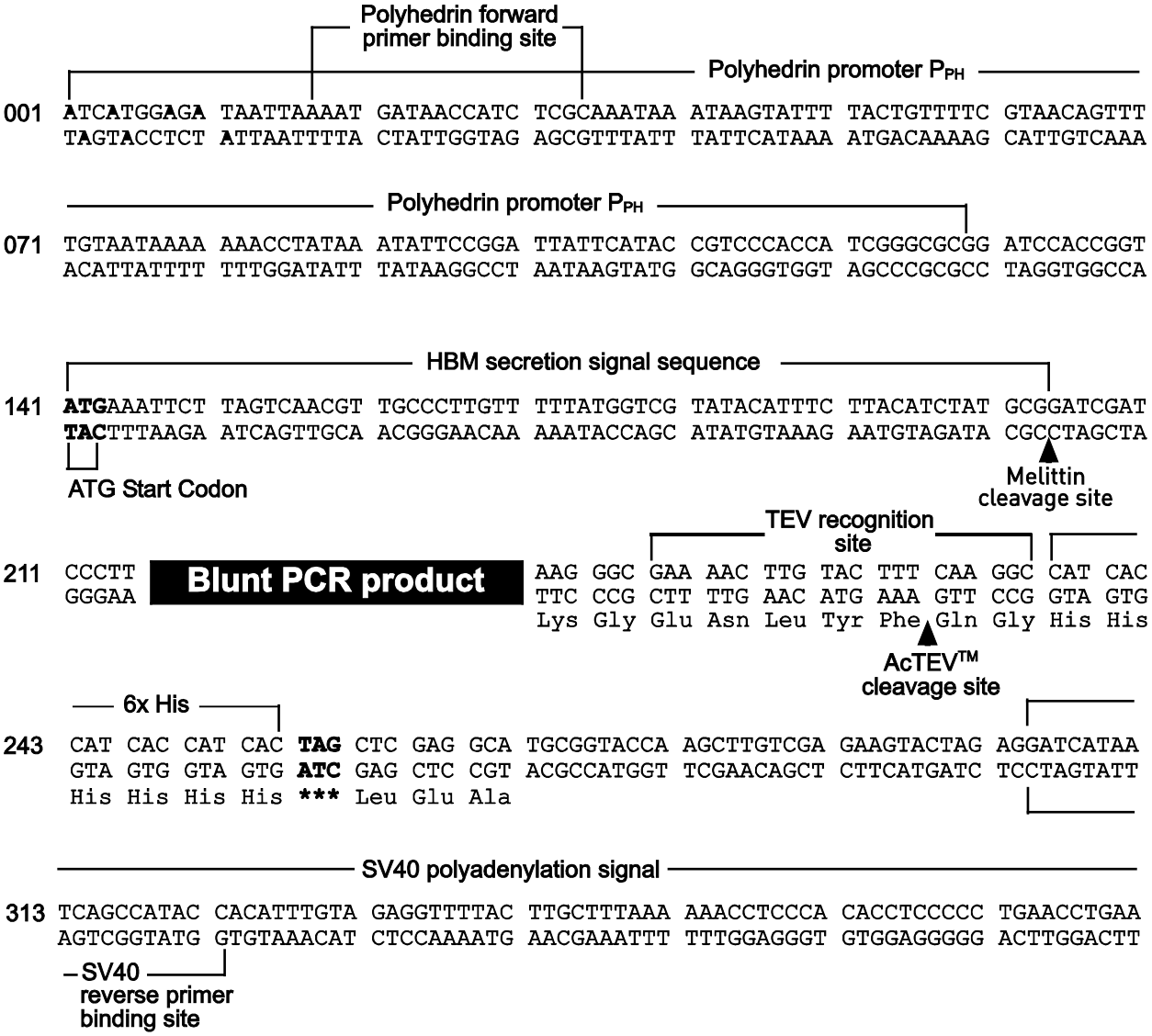
Cloning site of pFastBac™/ HBM-TOPO® vector

Below is the TOPO® Cloning site of the pFastBac™/HBM-TOPO® vector. **The vector sequence is available at www.lifetechnologies.com or by contacting Technical Support (page 40).**



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PCR reaction

After you have designed primers to amplify your gene of interest, you are ready to generate your PCR product for TOPO® Cloning into pFastBac™ TOPO® vectors.

Note: You must use a thermostable proofreading DNA polymerase such as Platinum® *Pfx* DNA Polymerase or AccuPrime™ *Pfx* DNA Polymerase to produce your blunt-end PCR product. *Taq* Polymerase has a terminal transferase activity that adds a single 3′-A overhang to each end of the PCR product, thus rendering it unsuitable for blunt-end TOPO® Cloning.

Materials needed

- Thermostable proofreading polymerase (see page 37)
- 10X PCR buffer appropriate for your polymerase
- Thermocycler
- DNA template and primers for your PCR product

Note: dNTPs (adjusted to pH 8) are provided in the kit.

