pDisplay™ Vector

for expression of proteins on the surface of mammalian cells

Catalog no. V660-20

Version D

110810
28-0148

Invitrogen™
life technologies

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Important Information

Contents

20 µg of pDisplay™, lyophilized in TE, pH 8.0.

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Product Qualification

The pDisplay™ vector is qualified by restriction digest. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes used to digest the vector and the expected fragments.

Restriction Enzyme	Expected Fragments (bp)
*Bam*H I	170, 5155
Bgl II	5325
Pst I	5325

Introduction

Overview

Introduction

pDisplay™ is a 5.3 kb mammalian expression vector that allows display of proteins on the cell surface. Proteins expressed from pDisplay™ are fused at the N-terminus to the murine Ig κ-chain leader sequence, which directs the protein to the secretory pathway, and at the C-terminus to the platelet derived growth factor receptor (PDGFR) transmembrane domain, which anchors the protein to the plasma membrane, displaying it on the extracellular side. Recombinant proteins expressed from pDisplay™ contain the hemagglutinin A and *myc* epitopes for detection by western blot or immunofluorescence. To get started with cloning into pDisplay™, see page 4.

Tested Applications of pDisplay™

The pDisplay™ vector has been used to express *c-jun* and a single chain antibody against phOx hapten (Chesnut *et al.*, 1996). Both proteins were shown to be correctly expressed at the cell surface by incubation with anti-*myc* antibody followed by incubation with a magnetic bead-conjugated secondary antibody. Transfected cells were selected by using a magnet.

Note

For your convenience, Anti-*myc* Antibody is available from Invitrogen, Catalog no. R950-25. In addition, Anti-*myc* Antibody is available as a horseradish peroxidase (HRP) conjugate or an alkaline phosphatase (AP) conjugate for one-step westerns (Catalog nos. R951-25 and R952-25, respectively).

Methods

Cloning into pDisplay™

Introduction

The following section provides general guidelines for cloning your gene of interest into the pDisplay™ vector. For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, please see *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Maintenance of pDisplay™

In order to propagate and maintain pDisplay™, we recommend that you resuspend the lyophilized vector in 20 µl sterile water to make a 1 µg/µl stock solution. Store at -20°C.

Use this stock solution to transform an *E. coli* strain that is recombination deficient (*rec*A) and endonuclease A deficient (*end*A) such as TOP10F´ (Catalog no. C615-00) or equivalent.

For your convenience, TOP10F´ is available from Invitrogen as chemically competent or electrocompetent cells.

Item	Quantity	Catalog no.
Electrocomp™ TOP10F´	5 x 80 µl	C665-55
Ultracomp™ TOP10F´ (chemically competent cells)	5 x 300 µl	C665-03
One Shot® TOP10F´ (chemically competent cells)	21 x 50 µl	C3030-03

Cloning into the pDisplay™ Vector

pDisplay™ vector is a fusion vector requiring that you clone your gene of interest in frame with the initiation ATG of the N-terminal Ig κ-chain leader sequence and the C-terminal *myc* epitope/PDGFR-TM. It may be necessary to use PCR to create a fragment with the appropriate restriction sites to clone in frame at both ends. Carefully inspect your gene and the multiple cloning site before cloning your gene of interest. A diagram of the multiple cloning site is included on the next page.

