

# TrueClone™

## Mouse Full-Length cDNA Clones

### APPLICATION GUIDE

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# PACKAGE CONTENTS AND STORAGE CONDITIONS

- “cDNA clone” as 10 ug lyophilized plasmid DNA in a 2-D bar-coded Matrix tube. Store at -20°C. Once DNA is resuspended in water, store at -20°C.
- Forward (VP1.5) and reverse (XL39) DNA vector sequencing primers; dried onto the bottom of screw cap tubes. Store at room temperature. Once DNA is resuspended in water or TE, store at -20°C.

## Other Suggested Reagents

- Competent cells
- LB agar + kanamycin plates
- LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH and autoclave)
- Kanamycin
- DNA purification reagents
- DNA sequencing reagents (for plasmid preparation confirmation)
- Sterile deionized water

## Related Products

HuSH shRNA plasmids: Potent 29mer shRNA with “knock-down guarantee”.  
<http://www.origene.com/rna>

Transfection Reagents: Transfection reagents recommended for all TrueClones  
<http://www.origene.com/cdna/transfection.msp>

GFC-Transfection Array: Functionally screen hundreds of genes simultaneously  
<http://www.origene.com/cdna/gfc-array/default.msp>

Validated Antibody: Useful for monitoring protein expression  
<http://www.origene.com/antibody/>

Functional Proteins:  
<http://www.origene.com/protein/>

# INTRODUCTION

## Production and Quality Assurance

The mouse full-length TrueClones™ were derived from mouse cDNA libraries. The libraries were constructed using the pCMV6-Kan/Neo vector: the cDNA inserts were cloned unidirectionally between the EcoR I and Not I sites.

Each mouse full-length cDNA TrueClone is fully sequenced and is assured to represent the specified reference sequence through strict BLAST requirements.

All OriGene mouse clones are purified by anion-exchange column chromatography to provide transfection ready plasmid DNA. The plasmids are lyophilized into 2-D barcoded tubes and provided as 10 ug of DNA plasmid.

OriGene's full-length cDNA clones are for research use only and are not intended for clinical use. As an "investigational tool," they may differ from the reference by acceptable single nucleotide polymorphisms at the published rate of ~0.1%. All cDNA libraries were generated using reverse transcriptase, without PCR amplification and thus have a low error rate. Even with the assumption that the annotated sequence in the public database is correct, which is not always the case, it remains impossible to distinguish between naturally occurring polymorphisms and mutations from unintentional errors that may exist in any molecular clone. It has to be recognized that, in using these molecular clones, there are some inherent uncertainties and, hence, each should be viewed as a product for discovery. In most cases, OriGene has a second independently derived clone of the same gene with small variations.

Vector primers were synthesized and validated by mass spectroscopy. After salt removal, the oligonucleotide concentrations were determined by  $A_{260}$  absorbance and 100 pmol were dried onto the bottom of each tube. Every lot of primer is quality-tested to provide clean end-sequences of OriGene TrueClones devoid of any (n-1) artifacts. These primers were also designed to not prime with any TrueClone insert, thus avoiding multiple priming sites. Additional aliquots can be obtained from OriGene.

## METHODS

### Protocol for Plasmid DNA Recovery and Sequencing

Carefully open the tube, and add 100  $\mu$ L sterile, deionized water to produce a concentration of 100 ng/ $\mu$ L. *NOTE: Dissolving the DNA in a lower volume is not recommended as an increased final EDTA concentration may affect some downstream applications.*

Close the tube and incubate for 10 minutes at room temperature, or 4°C overnight.

After vortexing for 10 seconds, touch spin the tube.

The DNA can be immediately used for:

- Transfection into mammalian cells
- Sequencing reactions
- PCR amplification or probe generation
- Transformation for the creation of a glycerol stock

DNA sequencing from the 5' end of the cDNA inserts should be performed with Vector Primer 1.5 (VP1.5). Its priming site is located approximately 120 bp upstream of the polylinker. Sequencing of the 3' end portion of the cDNA inserts should be performed using the XL39 vector primer. The priming site of XL39 is approximately 70 bp downstream of the polylinker sequence.