Generating blunt-end PCR products

Set up a 25 µL or 50 µL PCR reaction using the guidelines below:

- Follow the instructions and recommendations provided by the manufacturer of your thermostable proofreading polymerase to produce blunt-end PCR products.
- Use the cycling parameters suitable for your primers and template. Make sure to optimize PCR conditions to produce a single, discrete PCR product.
- Use a 7–30 minute final extension to ensure that all PCR products are completely extended.

After completing the PCR reaction, place the tube on ice or store at –20ºC for up to 2 weeks. Proceed to **Checking the PCR product**, below.

Checking the PCR product

After you have produced your blunt-end PCR product, use agarose gel electrophoresis to verify the quality and quantity of your PCR product.

Be sure you have a single, discrete band of the correct size. If you do not have a single, discrete band, follow the manufacturer's recommendations for optimizing your PCR with the polymerase of your choice.

Alternatively, you may gel-purify the desired product using the PureLink® Quick Gel Extraction Kit, available separately. We also offer the E-Gel® CloneWell™ SYBR® Safe gels, which allow the isolation of DNA bands without any additional gel purification steps. See page 37 for ordering information.

Blunt-end TOPO® Cloning reaction

Introduction

After you have produced the desired PCR product, you are ready to TOPO® Clone your blunt-end insert into the pFastBac™ TOPO® vector (pFastBac™/NT-TOPO®, CT-TOPO®, or HBM-TOPO®), and use the recombinant vector to transform competent *E. coli*.

It is important to have everything you need to set up the reaction so that you can obtain the best results. We suggest that you read this entire section and the next section about transformation before beginning.

If this is the first time you are TOPO® Cloning, perform the control reactions detailed on pages 34 and 35 in parallel with your samples.

Using Salt in the TOPO® Cloning reaction

Perform TOPO® Cloning in a reaction buffer containing salt (i.e., using the stock salt solution provided in the kit). **Note that the amount of salt added to the TOPO® Cloning reaction varies depending on whether you plan to transform chemically competent cells (provided) or electrocompetent cells.**

- If you are transforming One Shot® Mach1™ $T1^R$ Chemically Competent *E. coli* (included in the kit), use the stock Salt Solution as supplied, and set up the TOPO® Cloning reaction as directed on the next page.
- If you are transforming electrocompetent *E. coli* (available separately; see page 37), the amount of salt in the TOPO® Cloning reaction **must be reduced** to 50 mM NaCl, 2.5 mM $MgCl_2$ to prevent arcing during electroporation. Dilute the stock Salt Solution 4-fold with water to prepare a 300 mM NaCl, 15 mM $MgCl_2$ Dilute Salt Solution. Use the Dilute Salt Solution to set up the TOPO® Cloning reaction as directed on the next page.

Note

We have found that including salt (200 mM NaCl, 10 mM $MgCl_2$) in the TOPO® Cloning reaction increases the number of transformants 2- to 3-fold. In addition, incubating the reaction mixture for greater than 5 minutes in the presence of salt can also increase the number of transformants. In experiments **without salt**, the number of transformants decreases as the incubation time increases beyond 5 minutes.

Including salt in the TOPO® Cloning reaction allows for longer incubation times because it prevents topoisomerase I from rebinding and potentially nicking the DNA after ligating the PCR product and dissociating from the DNA. The result is more intact molecules leading to higher transformation efficiencies.

Continued on next page

Materials needed

- Your PCR product (freshly prepared)
- pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, or pFastBac™/HBM-TOPO® vector
- Salt Solution or Dilute Salt Solution (see previous page)
- Sterile Water

Performing the TOPO® Cloning reaction

1. Set up your TOPO® Cloning reaction (6 µL) as described in the table below. The best insert:vector ratio in a TOPO® Cloning reaction is 1:1 to 2:1.

   Note: The blue color of the TOPO® vector solution is normal.

Reagent	Chemically Competent *E. coli*	Electrocompetent *E. coli*
PCR Product	0.5 to 4 µL	0.5 to 4 µL
Salt Solution	1 µL	–
Dilute Salt Solution	–	1 µL
Sterile Water	To a total volume of 5 µL	To a total volume of 5 µL
pFastBac™ TOPO® vector	1 µL	1 µL
Final Volume	**6 µL**	**6 µL**

Note: Store all reagents at –20°C when finished. Salt solution and water can be stored at room temperature or 4°C.

2. Mix the reaction gently, and incubate it for 5 minutes at room temperature (22°–23°C).

   Note: For most applications, 5 minutes yields a sufficient number of colonies for analysis. The length of the TOPO® Cloning reaction can be increased from 30 seconds to several hours. For routine subcloning of PCR products, 30 seconds may be sufficient. For larger PCR products (>3 kb), increasing the reaction time, may yield more colonies.

3. Place the reaction on ice and proceed to **Transforming One Shot® Mach1™ T1^{R} Chemically Competent *E. coli***, next page.

