USER GUIDE

invitrogen™ by life technologies™

pTracer™-CMV/Bsd

Catalog numbers V883-01 and V883-20

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life technologies™

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Kit Contents and Storage

Introduction

This manual is supplied with the following products. Cat. no. V883-20 includes vectors only, while Cat. no. V883-01 includes vectors and the selection agent, blasticidin. See below for a detailed description of the contents of each kit.

Item	Cat. no.
pTracer™-CMV/Bsd Kit	V883-01
pTracer™-CMV/Bsd Vector	V883-20

Shipping and Storage

Cat. no. V883-01 is shipped at room temperature. Upon receipt, store the plasmids and the blasticidin powder at –20°C.

Cat. no. V883-20 is shipped at room temperature. Upon receipt, store the plasmids at –20°C.

Kit Contents

The pTracer™-CMV/Bsd vector and the pTracer™-CMV/Bsd/*lacZ* control plasmid are supplied with each catalog number. The pTracer™-CMV/Bsd kit also includes blasticidin antibiotic. The amount of each reagent provided is listed below.

Reagent	Composition	Amount
pTracer™-CMV/Bsd	0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.	20 µg
pTracer™-CMV/Bsd/*lacZ*	0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.	20 µg
Blasticidin antibiotic	powder	50 mg

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

Introduction

Product Overview

Description of the System

pTracer™-CMV/Bsd is a 6.0 kb mammalian expression vector designed for visual detection of transfected mammalian cells. The vector allows high-level expression of the gene of interest in mammalian cells, and can be used in both transient and stable expression studies. pTracer™-CMV/Bsd contains the following elements:

- Human cytomegalovirus (CMV) immediate-early promoter for high-level expression of your gene of interest in a wide range of mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987).
- Cycle 3-GFP, an improved GFP (Green Fluorescent Protein) gene (Crameri *et al.*, 1996) for non-invasive *in vivo* detection, fused to the blasticidin resistance gene (see below).
- Blasticidin resistance gene (Kimura *et al.*, 1994; Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965) for selection in both *E. coli* and mammalian cells (see page 12 for more information).
- Human elongation factor 1α-subunit (hEF-1α) promoter (Goldman *et al.*, 1996; Mizushima and Nagata, 1990) for expression of the cycle 3-GFP-blasticidin fusion gene in mammalian cells.

The control plasmid, pTracer™-CMV/Bsd/*lacZ*, is included for use as a positive control for transfection and expression.

Description of Cycle 3-GFP

The cycle 3-GFP gene used in this vector is described in Crameri *et al.*, 1996. In this paper, the codon usage of GFP was optimized for expression in mammalian cells and three cycles of DNA shuffling were used to generate a mutant form of GFP that has the following characteristics:

- Excitation and emission maxima that are the same as wild-type GFP (395 nm and 478 nm for primary and secondary excitation, respectively, and 507 nm for emission).
- >40-fold increase in fluorescent yield over wild-type GFP for visual detection of transformed cells.

The cycle 3-GFP gene is fused to the blasticidin resistance marker to correlate GFP fluorescence with resistance to blasticidin.

Continued on next page

Experimental Overview

The table below outlines the basic steps needed to clone and express your gene of interest in pTracer™-CMV/Bsd and to visually detect transformed or transfected cells.

Step	Action	Page
1	Develop a cloning strategy to ligate your gene into pTracer™-CMV/Bsd. Use the diagram of the multiple cloning site on page 4 to assist you.	3–5
2	Transform *E. coli* and select transformants.	5
3	Analyze transformants for the presence and orientation of the insert.	5
4	Isolate pure plasmid DNA and transfect your mammalian cell line.	6
5	Assay for fluorescence and estimate transfection efficiency.	7
6	Assay for optimal expression of your gene.	7
7	Optional: Select for stable cell lines using blasticidin.	8–9

Methods

Cloning into pTracer™-CMV/Bsd

Introduction

A diagram is provided on page 4 to help you ligate your gene of interest into pTracer™-CMV/Bsd. General considerations for cloning and transformation are discussed below.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: a Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

***E. coli* Strain**

Many *E. coli* strains are suitable for the propagation of the pTracer™-CMV/Bsd vector including TOP10, JM109, and DH5α™. We recommend that you propagate the pTracer™-CMV/Bsd vector in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 *E. coli* are available as chemically competent or electrocompetent cells for purchase (see page 16 for ordering information).