## Protocol for Primer Resuspension and DNA Sequencing

Carefully open the tube, and add 10  $\mu$ L of sterile, deionized  $H_2O$  to obtain a 10 $\mu$ M stock. Alternatively, a low TE solution (10mM Tris (8.0), 0.1mM EDTA) is advisable for long-term storage at  $-20^{\circ}C$ .

Close the tube and incubate for at least 10 minutes at room temperature (or overnight at  $4^{\circ}C$ ).

After vortexing for 10 seconds, touch spin the tube to bring the contents to the bottom of the tube.

The primer stock (10 $\mu$ M) is now ready to be added to a DNA sequencing reaction (1 $\mu$ L=10pmol).

To obtain a high quality sequencing signal, use 1 $\mu$ L of primer in an automated DNA sequencing reaction containing 100 ng of OriGene TrueClone plasmid DNA. OriGene used 1 $\mu$ L of Big Dye<sup>®</sup> v1.1 (Applied Biosystems; Foster City, CA) in a 10 $\mu$ L reaction volume to end-sequence the TrueClone collection.

The alignment of the sequences to either the NCBI reference or the TrueClone sequence published on the website will confirm that the correct full-length clone was obtained.

## Protocol for Introduction of cDNA Overexpression Plasmids into Mammalian Cells via Transient Transfection

### Step 1. Plate target cells

The day before transfection, passage cells into the desired cell container. Plate an amount of cells expected to achieve 50-80% confluency on the following day (see Table I for examples). Grow the cells overnight at  $37^{\circ}C$  in a 5%  $CO_2$  incubator.

**Table I. Seeding density of target cells 1 day prior to experiment**

Vessel Type	Seeding density of cells	Volume of Media
10 cm dish	$7 \times 10^5$ cells	12 mL
6 well plate	$10^5$ cells / well	2 mL / well
12 well plate	$5 \times 10^4$ cells / well	1 mL / well
24 well plate	$2 \times 10^4$ cells / well	500 $\mu$ L / well
96 well plate	$4 \times 10^3$ cells / well	50 $\mu$ L / well

### Step 2. Prepare transfection mixtures

Dilute the transfection reagent\* into serum-free medium without antibiotics (Invitrogen's OptiMEM solution is a good example). Do not let the transfection reagent come into contact with the side of the tube; instead, pipet the reagent

directly into the medium. Gently flick the tube or pipet up and down to mix. Incubate for 5 minutes at room temperature. Follow the manufacturer's recommendations for ratios and volumes of reagent and DNA (see Table II for examples).

\*A low toxicity serum compatible agent must be used. We routinely use MegaTran 1.0 (OriGene) but other transfection reagents such as FuGENE 6 (Roche) or the Lipofectamine family of transfection reagents are also suitable. A database with transfection results and recommendations for many established and primary cell lines is available on OriGene's web site (<http://www.origene.com/cdna/turbofectin.msp>).

Dilute the plasmid DNA into serum-free medium without antibiotics. Gently flick the tube or pipet up and down to mix. Combine the tube of reagent/medium with the tube of DNA/medium, and gently mix. Incubate for 15-45 minutes at room temperature.

**Table II. Volumes recommended for transfection reactions**

Vessel Type	OptiMEM	Transfection Reagent	OptiMEM	cDNA Expression Plasmid
10 cm dish	300 uL	18 uL	300 uL	6 ug
6 well plate	50 uL	3 uL	50 uL	1 ug
12 well plate	25 uL	1.5 uL	25 uL	0.5 ug
24 well plate	10 uL	0.6 uL	10 uL	0.2 ug
96 well plate	2.5 uL	0.15 uL	2.5 uL	0.05 ug

### **Step 3. Add transfection mixture to cells**

Remove culture vessel from incubator. For many transfection reagents, it is not necessary to change the medium to a serum-free solution prior to transfection, but check the manufacturer's recommendations for details. Slowly add the transfection mixture dropwise to the culture medium. Rock the plate gently to mix the solution into the media, then return the vessel to the incubator. For many transfection reagents, it is not necessary to change the media after transfection, but follow the manufacturer's instructions for your particular transfection reagent. Incubate the cells at 37°C in a 5% CO<sub>2</sub> incubator before testing for effects of overexpression (usually a minimum of 48-72 hours).