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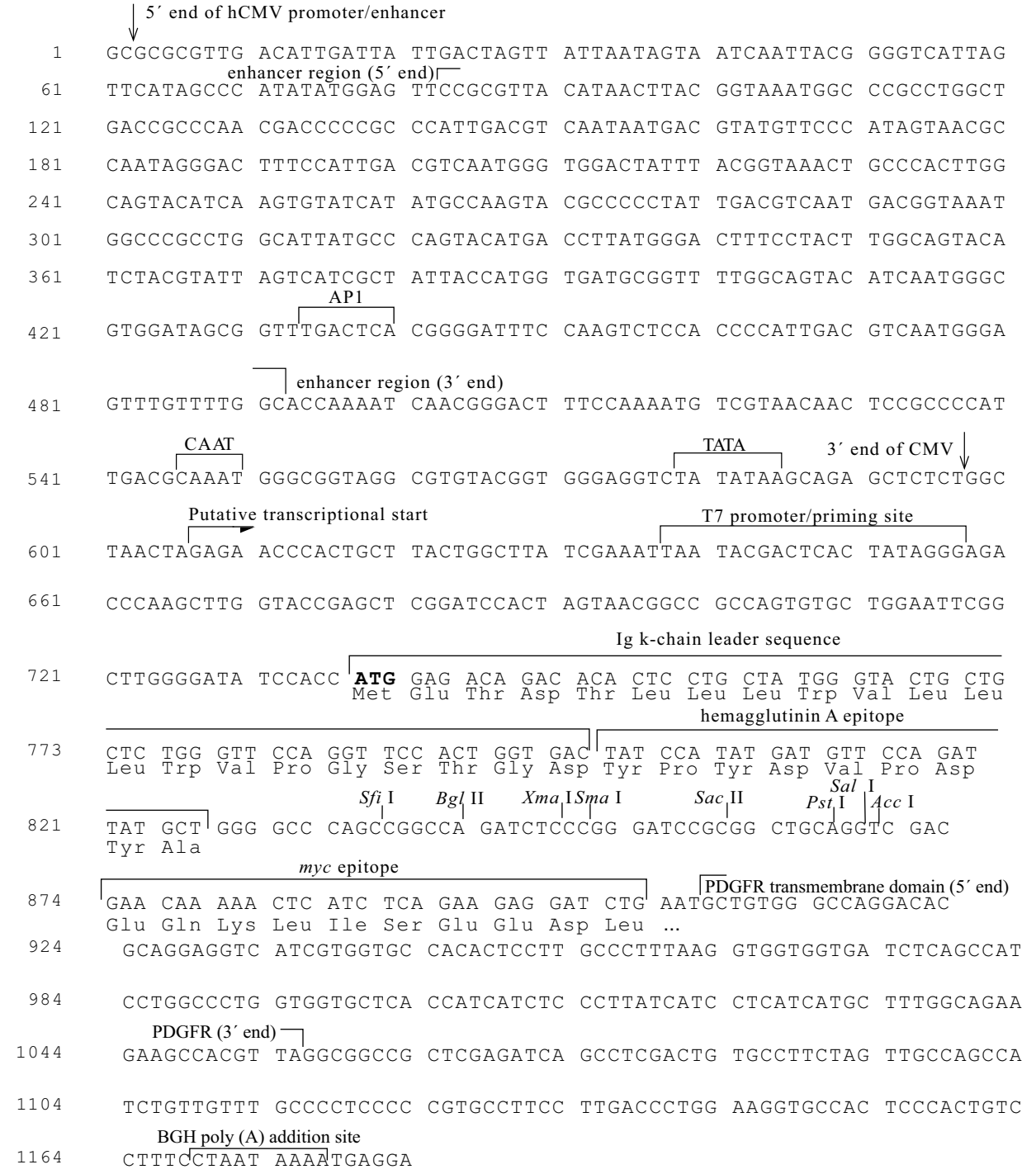
Cloning into pDisplay™, Continued

Multiple Cloning Site of pDisplay™

Below is the multiple cloning site for pDisplay™. Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.



General Guidelines for Transformation and Transfection

Introduction

The following guidelines and recommendations are provided for your convenience. If you need more details about the techniques discussed, please refer to the general molecular biology references in the **References** section.

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, TOP10F′, DH5α) and select on LB plates containing 50-100 µg/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the T7 Promoter (Catalog nos. N560-02) and a gene specific reverse primer to confirm that your gene is correctly fused to the Ig κ-chain leader sequence at the N-terminus and the *myc*/PDGFR-TM peptide at the C-terminus.

Plasmid Preparation

Once you have confirmed that your gene is correctly fused, prepare plasmid DNA for transfection. 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 lipids, decreasing transfection efficiency. We recommend using the S.N.A.P. Miniprep Kit (Catalog no. K1900-01) for isolation of 10 - 15 µg of plasmid DNA or CsCl gradient centrifugation for isolation of >15 µg of plasmid DNA.

Methods of Transfection

For established cell lines (e.g. HeLa, 293), please consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended 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). Invitrogen offers the Calcium Phosphate Transfection Kit (K2780-01) and a large variety of reagents for mammalian transfection. See our Web site at www.invitrogen.com for more details on transfection products.

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General Guidelines for Transformation and Transfection,
Continued

Geneticin® (G418) Activity

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen in both 1 g (Catalog no. 11811-023) and 5 g (Catalog no. 11811-031) quantities. Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Calculate concentration based on the amount of active drug (check the lot label).
- Use 100 to 800 µg/ml of Geneticin® in complete medium
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take up to 3 weeks of growth in selective medium. For more information, see Ausubel et *al*. (1994) unit 9.5.

Detection of Displayed Proteins

You may wish to confirm that your protein in being displayed on the cell membrane by using *in situ* immunofluorescent labeling of cells with antibodies to c-*myc* or to hemagglutinin A. Alternatively, you may use an indirect magnetic selection procedure with a magnetic bead-conjugated secondary antibody. For basic protocols, please see *Antibodies: A Laboratory Manual* (Harlow and Lane, 1988, p. 359-421)or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994, p. 14.6.1-14.6.2)

Troubleshooting

Introduction

The table below describes solutions to some possible problems.

Problem	Reason	Solution
No Expression	Method of transfection is not optimal.	Optimize transfection or change transfection method.
	Protein is not in frame with Ig k-chain leader sequence	Sequence your construct to check frame.
Protein is not displayed	Protein is not in frame with PDGFR-TM	Use antibody to hemagglutinin or *myc* to check for expression. Sequence your construct to check frame.

