

# pDisplay<sup>™</sup> Vector

for expression of proteins on the surface of mammalian cells

Catalog no. V660-20

**Version C**

032702

28-0148



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# Important Information

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

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

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**Limited Use Label License No. 49: BGH Polyadenylation Signal** The bovine growth hormone (BGH) polyadenylation sequence is licensed under U.S. Patent No. 5,122,458 for research purposes only. “Research purposes” means uses directed to the identification of useful recombinant proteins and the investigation of the recombinant expression of proteins, which uses shall in no event include any of the following: Any use in humans of a CLAIMED DNA or CLAIMED CELL; Any use in human of protein or other substance expressed or made at any stage of its production with the use of a CLAIMED DNA or a CLAIMED CELL; Any use in which a CLAIMED DNA or CLAIMED CELL would be sold or transferred to another party other than Invitrogen, its affiliate, or its sublicensee; Any use in connection with the expression or production of a product intended for sale or commercial use; or Any use for drug screening or drug development.

Inquiries for commercial use should be directed to: Bennett Cohen, Ph.D., Research Corporation Technologies, 101 North Wilmot Road, Suite 600, Tucson, AZ 85711-3335, Tel: 1-520-748-4400 Fax: 1-520-748-0025.

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**Limited Use Label License No. 28: CMV Promoter** The use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned and licensed by the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, Iowa 52242. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation. For further information, please contact the Associate Director of UIRF, at 319-335-4546

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**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)
<i>Bam</i> H I	170, 5155
<i>Bgl</i> II	5325
<i>Pst</i> I	5325

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# Introduction

## Overview

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### 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  $\kappa$ -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.

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### 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.

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#### 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).

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# Methods

## Cloning into pDisplay™

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### 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).

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### 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 (*recA*) and endonuclease A deficient (*endA*) 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

