# Sequence-Verified cDNA Clones

**Catalog nos. 97002.V, 97001.V, 97002.MmV**

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# Contents and Storage

## Shipping and Storage

The Sequence-Verified Human and Mouse cDNA Clones are supplied as glycerol stocks. The individual cDNA clones are shipped at room temperature and cDNA Library is shipped at -20°C.

Upon receipt, store the clones and library at -80°C.

## Types of Products

This manual is included with the following products:

| Product | Catalog no. |
|---|---|
| Sequence-Verified Human cDNA Clones | 97002.V |
| Sequence-Verified Human cDNA Library | 97001.V |
| Sequence-Verified Mouse cDNA Clones | 97002.MmV |

## Contents

**Clones**

Each tube of the Sequence-Verified cDNA Clone Collection contains the gene of interest in an appropriate vector (see page 3 for vector information) transformed into GeneHogs® *E. coli*. Each clone is supplied in 500 µl of LB media containing 8% glycerol and 100 µg/ml carbenicillin.

**Library**

Each well of the 96-well plate contains a distinct Sequence-Verified Human cDNA Clone in an appropriate vector (see page 3 for vector information) transformed into GeneHogs® *E. coli*. Each clone is supplied in 100 µl of LB media containing 8% glycerol and 100 µg/ml carbenicillin.

## Genotype of GeneHogs® *E. coli*

The genotype of GeneHogs® *E. coli* (also known as HS996) is:

$F^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *deo*R *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L ($Str^R$) *end*A1 *nup*G *fhu*A::IS2 (confers phage T1 resistance)

## Quality Control

The Sequence-Verified cDNA Clones are qualified by growing each clone in LB medium containing 100 µg/ml carbenicillin at 37°C overnight. The clone must exhibit growth under these conditions.

To verify the absence of phage contamination, 0.5-1 ml of *E. coli* are added to LB top agar and poured onto LB plates. A small aliquot of the clone culture is streaked on top agar. After overnight incubation, no plaques must be detected.

# Accessory Products

## Additional Products

Additional products that may be used with the Sequence-Verified Clones are available from Invitrogen. Ordering information is provided below.

| Item | Quantity | Catalog no. |
|---|---|---|
| BP Clonase™ Enzyme Mix | 20 reactions | 11789-013 |
| One Shot® TOP10 Chemically Competent *E. coli* | 10 reactions | C4040-10 |
| | 20 reactions | C4040-03 |
| One Shot® TOP10 Electrocompetent *E. coli* | 10 reactions | C4040-50 |
| | 20 reactions | C4040-52 |
| One Shot® MAX Efficiency® DH10B™ T1 Phage-Resistant Cells | 20 reactions | 12331-013 |
| imMedia™ Amp Agar | 20 pouches | Q601-20 |
| Ampicillin | 20 ml (10 mg/ml) | 11593-019 |
| Carbenicillin | 5 g | 10177-012 |
| pDONR™207 | 6 µg | 12213-013 |
| pDONR™221 | 6 µg | 12536-017 |
| cDNA Primer Pair | 25 µl (1 mM) | GF200.primer |
| S.N.A.P.™ MiniPrep Kit | 100 reactions | K1900-01 |
| S.N.A.P.™ MidiPrep Kit | 20 reactions | K1910-01 |
| T7 Primer | N560-02 | 327 pmoles |
| Sp6 Primer | N550-02 | 342 pmoles |

# Overview

## Introduction

The Sequence-Verified cDNA Clone Collection encompasses over 45,000 unique human clones and ~15,000 unique mouse clones. Clones in this collection are characterized to confirm identity and minimize redundancy. Based on the 3′ end sequence, one high-quality cDNA clone representing a unique gene is chosen from each NCBI UniGene cluster (see below). The clones are then sequence-verified at the 3′ end and re-transformed into a T1-phage resistant *E. coli* host, GeneHogs® (HS996) to eliminate identification errors and phage contamination.

The Sequence-Verified clones are cloned into a variety of vectors including the mammalian Gateway® expression vector, pCMV•SPORT6 (see page 3 for a complete list of vectors). The Gateway® Technology enables rapid transfer of genes into multiple gene expression systems (see page 3).