Appendix

pDisplay™ Vector

Features of pDisplay™

pDisplay™ (5325 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
ATG initiation codon	Permits initiation of translation of the pDisplay™ fusion protein
Murine Ig κ-chain leader sequence	Targets protein to secretory pathway (Coloma *et al.*, 1992)
Hemagglutinin A epitope tag (Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala)	Allows detection of the fusion protein by monoclonal antibody 12CA5 (Kolodziej and Young, 1991; Niman *et al.*, 1983)
Multiple cloning region with eight unique sites	Allows insertion of your gene and facilitates cloning
myc epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of pDisplay™ fusion protein with the Anti-*myc* Antibodies (Evan *et al.*, 1985)
Platelet-derived growth factor receptor transmembrane domain (PDGFR-TM)	Anchors the fusion protein to the plasma membrane for display (Gronwald *et al.*, 1988)
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
pUC origin	High-copy number replication and growth in *E. coli*
SV40 early promoter and origin	Permits expression of the kanamycin resistance gene for Geneticin® resistance in mammalian cells Allows episomal replication in cells containing SV40 large T antigen
Kanamycin resistance gene	Confers resistance to Geneticin® in mammalian cells
TK polyadenylation signal	Efficient transcription termination and polyadenylation of kanamycin resistance gene mRNA
Ampicillin resistance gene (β-lactamase)	Selection in *E. coli*
f1 origin	Allows rescue of single-stranded DNA

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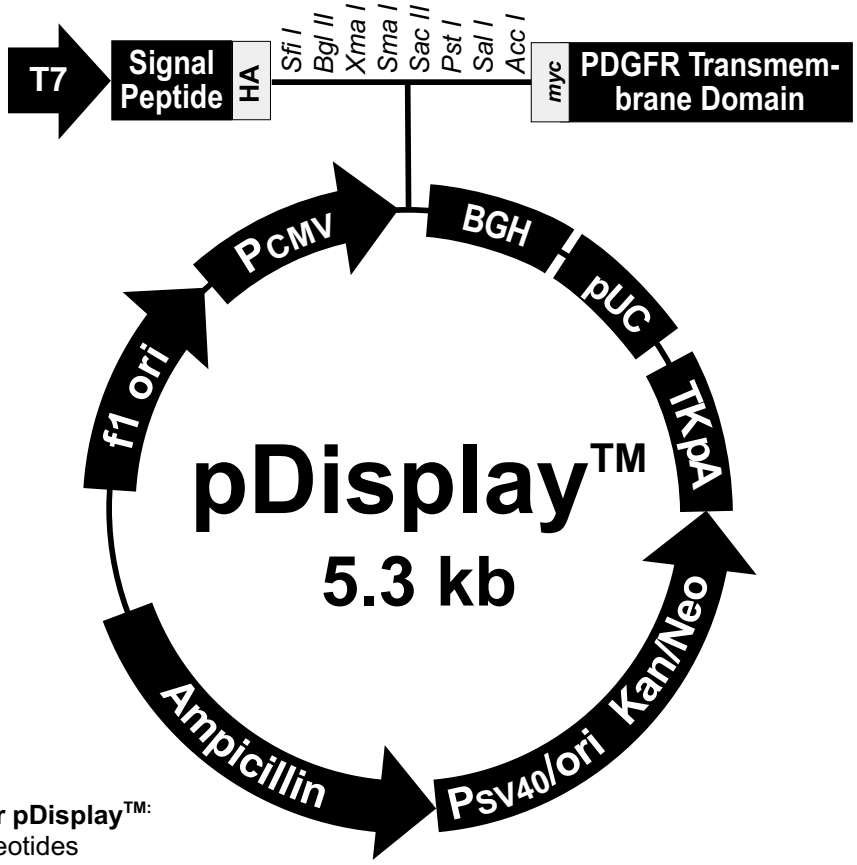
Map of pDisplay™

The figure below shows the features of pDisplay™. The complete sequence of pDisplay™ is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 9). Details of the multiple cloning site are shown on page 5.



Comments for pDisplay™:

5325 nucleotides

CMV promoter: bases 1-596
T7 promoter: bases 638-657
Murine Ig kappa-chain V-J2-C signal peptide: bases 737-799
Hemagglutinin A epitope: bases 800-826
Multiple Cloning Site: bases 827-873
myc epitope: bases 874-903
PDGFR transmembrane domain: bases 907-1056
Bovine growth hormone polyadenylation signal: bases 1069-1288
pUC origin: bases 1378-2051
Thymidine kinase polyadenylation site: bases 2458-2187
Neomycin/Kanamycin resistance gene: bases 3421-2366
SV40 origin and promoter: bases 3797-3456

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

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