   Note: You may store the TOPO® Cloning reaction overnight at –20°C.

Transforming One Shot® Mach1™ T1^R Chemically Competent *E. coli*

Introduction

Once you have performed the Blunt-End TOPO® Cloning reaction, you are ready to use your construct to transform competent *E. coli*. One Shot® Mach1™ T1^R Chemically Competent *E. coli* cells are included with the kit (Box 2). You may also transform electrocompetent cells (see page 37 for ordering information) if desired following the **One Shot® electroporation** protocol provided in the **Appendix** (see page 32).

Protocols for transforming chemically competent *E. coli* are provided in this section. For instructions on performing control reactions, see **Performing the control reactions** in the **Appendix**, page 34.

Note: Do not use One Shot® Mach1™ T1^R Chemically Competent *E. coli* for electroporation.

IMPORTANT!

If you are transforming One Shot® Mach1™-T1^R Chemically Competent *E. coli*, it is essential that selective plates are **pre-warmed to 37°** prior to spreading for optimal growth of cells.

Materials needed

- TOPO® Cloning reaction from **Performing the TOPO® Cloning reaction**, Step 2 (page 20)
- S.O.C. medium at room temperature
- 42°C water bath
- LB plates containing 100 µg/mL ampicillin, pre-warmed to 37°C
- 37°C shaking and non-shaking incubator
- Competent cells (thawed on ice)

One Shot® Mach1™ T1^R Chemical Transformation

1. Thaw **on ice** one vial of One Shot® Mach1™ T1^R Chemically Competent *E. coli* for each transformation.
2. Add 2 µL of the TOPO® Cloning reaction (Step 2, page 20) into a vial of One Shot® Mach1™ T1^R Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Immediately transfer the vial(s) to ice, and incubate them on ice for 2 minutes.
6. Add 250 µL of room temperature S.O.C. medium to each vial.
7. Cap the vial(s) tightly and shake them horizontally at 37°C for 1 hour (225 rpm).
8. Spread 25–100 µL from each transformation on a **pre-warmed** selective plate. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies.
9. Invert the plate(s) and incubate at 37°C. With ampicillin selection, visible colonies should appear within 8 hours.
10. Pick ~10 colonies for analysis (see **Analyzing positive clones**, page 22). An efficient TOPO® Cloning reaction produces several hundred colonies.

Analyzing positive clones

Introduction

After transforming your pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, or pFastBac™/HBM-TOPO® construct into *E. coli*, select and sequence several colonies using the specific primers included in the kit to determine the orientation of the insert.

Analyzing positive clones

1. Pick 10 overnight-grown colonies from the selective plates and culture them overnight in LB medium containing 100 µg/mL ampicillin.
2. Isolate plasmid DNA using your method of choice. If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend using the PureLink® HiPure Mini Plasmid Purification Kit (see page 37 for ordering information).
3. Analyze plasmid DNA by sequencing (see below).

Note

If you have used One Shot® Mach1™ T1^R Chemically Competent *E. coli* for your transformation, you can prepare plasmid DNA 4 hours after inoculating a single, overnight-grown colony in the selective media of choice. Note that this feature is not limited to ampicillin selection.

Sequencing

To confirm that your gene of interest is in the correct orientation, you may sequence your expression construct using the Polyhedrin forward and SV40 polyA reverse primers included with the kit. Refer to page 7 for the sequences of the primers and pages 13, 15, and 17 for the location of the primer binding sites of pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, and pFastBac™/HBM-TOPO®, respectively.

PCR analysis of transformants

You may also determine the orientation of your insert by PCR amplification using a pair of primers, where one primer binds outside the TOPO® Cloning site (e.g., polyhedrin forward primer), while the other is internal to your blunt-end PCR insert. The PCR product, the size of which will depend on the orientation of the insert, can then easily be visualized on an agarose gel.

Long-term storage

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage.

1. Streak the original colony out for single colonies on an LB plate containing 100 µg/mL ampicillin.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 100 µg/mL ampicillin.
3. Grow at 37°C with shaking until culture reaches stationary phase.
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol.
5. Vortex and transfer to a labeled cryovial.
6. Freeze the tube in liquid nitrogen or dry ice/ethanol bath and store at –80°C.

We also recommend that you store a stock of plasmid DNA at –20°C.

Next steps

Introduction

After you obtain the correct pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, or pFastBac™/HBM-TOPO® recombinant plasmid construct, create a recombinant bacmid to transfect into your insect cell line of choice to create a recombinant baculovirus. After amplifying and titering the baculovirus stock, you will be ready to use this stock to infect insect cells to express your protein of interest.

- For more information on generating bacmid DNA and intracellular protein expression in insect cells, refer to the Bac-to-Bac® TOPO® Expression System manual (part no. A10606).
- For more information on generating bacmid DNA and secreted protein expression in insect cells, refer to the Bac-to-Bac® HBM TOPO® Secreted Expression System manual (part no. A11341).