Transformation Method

You may use any method of choice for transformation. Chemical transformation is the most convenient for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintenance of Plasmids

The pTracer™-CMV/Bsd and pTracer™-CMV/Bsd/*lacZ* vectors contain the ampicillin resistance gene and the blasticidin resistance gene to allow selection of the plasmid using ampicillin or blasticidin. To propagate and maintain the pTracer™-CMV/Bsd and pTracer™-CMV/Bsd/*lacZ* plasmids, we recommend using the following procedure:

1. Use the supplied 0.5 µg/µL stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™, JM109, or equivalent.
2. Select transformants on LB plates containing 50–100 µg/mL ampicillin or 50 µg/mL blasticidin in Low Salt LB (see page 11 for a recipe).
3. Prepare a glycerol stock from each transformant containing plasmid for long-term storage (see page 5).

Continued on next page

Cloning Considerations

Your insert should contain a Kozak translation initiation sequence with an ATG start codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Note that other sequences are possible, but the G or A at position –3 and the G at position +4 are the most critical for function (shown in bold). The ATG initiation codon is shown underlined.

(**G/A**)NN<u>ATG</u>**G**

Your insert should also contain a stop codon.

Multiple Cloning Site of pTracer™-CMV/Bsd

Below is the multiple cloning site for pTracer™-CMV/Bsd. Restriction sites are labeled to indicate the cleavage site. Potential stop codons are underlined. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pTracer™-CMV/Bsd is available for downloading from www.lifetechnologies.com or from Technical Support (see page 17). For a map and a description of the features of pTracer™-CMV/Bsd, refer to the Appendix, pages 13–14.

```
                                                               enhancer region (3′ end)
 661   ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC
                                                                   CAAT
 721   AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG
       CMV Forward priming site     TATA      3′ end of hCMV    putative transcriptional start
 781   GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
                                  T7 Promoter/priming site              Nhe I
 841   CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
         Pme I                                              BstX I   EcoR I
 901   GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCACTAGT CCAGTGTGGT GGAATTCTGC
         EcoR V      BstX I   Not I         Xba I          Pme I
 961   AGATATCCAG CACAGTGGCG GCCGCTCGAG TCTAGAGGGC CCGTTTAAAC CCGCTGATCA
          BGH Reverse priming site
1021   GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC
                                               BGH polyadenylation signal
1081   TTGACCCTGG AAGGTGCCAC TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG
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Cloning into pTracer™-CMV/Bsd, Continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (*e.g.* TOP10, JM109, DH5α™) and select on LB agar plates containing 50 to 100 µg/mL ampicillin or Low Salt LB containing 50 µg/mL blasticidin (see page 11).

Blasticidin Selection in *E. coli*

To facilitate selection of blasticidin-resistant *E. coli*, the salt concentration of the medium must remain low (< 90 mM) and the pH must be 7.0. Prepare Low Salt LB broth and plates using the recipe in the Appendix, page 11.

