USER GUIDE

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Standard I.M.A.G.E. cDNA Clones

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Contents and Storage

Shipping and Storage

The Standard I.M.A.G.E. cDNA Clones are supplied as glycerol stocks. The individual cDNA clones are shipped at room temperature. Upon receipt, store the clones at –80°C.

Contents

Each tube of the Standard I.M.A.G.E. cDNA Clone contains the gene of interest in an appropriate vector (see page 3 for vector information) transformed into *E. coli*. Each clone is supplied in 1 ml of LB media containing 8% glycerol and the appropriate antibiotic.

Note

The clones in this clone collection have the following antibiotic resistance markers:

- ampicillin (carbenicillin) (clones in tubes with red caps)
- chloramphenicol (clones in tubes with purple caps)
- kanamycin resistant (clones in tubes with green caps)
- Zeocin™ resistant (clones in tubes with yellow caps)

Confirm antibiotic resistance for the clone on CloneRanger™ at **www.lifetechnologies.com/clones**.

E. coli Host

The Standard I.M.A.G.E. cDNA Clones are transformed into a wide variety of *E. coli* host strains. For more information on the host strain visit **www.lifetechnologies.com/clones** and search for the clone using clone ID, accession number, or keywords in CloneRanger™.

Product Use

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

Accessory Products

Additional Products

Additional products that may be used with the Standard I.M.A.G.E. cDNA Clones are available from Life Technologies. Ordering information is provided below.

Item	Quantity	Catalog no.
BP Clonase™ II Enzyme Mix	20 reactions 100 reactions	11789-020 11789-100
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions 20 reactions	C4040-10 C4040-03
One Shot® TOP10 Electrocomp™ *E. coli*	10 reactions 20 reactions	C4040-50 C4040-52
One Shot® MAX Efficiency® DH10B™ T1 Phage-Resistant Cells	20 reactions	12331-013
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Carbenicillin, Disodium Salt	5 g	10177-012
Kanamycin Sulfate	5 g	11815-024
Zeocin™	1 g	R250-01
pDONR™221	6 µg	12536-017
cDNA Primer Pair	25 µl (1 mM)	GF200.primer
PureLink® HiPure Plasmid MiniPrep Kit	25 preps	K2100-02
PureLink® HiPure Plasmid MidiPrep Kit	25 preps	K2100-04
ChargeSwitch®-Pro Plasmid Miniprep Kit	10 preps 50 preps 250 preps	CS30010 CS30050 CS30250
imMedia™ Amp Agar	20 pouches	Q601-20
imMedia™ Kan Agar	20 pouches	Q611-20
imMedia™ Zeo Agar	20 pouches	Q621-20

Overview

Introduction

A vast collection of standard, non-sequence-verified human, mouse, rat, *Xenopus*, and Zebrafish clones from the I.M.A.G.E. collection are available. These clones have been made available through the efforts of the I.M.A.G.E. consortium. The cDNA clones have been sequenced by Washington University and the information has been deposited with NCBI.

The Standard I.M.A.G.E. cDNA clones are cloned into a variety of vectors including the mammalian Gateway® expression vector, pCMV•SPORT6, pCMV•SPORT6.ccdb, pCMV•SPORT6.1.ccdb, and pOTB7 (see page 3 for a complete list of vectors). The Gateway® Technology enables rapid transfer of genes into multiple gene expression systems (see page 4).

I.M.A.G.E./ Consortium

For more information on Integrated Molecular Analysis of Genomes and their Expression (I.M.A.G.E.)/ Consortium, visit: **http://image.hudsonalpha.org**

For library information visit, **http://image.hudsonalpha.org/html/iresources.shtml**

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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Overview, continued

Note

The Standard I.M.A.G.E. cDNA Clones offered by Life Technologies were built by groups outside of Life Technologies. The quality of this collection is largely dependent on what was received from these groups. The Standard I.M.A.G.E. cDNA Clones are not guaranteed to exactly match GenBank sequences. If you have determined by sequencing that the clone is incorrect, contact Technical Support (see page 9).

The Standard I.M.A.G.E. cDNA Clones were not provided in a phage resistant *E. coli* host. Therefore, Life Technologies experiences a certain percentage of clones that do not grow due to phage contamination. Life Technologies will attempt the growth of clone orders. If the attempt fails we will notify you of the failure and your order will be cancelled.

In addition to possible phage contamination, there is a certain rate of misplating of the clones received from outside sources. Life Technologies has instituted quality procedures to ensure that clones are picked from the identified well in a plate. Due to the quality of the information provided to Life Technologies, the clone you receive might not match the expected clone. If this occurs, contact Technical Support (see page 9).