These manuals are available at **www.lifetechnologies.com/manuals** or by contacting Technical Support (page 40).

Bac-to-Bac® TOPO® Expression System

The Bac-to-Bac® TOPO® Expression System provides a rapid and efficient method of generating recombinant baculoviruses, based on site-specific transposition of an expression cassette into a baculovirus shuttle vector (bacmid) propagated in *E. coli*. The major components of the Bac-to-Bac® TOPO® Expression System include:

- pFastBac™/NT-TOPO® or pFastBac™/CT-TOPO® plasmid that allows generation of an expression construct containing the gene of interest.
- A competent *E. coli* host strain, MAX Efficiency® DH10Bac™, that contains a baculovirus shuttle vector (bacmid) and a helper plasmid, and that allows generation of a recombinant bacmid following transposition of your pFastBac™ TOPO® expression construct.
- pFastBac™ Gus control expression plasmid that allows production of a recombinant baculovirus which, when used to infect insect cells, expresses β-glucuronidase.
- An improved transfection reagent, Cellfectin® II, which provides high efficiency gene expression with minimal cytotoxicity across adherent and suspension cell lines.

Continued on next page

Bac-to-Bac® HBM TOPO® Secreted Expression System

The Bac-to-Bac® HBM TOPO® Secreted Expression System allows the rapid generation of recombinant baculoviruses for secreted protein expression. It contains the following major components:

- pFastBac™/HBM-TOPO® plasmid that allows generation of an expression construct containing the gene of interest in frame with the Honey Bee Melittin (HBM) secretion signal coding sequence for secretion of the cloned gene product into the extracellular medium
- A competent *E. coli* host strain, MAX Efficiency® DH10Bac™, that contains a baculovirus shuttle vector (bacmid) and a helper plasmid, and that allows generation of a recombinant bacmid following transposition of your pFastBac™/HBM-TOPO® expression construct.
- pFastBac™ Gus control expression plasmid that allows production of a recombinant baculovirus which, when used to infect insect cells, expresses β-glucuronidase.
- An improved transfection reagent, Cellfectin® II, which provides high efficiency gene expression with minimal cytotoxicity across adherent and suspension cell lines.

Appendix A: Troubleshooting

Troubleshooting

Introduction

The table below lists some potential problems solutions that may help you troubleshoot your TOPO® Cloning and expression of your gene of interest.

Observation	Possible cause	Solution
Few or no colonies obtained from sample reaction, but transformation control yielded colonies	Incomplete extension during PCR	Include a final extension step of 7–30 minutes during PCR. Longer PCR products need a longer extension time.
	Excess or dilute PCR product used in the TOPO® Cloning reaction	Reduce or concentrate the amount of PCR product.
	PCR primers contain 5' phosphates	Do not add 5' phosphates to your PCR primers.
	Large PCR product	• Increase the amount of PCR product used in the TOPO® Cloning reaction. • Increase the incubation time of TOPO® Cloning reaction from 5 minutes to 30, 60, or 120 minutes. Or incubate overnight. • Gel-purify the PCR product to remove primer-dimers or other artifacts.
	PCR reaction contains artifacts (i.e., not a single band on an agarose gel)	• Optimize your PCR conditions. • Gel-purify your PCR product.
	PCR product contains 3' A-overhangs because you used *Taq* polymerase	Use a thermostable proofreading DNA polymerase such as Platinum® *Pfx* DNA Polymerase or AccuPrime™ *Pfx* DNA Polymerase to produce your blunt-end PCR product.

Continued on next page

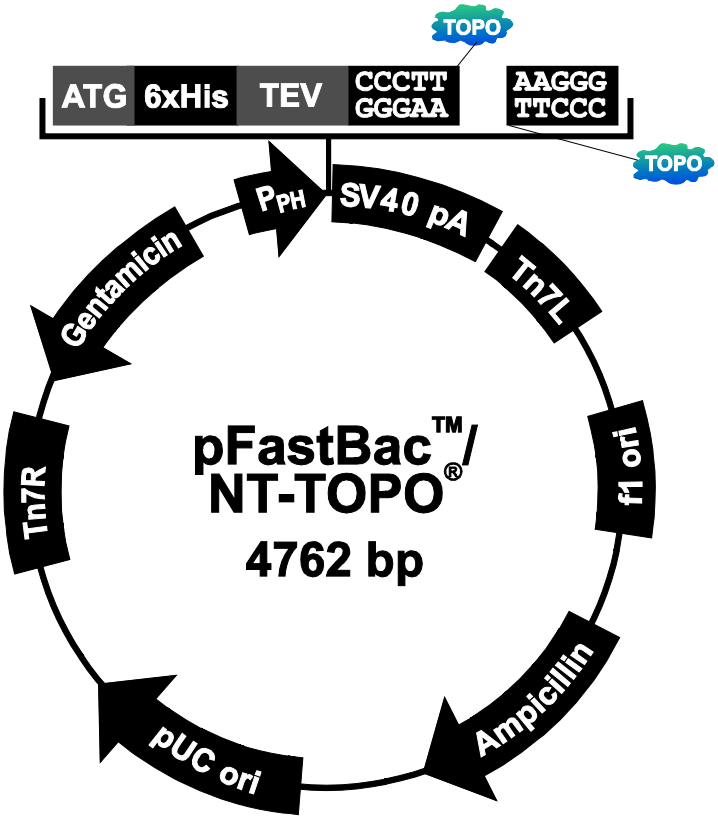
Problem	Possible Cause	Solution
Large number of incorrect inserts cloned	PCR cloning artifacts	• Gel-purify your PCR product to remove primer-dimers and other artifacts. • Optimize your PCR conditions. • Include a final extension step of 7–30 minutes during PCR.
High background and large number of satellite colonies after transformation.	Recovery period after transformation too long.	Reduce incubation period after transformation from 1 hour to 5–10 minutes at 37°C (225 rpm).
Few or no colonies obtained from sample reaction and the transformation control gave no colonies	One Shot® competent *E. coli* stored incorrectly	• Store One Shot® competent *E. coli* at –80°C. • If you are using another *E. coli* strain, follow the manufacturer's instructions.
	Insufficient amount of *E. coli* plated	Increase the amount of *E. coli* plated.
	Transformants plated on selective plates with the wrong antibiotic	Use LB ampicillin plates for selection.