## **Protocol for creating a stable cell line expressing a TrueClone in pCMV6-Kan/Neo**

*Stable integration of a TrueClone into a cell line allows you to study the effects of overexpression over a longer time course than transient transfection studies would allow. Stable cell lines can be clonally produced, assuring that every cell in the population contains the TrueClone plasmid.*

### **Step 1. Transfection**

Transfect the cells with the TrueClone plasmid DNA using your standard protocol for transient transfection. After transfection, do not change the medium until the cells are ready to be passaged.

### **Step 2. Selection**

Passage the transfected cells into a fresh vessel containing growth medium and 0.5 mg/ml a neomycin analog (such as G418). Continue to grow and passage the cells as necessary, maintaining selection pressure by keeping 0.5 mg/mL neomycin in the growth medium. After 1-2 weeks, a large number of the cells will be killed by the neomycin, indicating that they did not take up or have lost the plasmid with the neomycin resistance cassette. The cells that remain growing in the neomycin-containing medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells.

### **Step 3. Clonal selection**

Select clonal populations of cells by transferring a well-isolated single clump of cells (the clonal ancestor and cells divided from it) into a well of a 24 well plate; repeat to select 5-10 clonal populations. Continue growing these cells in selection medium for 1-2 additional passages. At this time, each well contains a clonal population of stably transfected cells, which can be maintained in normal growth medium without the selection pressure of neomycin (although you may wish to grow the cells under "light pressure", 0.2 mg/mL neomycin). These populations can be used for experiments or stored under liquid nitrogen in growth medium with 10% DMSO and 20% FBS for future use. You can verify the integration of the TrueClone by isolating total RNA from the cells and performing RT-PCR to amplify a portion of the cDNA insert.

## **Protocol for plasmid DNA Amplification in E. coli (OPTIONAL)**

*Transforming your constructs into competent cells allows you to create an eternal stock from which you can produce endless quantities of DNA for your transfection experiments. This simple protocol requires only 30 minutes of hands-on time to generate a glycerol stock and another 30 minutes to purify enough DNA for a transfection experiment.*

### **Step 1. Transformation**

Both electroporation and heat shock are appropriate methods of transformation for amplifying plasmid DNA; use the cells\* normally employed in your lab for routine transformations. Example protocols are given below for transformations using chemically competent cells and electrocompetent cells. Be sure to follow the specific recommendations of your competent cell manufacturer.

\*Most commercially available competent cells are appropriate for this purpose. Confirm the efficiency of your batch of cells by performing a parallel transformation with the supercoiled control DNA provided with the cells. OriGene recommends using cells with an efficiency of at least  $10^6$  CFU/ $\mu$ g DNA.

### Transformation with chemically competent cells

Briefly thaw on ice a tube of competent cells. Aliquot into prechilled microfuge tubes the appropriate volume of cells for individual transformations (e.g., 10 uL of cells for 1-5 ng supercoiled DNA). Add 1-5 ng of each expression plasmid to an aliquot of competent cells, stir gently with the pipet tip, and incubate on ice for 30 minutes. Perform the heat shock by incubating the mixture of DNA and cells at 42° C for exactly 30 seconds, then removing the cells to ice immediately. Add 250 uL of recovery medium (such as SOC) to the cells, and incubate at 37° C for 1 hour with agitation. Plate several dilutions of the transformation mixture onto separate LB-kan agar plates (try 1%, 10%, and 50% of the transformation reaction on separate plates, each diluted up to 100 uL in SOC). Incubate the plates overnight at 37° C. Store any remaining transformation solution at 4° C in case further dilutions and replating are necessary. The following day, inoculate 2-3 single bacterial colonies from each transformation into individual sterile culture tubes containing 5 mL of liquid medium with 60 ug/mL kanamycin (LB-kan). Incubate overnight at 37° C with agitation.