## NCBI UniGene Cluster

UniGene is an experimental system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location.

For more information on NCBI UniGene cluster, visit http://www.ncbi.nlm.nih.gov/UniGene/

## Clone Identification

The clone identification number (ID) is a code (a 5-8 digit number) assigned by the I.M.A.G.E. Consortium.

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### Specific Information on the Clone

Detailed information on each Sequence-Verified Clone including the clone ID, gene name, and sequence is available on CloneRanger™ from our Web site at www.invitrogen.com/clones. Clones are identified by the GenBank accession number or clone ID.

The data files for the Sequence-Verified Human and Mouse clones is available from:
ftp.resgen.com/pub/sv_libraries/, file name: RG_Hs_seq_ver_*date*.txt
ftp.resgen.com/pub/sv_libraries/, file name: RG_Mm_seq_ver_*date*.txt

These tab-delimited text files contain the following clone information:

- Vector
- Insert size
- Clone ID
- GenBank accession number
- Gene name (if known)
- Markers
- Antibiotic resistance

For more information on the clones, visit
http://www.ncbi.nlm.nih.gov/dbEST/index.html
Enter an EST search code (*e. g.*, 234589). This site includes the following information:

- Partial sequence
- Clone source
- GenBank accession number (3′ and/ or 5′)
- References
- Tissue and library information

**Note**

**The Sequence-Verified Clones are sequenced from the 3′ end only.**

The Sequence-Verified cDNA Clone Collection offered by Invitrogen was built by groups outside of Invitrogen. The quality of this collection is largely dependent on what was received from these groups. The Sequence-Verified cDNA Clones are not guaranteed to exactly match GenBank sequences. If you have determined by sequencing that the clone is incorrect, contact Technical Service (see page 7).

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# Overview, Continued

## Vector Information

The Sequence-Verified Human cDNA Clones are cloned into the following vectors:

- pCMV•SPORT2
- pCMV•SPORT4
- pCMV•SPORT6
- Lafmid BA
- pAmp1
- pAmp10
- pBluescriptSK-
- pT7T3D-Pac
- pSPORT1

The vector pCMV•SPORT6 contains *att*B1 and *att*B2 Gateway® recombination sites (see below).

For vector maps, visit ftp://ftp.resgen.com/pub/image_vectors/
For vector sequences, visit http://image.llnl.gov/image/html/vectors.shtml

## The Gateway® Technology

The Gateway® Technology is a universal cloning system that takes advantage of the site-specific recombination properties of bacteriophage lambda to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest using the Gateway® Technology, simply:

1. Recombine the Sequence-Verified cDNA Clone (in pCMV•SPORT6 vector only) containing your gene of interest with one of the pDONR™ vectors (see page vi) to generate an entry clone.
2. Generate an expression clone by performing a recombination reaction between the entry clone and a Gateway® destination vector of choice.
3. Introduce your expression clone into the appropriate host (*e.g.* bacterial, mammalian, yeast, insect) and express your recombinant protein.

For more information about the Gateway® Technology, refer to the Gateway® Technology manual available for downloading from our Web site at www.invitrogen.com or by contacting Technical Service (see page 7).

# Using Sequence-Verified Clones

## Introduction

General guidelines for using the Sequence-Verified cDNA Clones are described in this section.

## Preparing Glycerol Stocks

We recommend you prepare a set of master stocks prior to using the Sequence-Verified cDNA Clones. If you have ordered the Sequence-Verified Human Library, see next page for replica plating.

To prepare 5-10 glycerol master stocks for long-term storage:

1. Streak a small portion of the glycerol stock you received on a LB plate containing 100 µg/ml ampicillin.
2. Incubate the plate at 37°C overnight.
3. Isolate a single colony and inoculate into 5-10 ml of LB containing 100 µg/ml ampicillin.
4. Grow the culture to stationary phase ($OD_{600}$ = 1-2).
5. Mix 0.8 ml of culture with 0.2 ml of sterile glycerol and transfer to a cryovial.
6. Store at -80°C. Use one master stock to create working stocks for regular use. We also recommend that you isolate and store a stock of plasmid DNA at -20°C.

