USER GUIDE

invitrogen™
by life technologies™

Ultimate™ ORF Clones

Catalog Numbers HORF01, MORF01, HORF96

Documentation Part Number 25-0625
Publication Number MAN0000359
Revision 3.00

For Research Use Only. Not for diagnostic procedures.

life technologies™

Contents

Contents and Storage

Shipping and Storage

The Ultimate™ ORF (Open Reading Frame) Clones are shipped on dry ice. Upon receipt, store at –80°C. Products are guaranteed for six months from date of shipment when stored properly.

Types of Products

This manual is supplied with the following products:

Product	Catalog no.
Ultimate™ Human ORF Clones	HORF01
Ultimate™ Mouse ORF Clones	MORF01
Ultimate ORF Clones (96 well plate)	HORF96

Contents

Each tube of the Ultimate™ ORF clone contains the gene of interest in pENTR™221 vector transformed into a T1-Phage Resistant *E. coli*. Each clone is supplied in 1 ml LB medium containing 8% glycerol and 50 µg/ml kanamycin.
A 96 well plate format of clones is available and supplied with 100 µl of LB medium containing 8% glycerol and 50 µg/ml kanamycin. See our website www.lifetechnologies.com or contact Technical Support (page 16) for more information.

Genotype of *E. coli*

The genotype of the T1-Phage Resistant *E. coli* is:

F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G λ^- *ton*A (confers phage T1 and T5 resistance)

Introduction

Overview

Introduction

The Ultimate™ ORF (Open Reading Frame) Clones are human and mouse clones designed to provide the maximum flexibility in all of your research applications. The Ultimate™ ORF Clones are provided in a Gateway® entry vector, pENTR™221, allowing you to rapidly and efficiently transfer the ORF of interest to any expression (Gateway® destination) vector and perform gene analysis in your system of choice (see page 7 for information on Gateway® Technology).

Specific Information on the Clone

Detailed information on each Ultimate™ ORF Clone including the clone ID, sequence, and sequence description is available on our web site at http://orf.invitrogen.com/cgi-bin/ORF_Browser.

Important

The amino acid sequence (coding sequence) of each Ultimate™ ORF Clone is guaranteed to match the corresponding GenBank amino acid sequence. The Ultimate™ ORF Clones are not guaranteed to exactly match the GenBank base pair sequences.

Features of Ultimate™ ORF Clones

The Ultimate™ ORF Clones are:

- Full insert sequenced

  Each is sequenced for full-insert coverage and contains a Gap4 sequence quality of 40 or greater at each consensus base (representing < 1 error per 10,000 bases).
- Supplied as a Gateway®-compatible entry clone

  Enables rapid and efficient transfer of the ORF into any expression (Gateway® destination) vector for performing gene analysis in bacterial, mammalian, yeast, or insect system of choice (see **Note** on the next page).

Continued on next page

Note

The pENTR™221 vector contains a Kozak consensus sequence upstream of the ATG to provide optimal expression of the ORF after recombination with any eukaryotic Gateway® destination vector of choice (e.g., mammalian, insect, yeast). The pENTR™221 vector **does not** contain a Shine-Dalgarno sequence (RBS) for optimal expression in a prokaryotic system (Shine & Dalgarno, 1975). To express the ORF in a prokaryotic system, you need to recombine the entry clone with an appropriate destination vector containing an N-terminal fusion tag. The N-terminal fusion tag in some destination vectors for prokaryotic expression includes a Shine-Dalgarno sequence (RBS) optimally spaced from an ATG initiation codon for proper translation initiation in *E. coli*.

You may use the following destination vectors available from Life Technologies that contain an N-terminal fusion tag for prokaryotic expression.

Vector	Fusion Tag	Catalog no.
pDEST™15	Glutathione-S-transferase	11802-014
pDEST™17	6×His	11803-012
pBAD-DEST49	His-Patch Thioredoxin	12283-016

Note the expression of your protein with the N-terminal tag will increase the size of your recombinant protein. Be sure to account for any additional amino acids between the fusion tag and the start of your protein.

For details on the recombination site of pENTR™221, see page 9.

Continued on next page

Features of pENTR™221

The Ultimate™ ORF Clones are provided in a Gateway® entry vector, pENTR™221. The pENTR™221 vector contains the following elements:

- *rrn*B transcription termination sequences to prevent basal expression of the gene of interest in *E. coli*
- *att*L1 and *att*L2 sites for site-specific recombination of the entry clone with a Gateway® destination vector (for more information, refer to the Gateway® Technology manual)
- Kozak consensus sequence for efficient translation initiation in eukaryotic systems (Kozak, 1987; Kozak, 1990; Kozak, 1991)
- Kanamycin resistance gene for selection in *E. coli*
- pUC origin for high-copy replication and maintenance of the plasmid in *E. coli*

For a map of pENTR™221, see page 13.