### Transformation with electrocompetent cells

Briefly thaw on ice a tube of competent cells. Aliquot into prechilled microfuge tubes the appropriate volume of cells for individual transformations (e.g., 10 uL of cells for 1-5 ng supercoiled DNA). Add 1-5 ng of each expression plasmid to an aliquot of competent cells, stir gently with the pipet tip, and transfer the mixture to a pre-chilled electroporation cuvette. Incubate cuvette on ice for 30 minutes. Perform the electroporation with settings optimized for your electroporator, and note the Tau value returned. (This value represents the time required for the current of electricity to cross the membranes of your competent cells, and is a good indication of the efficiency of the transformation process.) Add 250 uL of recovery medium (such as SOC) to the cells, and incubate at 37° C for 1 hour with agitation. Plate several dilutions of the transformation mixture onto separate LB-kan agar plates (try 1%, 10%, and 50% of the transformation reaction on separate plates, each diluted up to 100 uL in SOC). Incubate the plates overnight at 37° C. Store any remaining transformation solution at 4° C in case further dilutions and replating are necessary. The following day, inoculate 2-3 single bacterial colonies from each transformation into individual sterile culture tubes each containing 5 mL of liquid medium with 60 ug/mL kanamycin (LB-kan). Incubate overnight at 37° C with agitation.

### **Step 2. Creating a glycerol stock (OPTIONAL)**

Remove 425 uL of each overnight liquid culture into a fresh microfuge tube. Add 75 uL sterile glycerol, and gently resuspend. Glycerol is quite viscous, so it's best to use a large bore pipet tip (you may even need to widen your pipet tips by cutting off the end with a sharp blade) or a transfer pipet. When the solution is homogenous, snap freeze the tube in liquid nitrogen or a dry-ice/ethanol bath. Store the glycerol stock at -80° C. If stored properly, this stock can be used for the next several years to inoculate a fresh liquid culture in order to amplify more DNA. Simply remove a small portion of the frozen glycerol stock (thawing the tube is not required) from the

tube by scraping the surface with a pipet tip, and deposit it in a sterile culture tube containing LB-kan. The culture should be incubated overnight at 37° C with agitation before proceeding to step 4.

### **Step 3. DNA preparation**

Miniprep method (for producing up to 40 ug plasmid from 5 mL liquid culture)  
Isolate DNA from the liquid cultures by using your preferred method. OriGene routinely uses Marligen's High Purity Plasmid Miniprep Kits or similar systems. Follow the manufacturer's protocol for isolation, and elute the DNA in 50 uL of TE [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]. Determine the concentration of each sample, and store the DNA at -20° C.

Midiprep method (for producing up to 500 ug plasmid from 100 mL liquid culture)  
Add 5 mL of liquid culture grown several hours or overnight to a sterile flask containing 95 mL of LB-kan. Incubate overnight at 37°C with agitation. Isolate DNA from the liquid culture using your preferred method. OriGene routinely uses Marligen's High Purity Plasmid MidiPrep Kits or similar systems. Follow the manufacturer's protocol for isolation, and elute the DNA in 4 mL of TE [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]. Determine the concentration of each sample, and store the DNA at -20°C.

## **FREQUENTLY ASKED QUESTIONS**

### **What is the advantage of your cDNA clones compared to clones derived from PCR amplification?**

Answer: PCR inevitably introduces mutations and creates experimental deviations in downstream applications. The fidelity of replication is the biggest concern for PCR-generated genes, as has been well documented in scientific literature. This translates into substantial mutations, deletions/additions, truncations and frameshifts of the gene products. Without knowing the template sequence, it is impossible to distinguish a PCR induced mutation from a naturally occurring SNP. Unlike cDNA clones from many other sources, OriGene's TrueClones are non-PCR generated, authentic clones obtained by brute-force sequencing or by Rapid-Screen, our proprietary cloning technology. Using a TrueClone as starting material provides assurance that downstream applications (e.g. protein expression, antibody production, functional assay or ligand search) will not be affected by unintended PCR-introduced mutations.