## Plasmid Preparation

To isolate plasmid DNA, you need to grow a culture of GeneHogs® *E. coli* containing your clone. Use LB medium containing 100 µg/ml ampicillin to select single colonies or to grow a culture. Use a culture volume appropriate for the amount of plasmid needed for your plasmid isolation method of choice.

We recommend isolating plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, see page vi), the S.N.A.P.™ MidiPrep Kit (10-200 µg, see page vi), or other resin-based methods.

**Note:** We recommend that you verify the clone by PCR, sequencing, or a method of choice (see next page).

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# Using Sequence-Verified Clones, Continued

## Replica-Plating

If you have ordered a Sequence-Verified Human cDNA Library, you need to produce a replica copy of the original microtiter plate. A replica copy can be generated by using replicating pins, a pin or liquid transfer robot, or a multichannel pipette.

Replica plating involves transferring a small aliquot of the clone from each well of the original microtiter plate into the corresponding well of another sterile microtiter plate containing fresh, sterile media with the appropriate antibiotic. The replica plate is incubated at an optimal temperature on an orbital shaker (to increase aeration) for at least 24 hours.

For more details on replica plating, refer to *Current Protocols in Molecular Biology* (Ausubel, *et al* 1994, New York: Greene Publishing Associates and Wiley-Interscience).

## Sequencing or PCR of Clones

**Sequence-Verified cDNA Clones are sequenced at 3′ end of the DNA only.** You may verify the clone by PCR, sequencing, or a method of choice.

A primer pair, GF200.primer (see page vi) was created for amplification or sequencing of most inserts of the I.M.A.G.E Consortium/LLNL collection of cDNA clones. The primers are designed for common vector elements. The primer sequence for GF200.primer pair is:

Forward: 5′-CTGCAAGGCGATTAAGTTGGGTAAC-3′

Reverse: 5′-GTGAGCGGATAACAATTTCACACAGGAAACAGC-3′

Use the GF200.primer pair for sequencing or PCR with all vectors in the Sequence-Verified cDNA Clone Collection, except the vectors: listed below:

- pCMV•SPORT2 (use T7 and Sp6 primers for sequencing and PCR)
- pCMV•SPORT4 (use T7 and Sp6 primers for sequencing and PCR)
- pCMV•SPORT6 (use T7 and Sp6 primers for sequencing and PCR)

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## Using Sequence-Verified Clones, Continued

### Expression of Cloned cDNA

The CMV promoter in pCMV•SPORT2, 4, and 6 vectors enable the **transient** expression of cloned cDNAs in mammalian cells.

**Note:** The pCMV•SPORT2, 4, and 6 vectors do **NOT** contain any eukaryotic origin of replication or antibiotic resistance marker for stable expression in mammalian cells. The Sequence-Verified cDNA Clones may not contain full-length sequences.

For expression of cDNA in multiple systems (prokaryotic, yeast, or insect), you need to transfer the cDNA insert to an appropriate expression vector using the Gateway® Technology (see below).

### Gateway® Cloning

The pCMV•SPORT6 vector contains the *att*B1 and *att*B2 recombination sites flanking the cloning site. For expression of cDNA in multiple systems, transfer the cDNA insert from pCMV•SPORT6 vector into other Gateway®-compatible vectors, by performing a BP recombination reaction with a pDONR™ vector (see page vi).

For more details on the Gateway® cloning technology and performing the BP recombination reaction, refer to the Gateway® Technology Manual available on our Web site at www.invitrogen.com or contact Technical Service (see next page).

# 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, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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# Technical Service, Continued

## MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

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# Purchaser Notification

**Information for European Customers**

These cells are genetically modified and contain a plasmid (see vectors listed on page 3). 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.

**Limited Use Label License No. 19: Gateway® Cloning Products**

The Gateway® Cloning Technology products and their use are the subject of one or more of U.S. Patent Nos. 5,888,732, 6,143,557, 6,171,861, 6,270,969, and 6,277,608 and/or other pending U.S. and foreign patent applications owned by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). No license is conveyed under the foregoing patents to use this product with any recombination sites other than those purchased from Invitrogen Corporation or its authorized distributor. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

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## Purchaser Notification, Continued

**Limited Use Label License No. 28: CMV Promoter**

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