The Gateway® Technology

The Gateway® Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) 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. Generate an expression clone by performing a LR recombination reaction between the Ultimate™ ORF entry clone and a Gateway® destination vector of choice.
2. 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 with Clonase™ II manual. This manual is available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (page 16).

Methods

Using Ultimate™ ORF Clones

Introduction

General guidelines for using the Ultimate™ ORF Clones are described in this section.

To perform the LR recombination reaction with a Gateway® destination vector, see page 10.

Preparing Glycerol Stocks

We recommend that you prepare a set of master stocks prior to using your Ultimate™ ORF clone.

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

1. Streak a small portion of the glycerol stock you received on an LB plate containing 50 µg/ml kanamycin.
2. Incubate the plate at 37°C overnight.
3. Isolate a single colony and inoculate into 5-10 ml of LB containing 50 µg/ml kanamycin.
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.

Plasmid Preparation

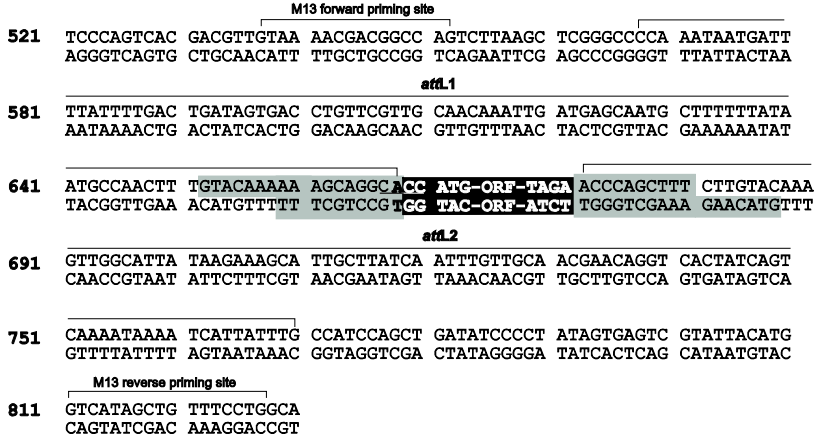
To isolate plasmid DNA, you need to grow a culture of T1-Phage Resistant *E. coli* containing your Ultimate™ ORF clone. Use LB medium containing 50 µg/ml kanamycin to select single colonies and to grow a culture. Use a culture volume appropriate for the amount of plasmid needed for your plasmid isolation method of choice. You will need ~150 ng of DNA for the LR recombination reaction (page 11).

We recommend isolating plasmid DNA using a resin based method such as the PureLink™ HiPure Plasmid Miniprep Kit or the PureLink™ HiPure Plasmid Midiprep Kit. See page 15 for ordering information.

Recombination Site of pENTR™221

Below is the recombination site for pENTR™221. Features are indicated as follows:

- The *att*L sites are properly spaced to indicate the correct reading frame for fusion of your gene of interest to an N-terminal tag following recombination with a destination vector.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the destination vector following recombination.
- Underlined sequence corresponds to the Kozak consensus sequence (CACC).



Note

The last nucleotide **T** in the *att*L1 sequence is changed to an **A** in Ultimate™ ORF Clones to generate the Kozak consensus sequence. This change in the nucleotide sequence does not affect the efficiency of the subsequent LR recombination reaction.

Performing the LR Reaction

Introduction

Each Ultimate™ ORF Clone is supplied as a Gateway®-compatible entry clone. To perform the LR recombination reaction, you will transfer the gene of interest into an *att*R-containing destination vector to create an *att*B-containing expression clone. Genes in the Ultimate™ ORF entry clone are transferred to the destination vector backbone by mixing the DNAs with the Gateway® LR Clonase™ II enzyme mix. The resulting recombination reaction is then transformed into *E. coli* and the expression clone is selected. Recombination between the *att*R (destination vector) and *att*L (Ultimate™ ORF entry clone) sites replace the *ccd*B gene in the destination vector with the gene of interest and results in the formation of *att*B sites in the expression clone.