### **How can OriGene clone difficult and rare cDNAs that others cannot?**

Answer: OriGene's clone isolation process is based on a patented method (US patent Number 6,316,193) for the rapid isolation of cDNA clones from arrayed libraries. This process allowed us to isolate rare and long clones in a high throughput fashion. It is an ongoing challenge and a commitment to collect every mouse full-length cDNA clone. OriGene has dedicated itself and has devoted major resources to achieve this goal and to deliver these novel cDNA clones through

the TrueClone Collection.

### **Where did these clones come from?**

Answer: The TrueClones were derived from mouse cDNA libraries from a variety of tissues. The inserts were cloned unidirectionally into the pCMV6-Kan/Neo vector between the EcoR I and Not I sites of the MCS.

### **How do I use the cDNA clone?**

Answer: Plasmid DNA containing an insert of the appropriate cDNA fragment is provided in a 2-D bar-coded tube, ready for immediate use. DNA can also be transformed to produce a glycerol stock for future amplification. The OriGene full-length cDNA fragment is cloned into an expression vector with the open reading frame located downstream from a eukaryotic transcriptional promoter capable of driving heterologous gene expression in a variety of mammalian cell lines in culture. The OriGene expression vector also contains a prokaryotic transcriptional promoter, which supports coupled transcription-translation of the cDNA sequence using an appropriate cell-free system. OriGene cDNA clones may also be used to validate RNAi studies or GFC-Transfection Array experiments, to generate hybridization probes, for DNA immunization to generate antibodies, and to search for polymorphisms and alternatively spliced forms.

### **Do you guarantee that your clone will express the gene?**

Answer: OriGene guarantees that the full-length open reading frame is contained in the expression vector, but cannot guarantee expression of this cDNA. There are examples in the literature suggesting post-transcriptional and/or translational regulation may affect gene expression, uncontrollable by either the strength or the specificity of the transcriptional promoter used. Examples include the effects of the presence of the 5' or 3' untranslated regions of the respective mRNA. Other factors can also affect expression of the cDNA clone, including the strength of the protein initiation site, and the properties of the gene product that the cDNA encodes.

### **Can I use Origene full-length cDNA for stable transfection?**

Answer: Yes. All Origene mouse full-length cDNAs are cloned into an expression vector with the neomycin resistant gene. Many mammalian cell lines can be transfected and selected for stable transfection using a neomycin analog (such as G418).

### **How have you determined the identity of each TrueClone?**

Answer: Each of OriGene's full-length clones has been fully sequenced and the resulting sequence matched to a corresponding known reference by BLAST analysis.

### **Why did you send sequencing primers with my clone?**

Answer: The DNA vector primers are included in this shipment for use in sequencing the ends of a TrueClone cDNA insert. Analysis of the end sequences of TrueClone inserts is the best method to confirm that you have received or have purified the correct full-length clone. It is best to first use the 5' primer for this purpose to avoid the difficulty of sequencing through a poly-A tail with the 3' primer. Do not use other

common sequencing primers such as T7 or M13rev as they are not always unique in the OriGene pCMV6 vector system.

**Why does the TrueClone that I sequenced match the correct gene, but contain some nucleotide changes?**

Answer: SNPs (single nucleotide polymorphisms) reflect the unique differences from genes expressed in different tissues and different individuals. Published references may represent a different SNP than the OriGene transcript. In addition, the sequence of cloned PCR products (such as those deposited in GenBank) can contain PCR-induced mutations that are not biologically relevant. The OriGene clones do not contain such mutations because only cDNA clones from cDNA libraries are provided. Should a specific SNP be required, this too can be contracted to OriGene.