Appendix B: Vectors

Map of pFastBac™/NT-TOPO®

Description

The map below shows the elements of the pFastBac™/NT-TOPO® vector. **The vector sequence is from www.lifetechnologies.com or by contacting Technical Support (page 40).**



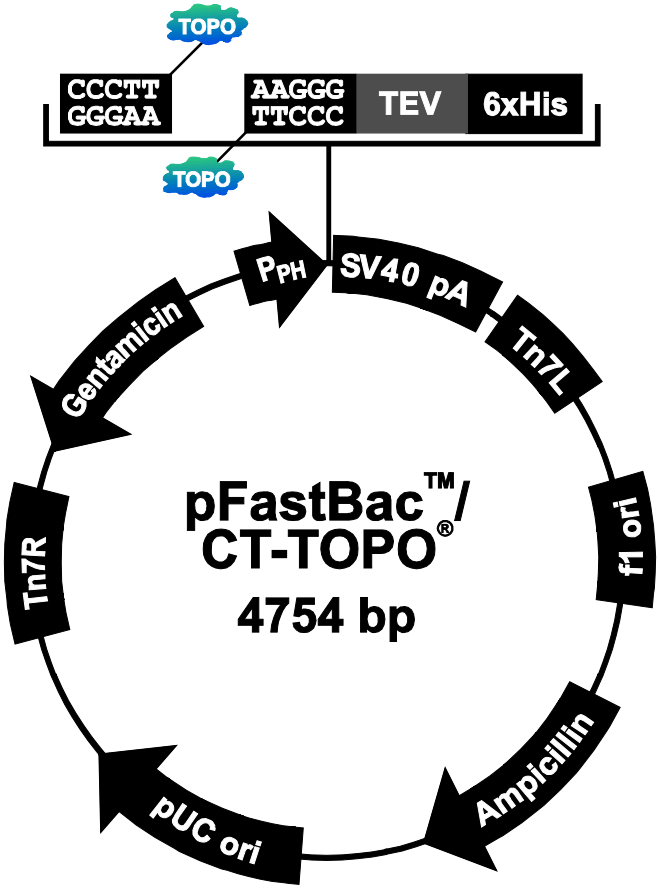
Comments for pFastBac™/NT-TOPO® vector
4762 nucleotides

Polyhedrin promoter (P_{PH}): bases 1-129
Initiation ATG: bases 147-149
6xHis tag: bases 159-179
TEV recognition site: bases 189-209
TOPO cloning site: bases 212-213
SV40 polyadenylation signal: bases 262-502
Tn7L: bases 531-696
f1 origin: bases 880-1334
Ampicillin resistance gene: bases 1465-2325
pUC origin: bases 2470-3143
Tn7R: bases 3389-3613
Gentamicin resistance gene: bases 3680-4208 (complementary strand)

Map of pFastBac™/CT-TOPO®

Description

The map below shows the elements of pFastBac™/CT-TOPO® vector. **The vector sequence is available from www.lifetechnologies.com or by contacting Technical Support (page 40).**