A brief protocol to perform the LR recombination reaction is provided below. For more details on the LR recombination reaction, refer to the Gateway® Technology with Clonase™ II manual. This manual is available for downloading from our website at www.lifetechnologies.com or by contacting Technical Support (page 15).

Important

For most applications, we recommend performing the LR recombination reaction using a:

- Supercoiled *att*L-containing entry clone
- Supercoiled *att*R-containing destination vector

Note: If your destination vector or entry clone is large (>10 kb), you may linearize either vector to increase recombinational efficiency. You may also relax the destination vector using topoisomerase I to increase efficiency. For details, refer to the Gateway® Technology with Clonase™ II manual.

Destination Vectors

A large selection of Gateway® destination vectors is available from Life Technologies to facilitate expression of your gene of interest in virtually any protein expression system. For more information about the vectors available, refer to our website (www.lifetechnologies.com) or contact Technical Support (page16).

If you wish to express your ORF in a prokaryotic system, see **Note** on page 6.

Continued on next page

E. coli Host

You may use any *rec*A, *end*A *E. coli* strain including TOP10, DH5α™, DH10B™or equivalent for transformation. **Do not** transform the LR reaction mixture into *E. coli* strains that contain the F′ episome (*e.g.* TOP10F′). These strains contain the *ccd*A gene and will prevent negative selection with the *ccd*B gene.

Materials Needed

You will need the following materials:

- Purified plasmid DNA of your Ultimate™ ORF entry clone (50–150 ng/µl in TE, pH 8.0)
- Destination vector of choice (150 ng/µl in TE, pH 8.0)
- LR Clonase™ II enzyme mix (see below); keep at –20°C until immediately before use)
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8, 1 mM EDTA)
- 2 µg/µl proteinase K solution (supplied with the LR Clonase™ II enzyme mix; thaw and keep on ice until use)
- Appropriate competent *E. coli* host and growth media
- S.O.C. Medium
- LB agar plates with the appropriate antibiotic to select for expression clones

LR Clonase™ II Enzyme Mix

Use LR Clonase™ II enzyme mix (Catalog no. 11791-020) to catalyze the LR recombination reaction. The LR Clonase™ II enzyme mix combines the proprietary enzyme formulation and 5X LR Clonase™ Reaction Buffer previously supplied as separate components in LR Clonase™ enzyme mix into an optimized single-tube format for easier set-up of the LR recombination reaction. Use the protocol below to perform the LR recombination reaction using LR Clonase™ II enzyme mix.

Note: You may perform the LR recombination reaction using LR Clonase™ enzyme mix, if desired. To use LR Clonase™ enzyme mix, follow the protocol provided with the product. **Do not** use the protocol for LR Clonase™ II enzyme mix as reaction conditions differ.

Continued on next page

Performing the LR Reaction, Continued

LR Recombination Reaction

1. Add the following components to 1.5 ml microcentrifuge tubes at room temperature and mix.

Component	Sample	Negative Ctrl
Entry clone (50–150 ng/rxn)	1–7 µl	1–7 µl
Destination Vector (150 ng/µl)	1 µl	1 µl
TE Buffer, pH 8.0	to 8 µl	to 10 µl

2. Remove the LR Clonase™ II enzyme mix from –20°C and thaw on ice for 2 minutes.
3. Vortex the LR Clonase™ II enzyme mix briefly twice (2 seconds each time).
4. To each sample (except the negative control), add 2 µl of LR Clonase™ II enzyme mix. Mix by vortexing briefly twice (2 seconds each time). Return the LR Clonase™ II enzyme mix to –20°C immediately after use.
5. Incubate reactions at 25°C for 1 hour.

   Note: One hour incubation generally yields a sufficient number of colonies for analysis; however, the length of the LR reaction can be extended up to 18 hours. For large plasmids (>10 kb), overnight incubation yields more colonies and is recommended.
6. Add 1 µl of the Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Transform 1–2 µl of the reaction into a suitable *E. coli* host and select for expression clones (refer to the Gateway® Technology with Clonase™ II manual on our website).

   Note: You may store the LR reaction at –20°C for up to 1 week before transformation, if desired.

Expected Results

If you use *E. coli* cells with a transformation efficiency of $\geq 1 \times 10^8$ cfu/µg, the LR reaction should yield > 5000 colonies if the entire reaction is transformed and plated.