**I got an unexpected restriction digestion pattern from a TrueClone - does this mean the clone is wrong?**

Answer: Not at all. Single nucleotide polymorphisms (SNPs) may exist between OriGene's cDNA clone and the reference sequence you used to predict the digestion pattern. These SNPs may give rise to novel cut sites, or may eliminate expected ones.

**Has every TrueClone been fully sequenced?**

Answer: Yes. All of OriGene mouse clone inserts have been fully sequenced and this sequence is posted on OriGene's website.

**Why is the clone longer than the reference sequence?**

Answer: OriGene's clone is a naturally occurring cDNA isolated from a mouse cDNA library. As a naturally occurring transcript, it contains untranslated regions (UTRs), which may differ in length from the UTR of the reference sequence. The reference sequence may represent a PCR product, or transcript from another tissue than that used to produce OriGene's clone, which would account for different UTR length.

**Why is there an extension to the reference sequence ID, for example NM\_024080.3? What does that imply?**

Answer: GenBank sequence records are subject to constant revision and updating. When a change to the sequence of a transcript is submitted as a new record to NCBI, the reference sequence number is updated to the new version, which is indicated by the extension. For clarity, OriGene specifies to which version of a reference sequence each TrueClone corresponds. You can view all versions of an NCBI reference sequence (current or earlier) by searching with this extension included in the sequence number.

**What does your disclaimer mean?**

Answer: OriGene's disclaimer for our TrueClones reads as follows: "Our molecular clone sequence data has been matched to the accession number below as a point of reference. Note that the complete sequence of our molecular clones may differ from the sequence published for this corresponding accession number, e.g., by representing an alternative RNA splicing form or single nucleotide polymorphism (SNP)."

The NCBI RefSeq mouse mRNA sequences are continuously being revised, as some may have been derived from aberrantly spliced transcripts or generated by incorrect prediction of intron-exon junctions in silico. These sequences are therefore used only as a “reference” and not as a “standard”. OriGene’s clones may differ from the reference sequence for this reason.

**Where can I find the sequence or insert size of my TrueClone?**

Answer: This information can be found on OriGene’s website. Just type in the catalog number, accession number, or name of your clone into the search box at the top of any page of the OriGene website (<http://www.origene.com>), and press “Go”. Then click on the catalog number that corresponds to your product of interest, and click on the orange “Details and Pricing” button. After entering a valid email address, you’ll be directed to a page containing the product’s list price and availability, related products, vector identity, insert size, sequence information and reference sequence information. You can also request this information through Technical Support at 888-267-4436 or 301-340-3188.

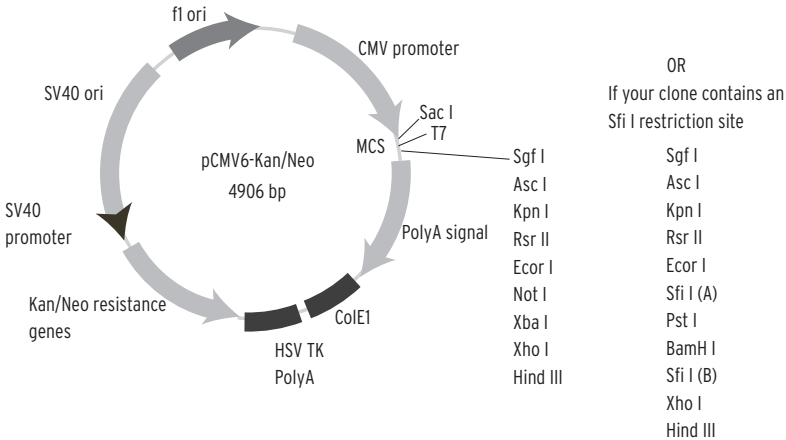
**What is the TrueClone Guarantee?**

Answer: OriGene warrants that the product will meet specifications listed. At OriGene’s discretion, free replacement of any non-conforming product will be made if OriGene is notified within 30 days of product receipt. If you experience any difficulty with any OriGene product, please contact our Technical Support Staff at 888-267-4436, or 301-340-3188 outside the US.