Note: Failure to lower the salt content of your LB medium will result in non-selection due to inhibition of the drug.

We recommend that you sequence your construct to confirm that your gene is in the correct orientation for expression and contains an ATG start codon and a stop codon. We suggest using the CMV Forward and BGH Reverse primer sequences. Alternatively, the T7 Promoter Primer is available for purchase for sequencing in the sense orientation (see page 16 for ordering). Refer to the diagram on page 4 for the sequences and location of the priming sites. For your convenience, we offer a custom primer synthesis service. For more information, refer to www.lifetechnologies.com or contact Technical Support (page 17).

Preparing a Glycerol Stock

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage. It is also a good idea to keep a DNA stock of your plasmid at -20°C.

1. Streak the original colony out on an LB plate containing 50 µg/mL ampicillin or 50 µg/mL blasticidin in Low Salt LB. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL ampicillin or 50 µg/mL blasticidin in Low Salt LB.
3. Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Transfection and Analysis

Introduction

At this point, you should have a positive clone with your fragment inserted in the correct orientation for expression in pTracer™-CMV/Bsd. The next step is to isolate purified plasmid DNA and transfect your cell line (see below). Before transfecting your cells, read **Detection of Fluorescence**, next page, for information about using fluorescence to detect transfected cells. After mammalian transfection, the cells may be harvested and assayed for transient expression, or split and selected for stable transformants using the appropriate concentration of blasticidin (see pages 8–9). Selection with blasticidin is not necessary for transient expression. It is only necessary when selecting for stable transfectants.

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating DNA using the PureLink® HiPure Miniprep Kit or the PureLink® HiPure Midiprep Kit (see page 16 for ordering information), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (*e.g.* HeLa, COS-1), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). For high efficiency transfection in a broad range of mammalian cells, use Lipofectamine® 2000 Reagent available for purchase (page 16). For more information on Lipofectamine® 2000 and other transfection reagents, visit www.lifetechnologies.com/support or contact Technical Support (see page 17).For more information about electroporation, refer to *Current Protocols in Molecular Biology*, Unit 9.3 (Ausubel *et al.*, 1994).

Positive Control

pTracer™-CMV/Bsd/*lacZ* is provided as a control vector for mammalian cell transfection and expression (see page 15) and may be used to optimize transfection conditions for your cell line. Successful transfection of the positive control vector results in β-galactosidase expression that can be easily detected by performing an ONPG assay or by staining with X-gal (see below).

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. The β-Gal Assay Kit and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression is available for purchase (see page 16 for ordering).

Detection of Fluorescence

Introduction

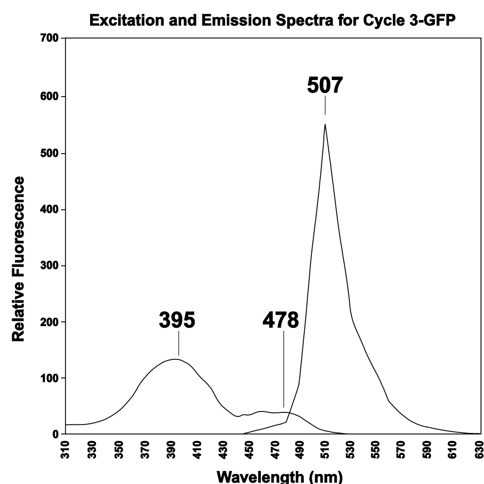