Specific Information on the Clone

Detailed information on each Standard I.M.A.G.E. cDNA Clone is available on CloneRanger™ from our website at **www.lifetechnologies.com/clones**. Clones are identified by the GenBank accession number or clone ID. The information on CloneRanger™ includes:

- Clone ID
- Sequence
- Sequence description
- Vector name
- Antibiotic resistance
- Host strain
- Library name and description
- Library species and development stage (if any)

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Overview, continued

Vector Information

The Standard I.M.A.G.E. cDNA Clones are cloned into the following vectors:

Lafmid BA	pAD-GAL4	pAMP1
pAMP10	pBK-CMV	pBluescribe (modified)
pBluescript (modified)	pBluescriptR	pBluescript-FL
pBluescript SK+	pBluescript SK-	pBluescript II KS+
pBSRN3	pcDNAI	pcDNAII
pcDNA™3.1	pCMV•SPORT	pCMV•SPORT2
pCMV•SPORT4	pCMV•SPORT6	pCMV•SPORT6.ccdb
pCMV•SPORT6.1.ccdb	pCR®2.1-TOPO®	pCS105
pCS107	pCS2+	pCS2G
pDNR-LIB	pDONR201	pME18S-FL3
pOTB7	pOTB7a	pSPORT1
pSPORT2	pT7T3D-Pac	pYX
pZErO®-2	pZL1	

The vectors pCMV•SPORT6, pCMV•SPORT6.ccdb, pCMV•SPORT6.1.ccdb , and pOTB7 contain *att*B1 and *att*B2 Gateway® recombination sites (see page 4). For vector sequences, visit **http://image.hudsonalpha.org/html/iresources.shtml**

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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 Standard I.M.A.G.E. cDNA Clone (in pCMV•SPORT6, pCMV•SPORT6.ccdb, pCMV•SPORT6.1.ccdb, and pOTB7 vectors 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 **www.lifetechnologies.com** or by contacting Technical Support (see page 9).

Using Standard I.M.A.G.E. cDNA Clones

Introduction

General guidelines for using the Standard I.M.A.G.E. cDNA Clones are described in this section.

Preparing Glycerol Stocks

We recommend you prepare a set of master stocks prior to using the Standard I.M.A.G.E. cDNA Clones.
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 the appropriate antibiotic.
2. Incubate the plate at 37°C overnight.
3. Isolate a single colony and inoculate into 5–10 ml of LB containing the appropriate antibiotic.
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.

Important

Clones that rely on ampicillin-based selective pressure have issues with long term storage due to the buildup of β-lactamase secreted into the growth media, which allows for background contaminations to take hold in the sample and eventually "over run" the desired clone. In order to avoid this situation from occurring, we recommend taking some (if not all) of the precautions described in **Applying Selective Pressure** on page 6.

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Using Standard I.M.A.G.E. cDNA Clones,
continued

Applying Selective Pressure

We recommend taking some (if not all) of the following precautions to prevent your clone from being "overrun" by background contaminants:

- **Use carbenicillin instead of ampicillin.** Carbenicillin is more stable than ampicillin, and allows for a longer period of selective pressure, thus preserving your clones longer.
- **Increase the antibiotic concentration.** More antibiotic means that your clones will not be overwhelmed by β-lactamase buildup.
- **Decrease growth times.** Minimize the amount of time that a clone can grow in the media so as to avoid β-lactamase buildup.
- **Periodically refresh plate media.** If you suspect that tubes/plates may be beginning to fail, spin them down, remove the old media, and replenish the wells with fresh LB media plus glycerol and antibiotic.
- **Streak clones on selective (preferably carbenicillin) LB agar plates.** After about 12 hours, isolate colonies for downstream usage. This will isolate your desired clones from potential background contaminants.

Plasmid Preparation

To isolate plasmid DNA, you need to grow a culture of the *E. coli* containing your clone. Use LB medium containing the appropriate antibiotic 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 a resin based method, such as the ChargeSwitch®-Pro Plasmid Miniprep Kit or PureLink® HiPure Plasmid Miniprep Kit or Midiprep Kits. See page vi for ordering information.

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Using Standard I.M.A.G.E. cDNA Clones,
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Sequencing or PCR of Clones

You may verify the Standard I.M.A.G.E. cDNA 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 GF.200 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 Standard I.M.A.G.E. cDNA Clones, except the vectors listed below:

- pCMV•SPORT2, pCMV•SPORT4, and pCMV•SPORT6: use primers to T7 and Sp6 sites for sequencing or PCR
- pME18S-FL3: use the following primers for sequencing or PCR
  Forward: 5′-CTTCT GCTCT AAAAG CTGCG-3′
  Reverse: 3′-CGACCTGCAGCTCGAGCACA-3′
- pDNR-LIB L3 and pOTB7: use the following primers for sequencing or PCR
  Forward: 5′-TGTAA AACGA CGGCC AGT-3′
  Reverse: 3′-CAGGA AACAG CTATG AC-3′

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Using Standard I.M.A.G.E. cDNA Clones, Continued

Expression of Cloned cDNA

The CMV promoter in pcDNA3.1, pcDNAI , pCMV•SPORT2, 4, and 6, pCMV•SPORT6.ccdb, and pCMV•SPORT6.1.ccdb vectors enable the **transient** expression of cloned cDNAs in mammalian cells.

Note: The pCMV•SPORT2, 4, and 6, pCMV•SPORT6.ccdb, pcDNAI, and pCMV•SPORT6.1.ccdb vectors do **NOT** contain any eukaryotic origin of replication or antibiotic resistance marker for stable expression in mammalian cells.

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 Gateway® Cloning).

Gateway® Cloning

The pCMV•SPORT6, pCMV•SPORT6.ccdb, pCMV•SPORT6.1.ccdb, and pOTB7 vectors contain the *att*B1 and *att*B2 recombination sites flanking the cloning site. For expression of cDNA in multiple systems, transfer the cDNA insert from these 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 website at **www.lifetechnologies.com** or contact Technical Support (see page 9).

Technical Support

Obtaining 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

Safety Data Sheets (SDS)

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

Certificate 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.

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Technical Support, Continued

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