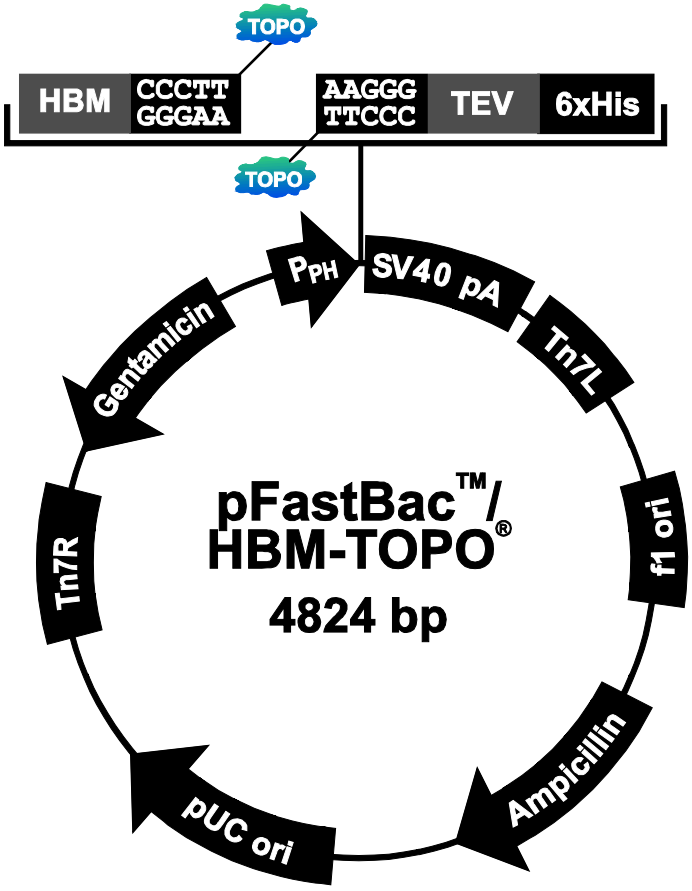
Comments for pFastBac™/CT-TOPO® vector
4754 nucleotides

Polyhedrin promoter (P_{PH}): bases 1-129
TOPO cloning site: bases 145-146
TEV recognition site: bases 152-169
6xHis tag: bases 173-190
SV40 polyadenylation signal: bases 235-475
Tn7L: bases 504-669
f1 origin: bases 853-1307
Ampicillin resistance gene: bases 1438-2298
pUC origin: bases 2443-3116
Tn7R: bases 3362-3586
Gentamicin resistance gene: bases 3653-4186 (complementary strand)

Map of pFastBac™/HBM-TOPO®

Description

The map below shows the elements of pFastBac™/HBM-TOPO® vector. **The vector sequence is available from www.lifetechnologies.com or by contacting Technical Support (page 40).**



Comments for pFastBac™/HBM-TOPO® vector 4824 nucleotides

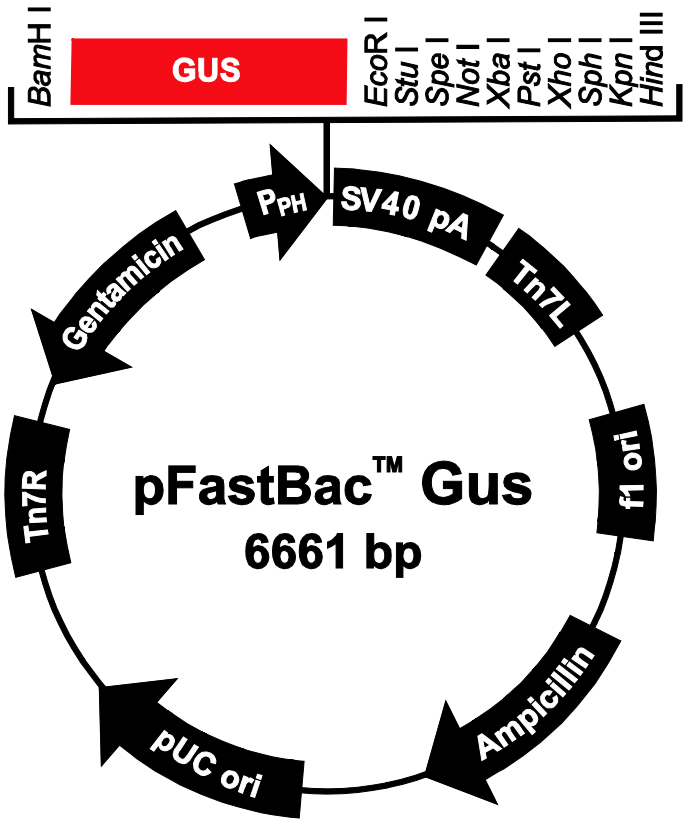
Polyhedrin promoter (P_{PH}): bases 1-129
Honey Bee Mellitin (HBM) secretion signal: 141-210
TOPO cloning site: bases 215-216
TEV recognition site: bases 222-242
6xHis tag: bases 243-260
SV40 polyadenylation signal: bases 305-545
Tn7L: bases 574-739
f1 origin: bases 923-1377
Ampicillin resistance gene: bases 1508-2368
pUC origin: bases 2513-3186
Tn7R: bases 3432-3656
Gentamicin resistance gene: bases 3723-4256 (complementary strand)

Map of pFastBac™ Gus control plasmid

Description

pFastBac™ Gus is a 6,661 bp control vector that contains the *Arabidopsis thaliana* gene for β-glucuronidase (Gus) (Kertbundit *et al.*, 1991). The molecular weight of β-glucuronidase is 68.5 kDa.