After transfecting your cells, you can monitor for fluorescence of cycle 3-GFP using fluorescence microscopy. Only transfected cells will emit a green fluorescent signal upon illumination, and the fluorescence can be used to estimate the transfection efficiency.

Detection of Fluorescence

To detect fluorescent cells, it is important to pick the best filter set to optimize detection. The primary excitation peak of cycle 3-GFP is at 395 nm. There is a secondary excitation peak at 478 nm. Excitation at these wavelengths yields a fluorescent emission peak with a maximum at 507 nm (see below).

Use of the best filter set will ensure that the optimal regions of the cycle 3-GFP spectra are excited and passed (emitted). For example, the FITC filter set that we use excites cycle 3-GFP with light from 460 to 490 nm, which covers the secondary excitation peak. The filter set passes light from 515 to 550, allowing detection of most of the GFP fluorescence. Standard FITC filters easily suit most purposes; however, it is important to keep in mind that fluorescence will be affected by the sample assayed and the filter you choose.

For general information about GFP fluorescence and detection, refer to *Current Protocols in Molecular Biology*, pages 9.7.22 to 9.7.28 (Ausubel *et al.*, 1994)



Detection of Transfected Cells

After transfection, allow the cells to recover for 24 to 48 hours before assaying for fluorescence. Most media fluoresce because of the presence of riboflavin (Zylka and Schnapp, 1996) and may interfere with detection of cycle 3-GFP fluorescence. Medium can be removed and replaced with PBS during viewing to alleviate this problem. Be sure to replace PBS with fresh medium if you wish to continue growing the cells.

You can use fluorescence to estimate the transfection efficiency and normalize any subsequent assay for your gene of interest. Estimate the total number of cells before assaying for fluorescence. Then check your plate for fluorescent cells.

Note: Cells can be incubated further in order to optimize expression of your gene of interest.

Creation of Stable Cell Lines

Introduction

Once you have established that your construct can be expressed in your cell line of choice, you may wish to generate a stable cell line expressing your protein. To generate a stable cell line, you may transfect the pTracer™-CMV/Bsd construct into your mammalian host cell line and select with blasticidin. Before transfection, we recommend that you first test the sensitivity of your mammalian host cell to blasticidin as natural resistance varies among cell lines.

Determination of Blasticidin Sensitivity

To generate a stable cell line expressing pTracer™-CMV/Bsd, you need to determine the minimum concentration of blasticidin required to kill your untransfected host cell line. Typically, concentrations between 2 and 10 µg/mL blasticidin are sufficient to kill the untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line. For more information about blasticidin and instructions for use, refer to the Appendix, page 12.

- Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare 6 plates of cells.
- The next day, substitute culture medium with medium containing varying concentrations of blasticidin (*e.g.* 0, 1, 3, 5, 7.5, and 10 µg/mL).
- Replenish the selective medium every 3–4 days. Cells sensitive to blasticidin will round up and detach from the plate. Dead cells will accumulate in the medium.
- Count the number of viable cells at regular intervals to determine the appropriate concentration of blasticidin that prevents growth within 1–2 weeks after addition of blasticidin.