Expressing your Recombinant Protein

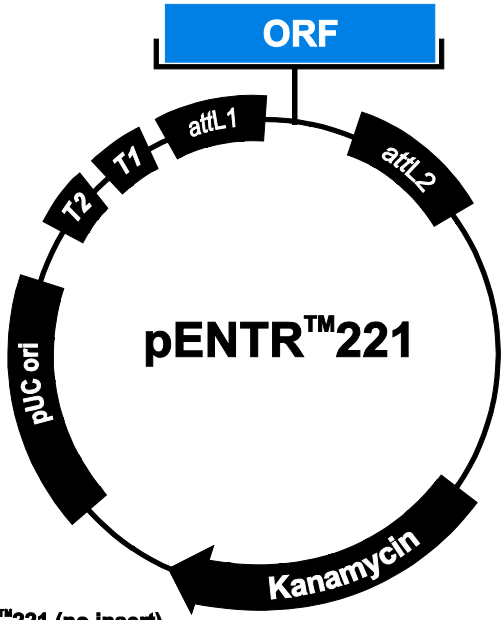
Once you have obtained an expression clone, you are ready to express your recombinant protein. Refer to the manual for the destination vector you are using for guidelines and instructions to express your recombinant protein in the appropriate system. Manuals for all Gateway® destination vectors are available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (page 16).

Appendix

Map of pENTR™221

Map of pENTR™221

The map below shows the elements of pENTR™221. **The complete sequence of pENTR™221 is available for downloading from our website (www.lifetechnologies.com) or by contacting Technical Support (page16).**



Comments for pENTR™221 (no insert)
2546 nucleotides

*rrn*B T2 transcription termination sequence: bases 268-295
*rrn*B T1 transcription termination sequence: bases 427-470
M13 forward (-20) priming site: bases 537-552
*attL*1: bases 569-667 (complementary strand)
ORF insertion site: bases 668-669
*attL*2: bases 671-770
M13 reverse priming site: bases 811-827
Kanamycin resistance gene: bases 940-1749
pUC origin: bases 1870-2543

Features of pENTR™221

Features of pENTR™221

pENTR™221 contains the following elements. All features have been functionally tested.

Feature	Benefit
*rrn*B T1 and T2 transcription termination sequences	Protects the cloned gene from expression by vector-encoded promoters, thereby reducing possible toxicity (Orosz *et al.*, 1991)
M13 forward (-20) priming site	Allows sequencing in the sense orientation
*att*L1 and *att*L2 sites	Allows site-specific recombination of the entry clone with a Gateway® destination vector (Landy, 1989)
M13 reverse priming site	Allows sequencing in the anti-sense orientation
Kanamycin resistance gene	Allows selection of the plasmid in *E. coli*
pUC origin	Allows high-copy number replication and growth in *E. coli*

Accessory Products

Additional Products

Additional products that may be used with the Ultimate™ ORF Clones are available from Life Technologies. Ordering information is provided below.

Item	Quantity	Catalog no.
Gateway® LR Clonase™ II Enzyme Mix	20 reactions 100 reactions	11791-020 11791-100
One Shot® TOP10 Chemically Competent *E. coli*	20 reactions	C4040-03
One Shot® TOP10 Electrocompetent *E. coli*	20 reactions	C4040-52
One Shot® MAX Efficiency® DH10B™-T1 Phage-Resistant *E. coli*	20 reactions	12331-013
Kanamycin Sulfate	5 g 25 g	11815-024 11815-032
Kanamycin Sulfate (100X), liquid	100 ml	15160-054
Gateway® Vector Conversion System	20 reactions	11828-019
Lipofectamine™ 2000 Reagent	0.75 ml 1.5 ml	11668-027 11668-019
PureLink™ HiPure Plasmid MiniPrep Kit	25 preps	K2100-02
PureLink™ HiPure Plasmid MidiPrep Kit	25 preps	K2100-04

Gateway® Destination Vectors

A large selection of Gateway® destination vectors is available from Life Technologies to facilitate expression of your gene of interest in virtually any protein expression system. For more information about the vectors available, refer to our website (www.lifetechnologies.com) or contact Technical Support (page 16).

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

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Purchaser Notification

Information for European Customers

These cells are genetically modified and contain the pUC-derived plasmid, pENTR™221. As a condition of sale, use of 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.

Gateway® Clone Distribution Policy Limited Use Label License No. 220: Ultimate™ ORF

For additional information about Life Technologies's policy for the use and distribution of Gateway® clones, see the section entitled **Gateway® Clone Distribution Policy**, page 19.