The map below shows the elements of pFastBac™ Gus control plasmid. **The vector sequence is available from www.lifetechnologies.com or by contacting Technical Support (page 40).**



Comments for pFastBac™ Gus vector 6661 nucleotides

f1 origin: bases 2-457
Ampicillin resistance gene: bases 589-1449
pUC origin: bases 1594-2267
Tn7R: bases 2511-2735
Gentamicin resistance gene: bases 2802-3335 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 3904-4032
GUS ORF: bases 4081-5892
SV40 polyadenylation signal: bases 6047-6287
Tn7L: bases 6315-6480

Appendix C: Support protocols

Recipes

Pre-mixed media

We carry pre-mixed growth media, such as imMedia™, in convenient pouches or in bulk. imMedia™ is pre-mixed and pre-sterilized for convenient preparation of liquid medium or agar plates for *E. coli* growth, and it is available with or without IPTG and X-gal and a choice of three antibiotics: ampicillin, kanamycin, or Zeocin™ selection agent. Refer to page 37 for ordering information.

LB (Luria-Bertani) medium and plates

Composition:

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic if needed.
4. Store at room temperature or at 4°C.

LB agar plates

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic if needed, and pour into 10 cm plates.
4. Let harden, then invert and store at 4°C.

One Shot® electroporation

Introduction

Although chemical transformation of *E. coli* is the most convenient method, electroporation is more efficient, and it is the method of choice for large plasmids. This section provides an optional protocol if you prefer to use electroporation.

We offer a variety of high transformation-efficiency electrocompetent cells that are suitable for use with your recombinant pFastBac™ TOPO® vector. For more information on electrocompetent *E. coli* cells, refer to **www.lifetechnologies.com** or contact Technical Support (page 37).

This section provides a protocol for transforming **One Shot® Electrocomp™ *E. coli*** with your recombinant pFastBac™ TOPO® vector.

IMPORTANT!

Do not use One Shot® Mach1™ T1^{R} Chemically Competent *E. coli* for electroporation.

Materials needed

In addition to general microbiological supplies (e.g., plates, spreaders), you will need the following reagents and equipment.

- TOPO® Cloning reaction from **Performing the TOPO® Cloning reaction**, Step 2 (page 20)
- Electroporator
- S.O.C. medium at room temperature
- LB plates containing 100 µg/mL ampicillin, pre-warmed to 37°C.
- Electrocompetent cells (thawed on ice)

Continued on next page

One Shot® electroporation protocol

1. Add 2 µL of the TOPO® Cloning reaction to a vial (50 µL) of One Shot® Electrocomp™ *E. coli* and mix gently. **Do not mix by pipetting up and down. Avoid formation of bubbles.**
2. Carefully transfer cells and DNA to a chilled 0.1 cm cuvette.
3. Electroporate your samples using your own protocol and electroporator.

   Note: If you have problems with arcing, see the next page.
4. Immediately add 250 µL of room temperature S.O.C. medium to the cuvette.
5. Transfer the solution to a 15 mL snap-cap tube (e.g., Falcon®) and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance genes.
6. Spread 10–50 µL from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µL of S.O.C. medium. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. Incubate plates overnight at 37°C.
7. Pick ~10 colonies for analysis (see **Analyzing positive clones**, page 22). An efficient TOPO® Cloning reaction produces several hundred colonies.

Note

Addition of the Dilute Salt Solution in the **TOPO® Cloning reaction** brings the final concentration of NaCl and $MgCl_2$ in the TOPO® Cloning reaction to 50 mM and 2.5 mM, respectively. To prevent arcing of your samples during electroporation, the volume of cells should be between 50 µL and 80 µL (0.1 cm cuvettes) or 100 µL to 200 µL (0.2 cm cuvettes).

If you experience arcing during transformation, try **one** of the following suggestions:

- Reduce the voltage normally used to charge your electroporator by 10%
- Reduce the pulse length by lowering the load resistance to 100 ohms
- Precipitate the TOPO® Cloning reaction and resuspend in water prior to electroporation

Performing the control reactions

Introduction

We recommend performing the following control TOPO® Cloning reactions the first time you use the kit to help you evaluate your results. Performing the control reactions involves generating a 750 bp control PCR product and using the PCR product directly in a TOPO® Cloning reaction.

Producing the control PCR product

1. Set up the following reaction in a 50 µL volume.

Reagent	Amount
Control PCR Template	1 µL
10X PCR Buffer	5 µL
50 mM dNTPs	0.5 µL
Control PCR Primers (0.1 µg/µL each)	1 µL each
Sterile Water	40.5 µL
Thermostable Proofreading DNA Polymerase (e.g., Platinum® *Pfx* DNA Polymerase)	1 µL
Total Volume	**50 µL**

2. Amplify the control PCR product using the following cycling parameters:

Step	Time	Temp.	Cycles
Initial Denaturation	2 min	94°C	1X
Denaturation	1 min	94°C	25X
Annealing	1 min	55°C	
Extension	1 min	72°C	
Final Extension	7 min	72°C	1X

3. Remove 10 µL from the reaction and analyze by gel electrophoresis on a 0.8% agarose gel. A discrete 750 bp band should be visible.