Possible Sites for Linearization

To obtain stable transfectants, you may choose to linearize the pTracer™-CMV/Bsd plasmid before transfection. While linearizing your vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts the gene of interest or other elements required for mammalian expression. The table below lists unique sites that may be used to linearize pTracer™-CMV/Bsd prior to transfection. **Other restriction sites are possible.** Be sure that your insert does not contain the restriction site you wish to use to linearize your vector.

Enzyme	Restriction Site (bp)	Location	Supplier
Sap I	4117	Backbone	New England Biolabs
*Eam*1105 I	5126	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	5348	Ampicilllin gene	Many
Sca I	5606	Ampicillin gene	Many
Ssp I	5930	*bla* promoter	Many

*Angewandte Gentechnologie Systeme

Continued on next page

Creation of Stable Cell Lines, Continued

Selection of Stable Integrants

Once you have determined the appropriate blasticidin concentration to use (see previous page), you can generate a stable cell line with your pTracer™-CMV/Bsd construct. Use fluorescence to monitor development of foci and ensure a homogeneous population of cells.

1. Transfect the cell line of choice with your pTracer™-CMV/Bsd construct using the desired protocol. Include a sample of untransfected cells as a negative control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing blasticidin at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent. If the cells are too dense, the blasticidin will not kill the untransfected cells.
4. Replenish selective medium every 3–4 days until blasticidin-resistant colonies are detected. Typically, blasticidin selection takes 7–10 days.
5. Pick and expand at least 20 colonies.
6. Test clones for expression of your protein. Positive clones can be expanded further into large microtiter wells and then into flasks or plates as desired and re-tested to confirm expression.

Troubleshooting Guidelines

Introduction

The pTracer™-CMV/Bsd vector was tested in human embryonic kidney (HEK-293) cells. Although both fluorescence and expression of a heterologous gene are easily detected within 72 hours in this cell line, expression of the cycle 3-GFP-blasticidin fusion gene and your gene may vary from cell line to cell line. Basic guidelines to troubleshoot any unexpected results are provided below.

Low or No Fluorescence

- High background fluorescence due to riboflavin in the culture medium. Replace medium with 1X PBS to eliminate background fluorescence.
- A filter set was used that did not allow excitation at the optimal wavelength or permit detection of the emitted fluorescence. Check the filter set you are using.
- Transfection efficiency is too low to allow detection of transfected cells. Optimize your transfection conditions or try another method.
- Expression of cycle 3-GFP may be low depending on the cell line used. In HEK-293 cells, maximum fluorescence was observed 72 hours posttransfection.

No Transient Expression

Make sure there is an initiation codon in a proper Kozak consensus sequence (see page 4) for eukaryotic expression. Be sure that your insert also contains a stop codon.

No Stable Expression

- Confirm integration of your construct by either isolating genomic DNA and performing a Southern blot or PCR to see if your gene is present.