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). The purchase of this product does not convey a license under any method claims in the foregoing patents or patent applications, or to use this product with any recombination sites other than those purchased from Life Technologies Corporation or its authorized distributor. The right to use methods claimed in the foregoing patents or patent applications with this product for research purposes only can only be acquired by the use of ClonaseTM purchased from Life Technologies Corporation or its authorized distributors. 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 by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the employment 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. Notwithstanding the preceding, any buyer who is employed in an academic or government institution may transfer materials made with this product to a third party who has a license from Life Technologies under the patents identified above to distribute such materials. Transfer of such materials and/or information to collaborators does not convey rights to practice any methods claimed in the foregoing patents or patent applications.

Continued on next page

Purchaser Notification, Continued

Limited Use Label License No. 220: Ultimate™ ORF, continued

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. Life Technologies 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 none of (i) this product, (ii) any of its components, or (iii) a method claim of the foregoing patents, was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to use this product for purposes other than those permitted above, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200.

Disclaimer

Life Technologies disclaims any warranty that the DNA inserted into the clones or the manufacture, use, or sale of those inserts or their sequences are free from third party intellectual property claims. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For more information, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning Life Technologies's policy for the use and distribution of cloned nucleic acid fragments, including open reading frames, created using Life Technologies's commercially available Gateway® Technology.

Gateway® Entry Clones

Life Technologies understands that Gateway® entry clones, containing *att*L1 and *att*L2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Life Technologies.

Gateway® Expression Clones

Life Technologies also understands that Gateway® expression clones, containing *att*B1 and *att*B2 sites, may be generated by academic and government researchers for the purpose of scientific research. Life Technologies agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Life Technologies. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee payable to Life Technologies.

Additional Terms and Conditions

We would ask that such distributors of Gateway® entry and expression clones indicate that such clones may be used only for research purposes, that such clones incorporate the Gateway® Technology, and that the purchase of Gateway® Clonase™ from Life Technologies is required for carrying out the Gateway® recombinational cloning reaction. This should allow researchers to readily identify Gateway® containing clones and facilitate their use of this powerful technology in their research. Use of Life Technologies's Gateway® Technology, including Gateway® clones, for purposes other than scientific research may require a license and questions concerning such commercial use should be directed to Life Technologies's licensing department at 760-603-7200.

References

Chen, C., and Okayama, H. (1987) High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. Mol. Cell. Biol. *7*, 2745-2752

Chu, G., Hayakawa, H., and Berg, P. (1987) Electroporation for the Efficient Transfection of Mammalian Cells with DNA. Nucleic Acids Res. *15*, 1311-1326

Felgner, P. L., Holm, M., and Chan, H. (1989) Cationic Liposome Mediated Transfection. Proc. West. Pharmacol. Soc. *32*, 115-121

Felgner, P. L. a., and Ringold, G. M. (1989) Cationic Liposome-Mediated Transfection. Nature *337*, 387-388

Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R. (1977) Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. J. Gen. Virol. *36*, 59-74

Kozak, M. (1987) An Analysis of 5´-Noncoding Sequences from 699 Vertebrate Messenger RNAs. Nucleic Acids Res. *15*, 8125-8148

Kozak, M. (1990) Downstream Secondary Structure Facilitates Recognition of Initiator Codons by Eukaryotic Ribosomes. Proc. Natl. Acad. Sci. USA *87*, 8301-8305

Kozak, M. (1991) An Analysis of Vertebrate mRNA Sequences: Intimations of Translational Control. J. Cell Biology *115*, 887-903

Kozarsky, K. F., and Wilson, J. M. (1993) Gene Therapy: Adenovirus Vectors. Curr. Opin. Genet. Dev. *3*, 499-503

Krougliak, V., and Graham, F. L. (1995) Development of Cell Lines Capable of Complementing E1, E4, and Protein IX Defective Adenovirus Type 5 Mutants. Hum. Gene Ther. *6*, 1575-1586

Landy, A. (1989) Dynamic, Structural, and Regulatory Aspects of Lambda Site-specific Recombination. Ann. Rev. Biochem. *58*, 913-949

Orosz, A., Boros, I., and Venetianer, P. (1991) Analysis of the Complex Transcription Termination Region of the *Escherichia coli rrnB* Gene. Eur. J. Biochem. *201*, 653-659

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

Continued on next page

References, Continued

Shine, J., and Dalgarno, L. (1975) Terminal-Sequence Analysis of Bacterial Ribosomal RNA. Correlation Between the 3'-Terminal-Polypyrimidine Sequence of 16-S RNA and Translational Specificity of the Ribosome. Eur. J. Biochem. *57*, 221-230

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

Notes

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit
www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

20 August 2012