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### 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.

```

      ↓ 5' end of hCMV promoter/enhancer
1   GCGCGCGTTG ACATTGATTA TTGACTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG
      enhancer region (5' end)
61  TTCATAGCCC ATATATGGAG TTCCGCGTTA CATAACTTAC GGTAATGGC CCGCCTGGCT
121 GACCGCCCAA CGACCCCGC CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC
181 CAATAGGGAC TTTCCATTGA CGTCAATGGG TGGACTATTT ACGGTAAACT GCCCACTTGG
241 CAGTACATCA AGTGTATCAT ATGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT
301 GGCCCGCCTG GCATTATGCC CAGTACATGA CTTTATGGGA CTTTCTACT TGGCAGTACA
361 TCTACGTATT AGTCATCGCT ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC
      API
421 GTGGATAGCG GTTT[ GACTCA ] CGGGGATTTT CAAGTCTCCA CCCCATTGAC GTCAATGGGA
      enhancer region (3' end)
481 GTTTGT[TTT]TGCACCAAAT CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCAT
      CAAT
541 TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCTCTGGC
      TATA
      3' end of CMV ↓
601 TAACTAGAGA ACCCACTGCT TACTGGCTTA TCGAAAT[TA] TACGACTCAC TATAGGGAGA
      Putative transcriptional start
      T7 promoter/priming site
661 CCCAAGCTTG GTACCGAGCT CGGATCCACT AGTAACGGCC GCCAGTGTGC TGGAATTCGG
      Ig k-chain leader sequence
721 CTTGGGGATA TCCACC [ ATG GAG ACA GAC ACA CTC CTG CTA TGG GTA CTG CTG
      Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu
      hemagglutinin A epitope
773 CTC TGG GTT CCA GGT TCC ACT GGT GAC [ TAT CCA TAT GAT GTT CCA GAT
      Leu Trp Val Pro Gly Ser Thr Gly Asp Tyr Pro Tyr Asp Val Pro Asp
      Sfi I Bgl II Xma I Sma I Sac II Pst I Sal I Acc I
821 TAT GCT [ GGG GCC CAGCCGGCCA GATCTCCCGG GATCCGCGG CTGCAGGTC GAC
      Tyr Ala
      myc epitope
874 [ GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG ] AATGCTGTGG GCCAGGACAC
      Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu ...
      PDGFR transmembrane domain (5' end)
924 GCAGGAGGTC ATCGTGGTGC CACACTCCTT GCCCTTTAAG GTGGTGGTGA TCTCAGCCAT
984 CCTGGCCCTG GTGGTGCTCA CCATCATCTC CTTATCATC CTCATCATGC TTTGGCAGAA
      PDGFR (3' end)
1044 GAAGCCACGT TAGGCGGCCG CTCGAGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA
1104 TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC TCCCCTGTCT
      BGH poly (A) addition site
1164 CTTTCTAAT AAAATGAGGA
  
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# General Guidelines for Transformation and Transfection

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## 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.

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## *E. coli* Transformation



Transform your ligation mixtures into a competent *recA*, *endA* *E. coli* strain (e.g. TOP10, TOP10F', DH5 $\alpha$ ) and select on LB plates containing 50-100  $\mu$ g/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

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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  $\kappa$ -chain leader sequence at the N-terminus and the *myc*/PDGFR-TM peptide at the C-terminus.

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## 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  $\mu$ g of plasmid DNA or CsCl gradient centrifugation for isolation of >15  $\mu$ g of plasmid DNA.

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## 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](http://www.invitrogen.com) for more details on transfection products.

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# General Guidelines for Transformation and Transfection,

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## Geneticin<sup>®</sup> (G418) Activity

Geneticin<sup>®</sup> 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 Tn5, in mammalian cells results in detoxification of Geneticin<sup>®</sup> (Southern and Berg, 1982).

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## Geneticin<sup>®</sup> Selection Guidelines

Geneticin<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> in complete medium
- Test varying concentrations of Geneticin<sup>®</sup> on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin<sup>®</sup>

Cells will divide once or twice in the presence of lethal doses of Geneticin<sup>®</sup>, 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.

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## Detection of Displayed Proteins

You may wish to confirm that your protein is 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)

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# Troubleshooting

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## Introduction

The table below describes solutions to some possible problems.

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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 <i>myc</i> to check for expression. Sequence your construct to check frame.

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## Appendix

### pDisplay™ Vector

#### Features of pDisplay™

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

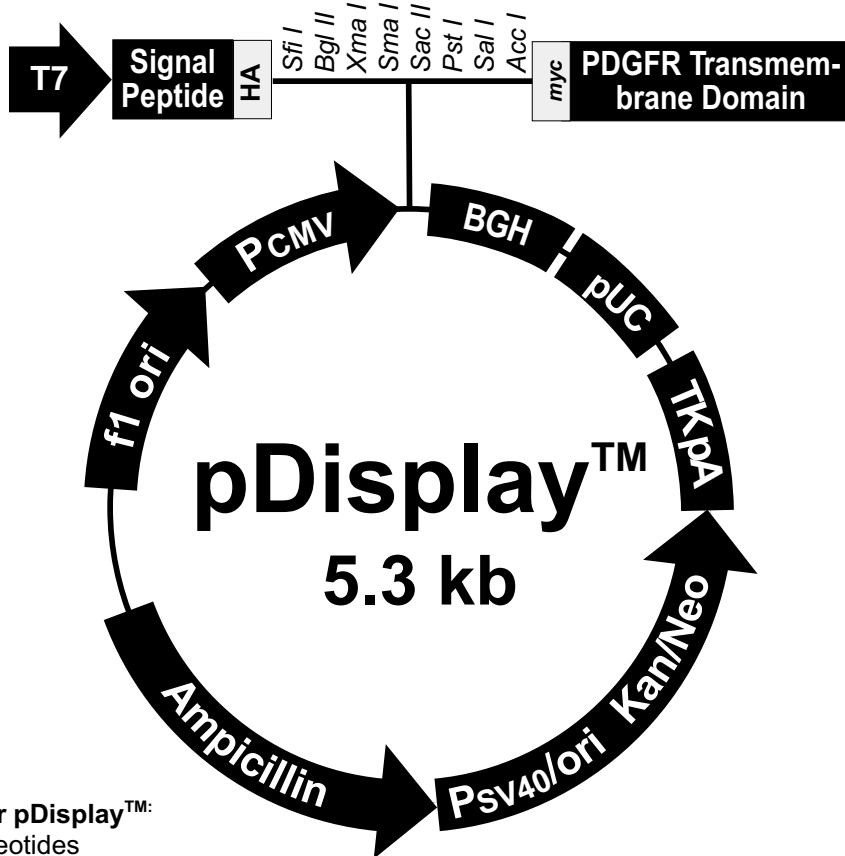
Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987)
T7 promoter/priming site	Allows for <i>in vitro</i> transcription 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 <i>et al.</i> , 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 <i>et al.</i> , 1983)
Multiple cloning region with eight unique sites	Allows insertion of your gene and facilitates cloning
<i>myc</i> epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of pDisplay™ fusion protein with the Anti- <i>myc</i> Antibodies (Evan <i>et al.</i> , 1985)
Platelet-derived growth factor receptor transmembrane domain (PDGFR-TM)	Anchors the fusion protein to the plasma membrane for display (Gronwald <i>et al.</i> , 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 <i>E. coli</i>
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 <i>E. coli</i>
f1 origin	Allows rescue of single-stranded DNA

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## pDisplay™ Vector, Continued

### 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](http://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

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## 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!

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## Contact Us

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## Technical Service, Continued

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## References

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- Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989). Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. *J. Biol. Chem.* *264*, 8222-8229.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). *Current Protocols in Molecular Biology* (New York: Greene Publishing Associates and Wiley-Interscience).
- Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985). A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. *Cell* *41*, 521-530.
- Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. *Mol. Cell. Biol.* *7*, 2745-2752.
- Chesnut, J. D., Baytan, A. R., Russell, M., M.-P.Chang, Bernard, A., Maxwell, I. H., and Hoeffler, J. P. (1996). Selective Isolation of Transiently Transfected Cells from a Mammalian Cell Population with Vectors Expressing a Membrane Anchored Single-Chain Antibody. *J. Imm. Methods* *193*, 17-27.
- Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. *Nuc. Acids Res.* *15*, 1311-1326.
- Coloma, M. J., Hastings, A., Wims, L. A., and Morrison, S. L. (1992). Novel Vectors for the Expression of Antibody Molecules Using Variable Regions Generated by Polymerase Chain Reaction. *J. Imm. Methods* *152*, 89-104.
- Evan, G. I., Lewis, G. K., Ramsay, G., and Bishop, V. M. (1985). Isolation of Monoclonal Antibodies Specific for *c-myc* Proto-oncogene Product. *Mol. Cell. Biol.* *5*, 3610-3616.
- Felgner, P. L., Holm, M., and Chan, H. (1989). Cationic Liposome Mediated Transfection. *Proc. West. Pharmacol. Soc.* *32*, 115-121.
- Felgner, P. L., and Ringold, G. M. (1989). Cationic Liposome-Mediated Transfection. *Nature* *337*, 387-388.
- Goodwin, E. C., and Rottman, F. M. (1992). The 3'-Flanking Sequence of the Bovine Growth Hormone Gene Contains Novel Elements Required for Efficient and Accurate Polyadenylation. *J. Biol. Chem.* *267*, 16330-16334.
- Gronwald, R. G., Grant, F. J., Haldeman, B. A., Hart, C. E., O'Hara, P. J., Hagen, F. S., Ross, R., Bowen-Pope, D. F., and Murray, M. J. (1988). Cloning and Expression of a cDNA Coding for the Human Platelet-Derived Growth Factor Receptor: Evidence for More Than One Receptor Class. *Proc. Natl. Acad. Sci. USA* *85*, 3435-3439.
- Harlow, E., and Lane, D. (1988). *Antibodies: A Laboratory Manual* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory).
- Kolodziej, P. A., and Young, R. A. (1991). Epitope Tagging and Protein Surveillance. *Meth. Enzymol.* *194*, 508-519.
- Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987). Negative and Positive Regulation by a Short Segment in the 5'-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. *Mol. Cell. Biol.* *7*, 4125-4129.
- Niman, H. L., Houghten, R. A., Walker, L. E., Reisfeld, R. A., Wilson, I. A., Hogle, J. M., and Lerner, R. A. (1983). Generation of Protein-reactive Antibodies by Short Peptides is an Event of High Frequency: Implications for the Structural Basis of Immune Recognition. *Proc. Natl. Acad. Sci. USA* *80*, 4949-4953.

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## References, Continued

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Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Shigekawa, K., and Dower, W. J. (1988). Electroporation of Eukaryotes and Prokaryotes: A General Approach to the Introduction of Macromolecules into Cells. *BioTechniques* 6, 742-751.

Southern, P. J., and Berg, P. (1982). Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter. *J. Molec. Appl. Gen.* 1, 327-339.

Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C., and Axel, R. (1977). Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells. *Cell* 11, 223-232.

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