- Confirm transcription by isolating mRNA and performing a Northern or RT-PCR to test for the expression of your gene.

  Note: Be sure that the plasmid is not being maintained episomally.
- Isolate at least 50 independent foci as the location of integration may affect expression from both promoters.

Appendix

Recipe

Low Salt LB Medium with Blasticidin

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 pH to 7.0 with 1 N NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 psi and 121°C for 20 minutes.
3. Allow the medium to cool to at least 55°C before adding the blasticidin to 50 µg/mL final concentration.
4. Store plates at 4°C in the dark. Plates containing blasticidin are stable for up to 2 weeks.

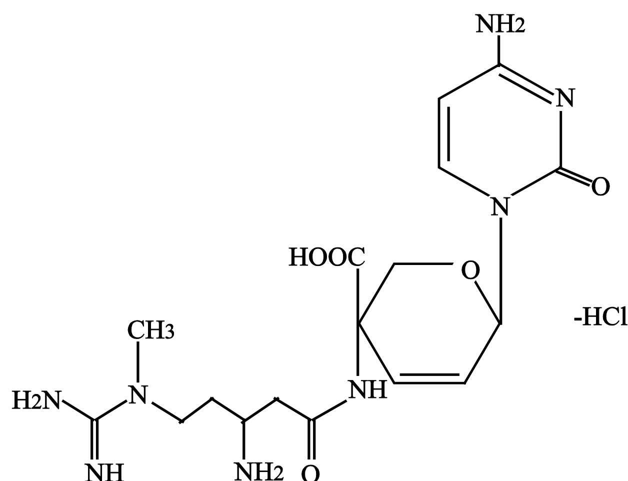
Blasticidin

Blasticidin

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two blasticidin S deaminase genes: *BSD* from *Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

Molecular Weight, Formula, and Structure

The formula for blasticidin S is $C_{17}H_{26}N_8O_5$-HCl, and the molecular weight is 458.9. The diagram below shows the structure of blasticidin.



Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (*e.g.* a laboratory coat) when handling blasticidin. Weigh out blasticidin and prepare solutions in a hood.

Preparing and Storing Stock Solutions

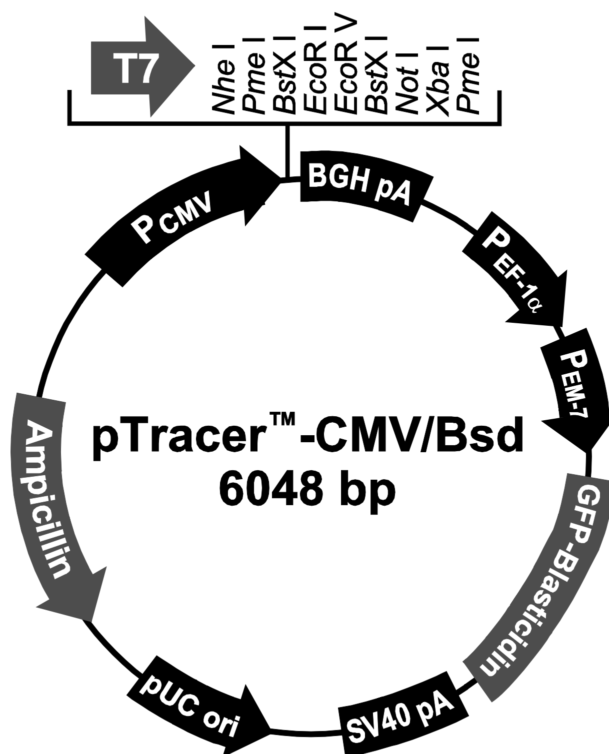
Blasticidin is available for purchase in 50 mg aliquots (see page 16 for ordering). Blasticidin is soluble in water. Sterile water is generally used to prepare stock solutions of 5–10 mg/mL.

- Dissolve blasticidin in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one time use (see next to last point below) and freeze at –20°C for long-term storage or store at 4°C for short-term storage.
- Aqueous stock solutions are stable for 1–2 weeks at 4°C and 6–8 weeks at -20°C.
- pH of the aqueous solution should be 7.0 to prevent inactivation of blasticidin.
- Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
- Upon thawing, use what you need and store the stock solution at 4°C for up to 2 weeks.
- Medium containing blasticidin may be stored at 4°C for up to 2 weeks.

Map of pTracer™-CMV/Bsd Vector

Map of pTracer™-CMV/Bsd

The figure below summarizes the features of the pTracer™-CMV/Bsd vector. The vector sequence for pTracer™-CMV/Bsd is available for downloading from www.lifetechnologies.com or from Technical Support (see page 17). See the next page for a description of the features of the vector.



Comments for pTracer™-CMV/Bsd
6048 nucleotides

Human cytomegalovirus (CMV) promoter: bases 235-822
CMV forward priming site: bases 769-789
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
BGH polyadenylation sequence: bases 1028-1223
BGH reverse priming site: bases 1022-1039
Human EF-1α promoter: bases 1307-2485
EM7 promoter: bases 2509-2575
Cycle 3-GFP-blasticidin fusion gene
  Cycle 3-GFP gene: bases 2576-3280
  Blasticidin resistance gene (no ATG): bases 3281-3679
SV40 early polyadenylation sequence: bases 3721-3851
pUC origin: bases 4234-4907 (complementary strand)
bla promoter: bases 5913-6011 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 5052-5912 (complementary strand)

Continued on next page

Features of pTracer™-CMV/Bsd Vector

Features of pTracer™-CMV/Bsd

The table below describes the relevant features of pTracer™-CMV/Bsd. All features have been functionally tested and the vector has been fully sequenced.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