Continued on next page

Control TOPO® Cloning reactions

1. Using the control PCR product generated in the steps above and the control vector, set up two 6 µL TOPO® Cloning reactions as described below:

Reagent	Vector Only	Vector + PCR Insert
Control PCR Product	–	1 µL
Sterile Water	4 µL	3 µL
Salt Solution or Dilute Salt Solution	1 µL	1 µL
pFastBac™/NT-TOPO®, pFastBac™/CT-TOPO®, or pFastBac™/HBM-TOPO®	1 µL	1 µL

2. Incubate the reactions at room temperature for 5 minutes, and place them on ice.
3. Use 2 µL of the reaction to transform two separate vials of One Shot® competent cells using the procedure on page 21.
4. Spread 10–50 µL of each transformation mix onto LB plates containing 100 µg/mL ampicillin. When plating small volumes, add 20 µL of S.O.C. medium to ensure even spreading. Be sure to plate two different volumes to ensure that at least one plate has well-spaced colonies.
5. Incubate plates overnight at 37°C.

What you should see

The vector + PCR insert reaction should produce hundreds of colonies. 95% of these colonies should contain the 750 bp insert when analyzed by restriction digestion and agarose gel electrophoresis.

The vector-only reaction should yield very few colonies (<15% of the vector + PCR insert plate).

Transformation control

The pUC19 plasmid is included to check the transformation efficiency of the One Shot® Mach1™ T1^R chemically competent cells.

1. Transform one vial of One Shot® Mach1™ T1^R cells with 10 pg of pUC19 using the protocol on page 21.
2. Plate 10 µL of the transformation reaction plus 20 µL of S.O.C. on LB plates containing 100 µg/mL ampicillin.

The transformation efficiency should be 1×10^9 cfu/µg DNA.

Control for protein expression in insect cells

Assay for β-glucuronidase

If you include the pFastBac™ Gus baculoviral control construct in your expression experiment, you may assay for β-glucuronidase expression. To assess β-glucuronidase expression in a rapid manner, mix a small amount of media from the infected cells with the chromogenic indicator X-glucuronide, and observe the development of blue color.

1. Mix 5 µL of 20 mg/mL X-glucuronide solution (in DMSO or dimethylformamide) with 50 µL of cell-free medium.
2. Monitor for development of blue color within 2 hours.

Note: Other methods are also suitable.

Appendix D: Ordering information

Accessory products

Additional products

The table below lists additional products that may be used with Bac-to-Bac® TOPO® Cloning Kits. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (page 40).

Product	Amount	Cat. no.
Platinum® *Pfx* DNA Polymerase	100 units 250 units 500 units	11708-013 11708-021 11708-039
AccuPrime™ *Pfx* DNA Polymerase	200 reactions 1000 reactions	12344-024 12344-032
Pfx50™ DNA Polymerase	100 reactions 500 reactions	12355-012 12355-036
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions 20 reactions	C4040-10 C4040-03
One Shot® Mach1™-T1^R Chemically Competent *E. coli*	20 reactions	C8620-03
One Shot® TOP10 Electrocompetent *E. coli*	10 reactions 20 reactions	C4040-50 C4040-52
PureLink® PCR Purification Kit	50 preps	K3100-01
PureLink® Quick Gel Extraction System	1 kit	K2100-12
PureLink® HiPure Plasmid Miniprep Kit	25 preps 100 preps	K2100-02 K2100-03
E-Gel® CloneWell™ 0.8% SYBR Safe™, E-Gel® iBase™ & E-Gel® Safe Imager™ Starter Kit	1 kit	G6500ST
E-Gel® CloneWell™ 0.8% SYBR® Safe gels, 18-Pak	18 gels	G6618-08
E-Gel® 1.2% Starter Pak (6 gels + Powerbase™)	1 kit	G6000-01
E-Gel® 1.2% 18 Pak	18 gels	G5018-01
PCR Optimizer™ Kit	1 kit	K1220-01
AcTEV™ Protease	1000 Units 10,000 Units	12575-015 12575-023
imMedia™ Amp Liquid	20 pouches (200 mL medium)	Q600-20
imMedia™ Amp Agar	20 pouches (8–10 plates)	Q601-20
LB Broth (1X), liquid	500 mL	10855-021
S.O.C. Medium	10 × 10 mL	15544-034
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Gentamicin, liquid	10 mL (50 mg/mL)	15750-060

Appendix E: Safety

Chemical safety

WARNING!

GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING!

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Obtaining support

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining SDS

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Technical support

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

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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