# APPENDIX

## Physical Map of pCMV6-Kan/Neo

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.



The **pCMV-Kan/Neo** is 4906 bp in size. The cDNA library inserts are directionally cloned between the EcoR I and Not I sites. The CMV promoter (used to express the cloned cDNA) is upstream of the cDNA insert, which is followed by the hGH (human growth hormone) polyA signal. The ColE1 ori is the bacterial origin of replication, the SV40 ori allows for replication in mammalian cells and the f1 ori is the filamentous phage origin of replication, which allows for the recovery of single-stranded plasmids. Selection of the plasmid in *E. coli* is conferred by the kanamycin resistance gene.

The T7 RNA polymerase can be used for generating transcripts of the cDNA by *in vitro* transcription. The T7 promoter site is located within the polylinker sequence in the pCMV-Kan/Neo vector.

## Polylinker Sequence(s) of pCMV6-Kan/Neo

Vector Primer v1.5 >

TTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGGTACG  
AAAGGTTTTACAGCATTATTGGGGCGGGCAACTGCGTTTACCCGCCATCCGCACATGC

**T7 promoter >**

Sac I

(Not for sequencing)

GTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGAATTTTGTAATACGAC  
CACCTCCAGATATATTCTGCTCTCGAGCAAATCACAAGGCAGTCTTAAACATTATGCTG

Sgf I

Asc I

Kpn I

Rsr II

EcoR I

TCACTATAGGCGATCGCGGCGCGCCGTACCCGACCGGAATTCCCGGGATATCGTCTG  
AGTGATATCCCGTAGCGCCGCGGCCATGGCCTGGCCTTAAGGGCCATAGCAGC

Not I

Xba I

Xho I

ACCCACGCTCCC cDNA insert GGGCGGCGCTCTAGAGTATCCCTCGAGGGCCC  
TGGGTGCGAGGG cDNA insert CCCGCGGCGAGATCTCATAGGAGCTCCCGGG

Hind III

AAGCTTACGCGTACGCGGCCACTCGAGCAGAACTCATCTCAGAAGAGGATCTGGCAA  
TTCAATGCGCATGCGCCGGTGAAGTCTGCTTTGAGTAGAGTCTTCTCTAGACCGTTT

TGATATCCTGGTTTAAACGGCCGCGCGGTATAGCTGTTTCTGAAACATGTGATCCC  
ACTATAGGACCAAATTTGCCGGCCGCGCCAGTATCGACAAAGGACTTGTACACTAGGG

GGGTGGCATCCCTGTGACCCCTCCCACTGCCTCTCTGGCCCTGGAAGTTGCCACTCC  
CCCACCGTAGGGACTGGGGAGGGGTCACGGAGAGGACCGGGACCTTCAACGGTGAGG

AGTGCCACCAGCCTTGTCTTAATAAAA

TCACGGGTGGTCGGAACAGGATTATTT

< Vector Primer XL39

**In cases where your gene contains the cloning site(s) Sfi I, use the diagram below**

Vector Primer v1.5 >

TTTGGCACAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCG  
GTAGGCGGTACG  
AAACCGTGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTATTGGGGCGGGCAACTGCGTTTACCCGC  
CATCCGCACATGC

**T7 promoter >**

Sac I

(Not for sequencing)

GTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGAATTTTGTAATACGAC  
CACCTCCAGATATATTCTGCTCTCGAGCAAATCACAAGGCAGTCTTAAACATTATGCTG

Sgf I

Asc I

Kpn I

Rsr II

EcoR I

TCACTATAGGCGATCGCGGCGCGCCGTACCCGACCGGAATTCGGCCATTACGGCCT  
AGTGATATCCCGTAGCGCCGCGGCCATGGCCTGGCCTTAAGCCGTAATGCCGA