CMV Forward priming site	Allows sequencing through the insert
T7 Promoter/priming site	Allows sequencing through the insert
Multiple cloning site	Allows insertion of your gene for expression
BGH Reverse priming site	Allows sequencing through the insert
Bovine growth hormone (BGH) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
Human EF-1α promoter	Allows high-level expression of the cycle 3-GFP-blasticidin resistance gene fusion in mammalian cells (Goldman *et al.*, 1996; Mizushima and Nagata, 1990)
EM7 promoter	Synthetic prokaryotic promoter for efficient expression of the cycle 3-GFP-blasticidin resistance gene fusion in *E. coli*
Cycle 3 GFP-Blasticidin resistance gene fusion	Allows visual detection of transfected mammalian cells using fluorescence microscopy Allows selection of stable transfectants in mammalian cells
SV40 polyadenylation signal	Allows polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin gene
Ampicillin resistance gene (β-lactamase)	Allows selection of vector in *E. coli*

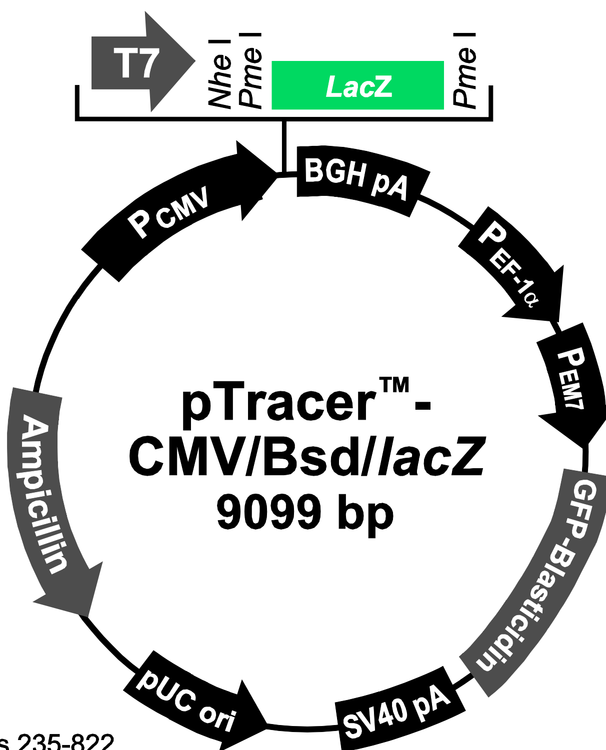
Map of pTracer™-CMV/Bsd/*lacZ* Vector

Description

pTracer™-CMV/Bsd/*lacZ* is a 9099 bp control vector containing the gene for β-galactosidase. A 3.1 kb fragment containing the *lacZ* gene was ligated into the pTracer™-CMV/Bsd vector.

Map of pTracer™-CMV/Bsd/*lacZ*

The figure below summarizes the features of the pTracer™-CMV/Bsd/*lacZ* vector. The vector sequence for pTracer™-CMV/Bsd/*lacZ* is available for downloading from www.lifetechnologies.com or from Technical Support (see page 17).



Comments for pTracer™-CMV/Bsd/*lacZ*
9099 nucleotides

Human cytomegalovirus (CMV) promoter: bases 235-822
CMV forward priming site: bases 769-789
T7 promoter/priming site: bases 863-882
LacZ ORF: bases 972-4046
BGH polyadenylation sequence: bases 4079-4274
BGH reverse priming site: bases 4073-4090
Human EF-1α promoter: bases 4358-5536
EM7 promoter: bases 5560-5626
Cycle 3-GFP-blasticidin fusion gene
  Cycle 3-GFP gene: bases 5627-6331
  Blasticidin resistance gene (no ATG): bases 6332-6730
SV40 early polyadenylation sequence: bases 6772-6902
pUC origin: bases 7285-7958 (complementary strand)
bla promoter: 8964-9062 (complementary strand)
Ampicillin (*bla*) resistance gene: 8103-8963 (complementary strand)

Accessory Products

Additional Products

The following additional products may be used with the pTracer™-CMV/Bsd © vectors. For more information, visit www.lifetechnologies.com or contact Technical Support (see page 17).

Item	Quantity	Cat. no.
One Shot® TOP10 Chemically Competent *E. coli*	20 reactions	C4040-03
One Shot® Electrocomp™ TOP10 (electrocompetent *E. coli*)	20 reactions	C4040-52
Electrocomp™ TOP10 (electrocompetent *E. coli*)	5 × 80 µL	C664-55
T7 Promoter Primer	2 µg	N560-02
Lipofectamine® 2000 Reagent	1.5 mL	11668-019
β–Gal Assay Kit	1 kit	K1455-01
β–Gal Staining Kit	1 kit	K1465-01
Blasticidin	50 mg, powder	R210-01

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, 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)

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

Certificate of Analysis

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The (cycle 3) mutant GFP gene was produced by Maxygen, Inc. using the DNA shuffling technology. Crameri, A., Whitehorn,E.A., and Stemmer, W.P.C. (1996) Improved Green Fluorescent Protein by Molecular Evolution Using DNA Shuffling. *Nature Biotechnology*, 14: 315-319.

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