TrueClone™

Human Full-Length cDNA Clones

APPLICATION GUIDE

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Revision 08.09.TS

PACKAGE CONTENTS AND STORAGE CONDITIONS

- cDNA clone as 10 ug lyophilized plasmid in a 2-D bar-coded matrix tube. The lyophilized plasmid is stable for up to one year when stored at ambient temperature. Following resuspension in 100 ul dH_2O, store at -20° C.
- Forward (VP1.5) and reverse (XL39) cDNA sequencing primers; lyophilized onto the bottom of screw-cap tubes. Lyophilized primers are stable for up to one year when stored at ambient temperature. Following resuspension in 10 ul dH_2O, store at -20° C.

Other Recommended reagents

- Competent *E. coli* (NEB DH5α recommended)
- LB-agar plus ampicillin plates (100 ug/ml)
- LB-broth (10 g/L Tryptone, 5 g/L yeast extract, 10 g/L NaCl. Adjust pH to 7.0 with 1N NaOH and autoclave. When cooled, add ampicillin to 100 ug/ml.)
- Ampicillin
- DNA purification reagents
- DNA sequencing reagents (for plasmid preparation and confirmation)
- Sterile deionized water

Related Products

TrueORF™ Tagged ORF Clones http://www.origene.com/orf/
HuSH™ shRNA Plasmids http://www.origene.com/rnai/
VERIFY™ Tagged Antigens http://www.origene.com/lysate/
Validated Antibodies http://www.origene.com/antibody/
Functional Proteins http://www.origene.com/protein/
Transfection Reagents http://www.origene.com/cdna/transfection.mspx

INTRODUCTION

Overview

Many 'known' genes have numerous redundant and overlapping sequences in the public databases. The National Center for Biotechnology Information (NCBI) RefSeq sequences represent the best effort in defining the most complete mRNA sequence, with an appropriate or putative open reading frame and flanking nucleotide sequences of that gene. Each of OriGene's full-length clones has been sequenced at both its 5' and 3' ends, and the resulting sequences are confirmed to align upstream and downstream of the start and stop codons, respectively. All OriGene Custom Clones are fully sequenced to provide a non-variant match to the expected reference without frameshifts.

Plasmid DNA containing an insert of the appropriate cDNA fragment is provided in a 2-D bar-coded Matrix tube, ready for immediate use. DNA can also be transformed to produce a glycerol stock for future amplification. The full-length cDNA fragment is present in an expression vector downstream of a CMV promoter capable of driving

heterologous gene expression in a variety of mammalian cell lines in culture and of supporting heterologous gene expression in a variety of tissues in transgenic mice. The vector also contains a prokaryotic transcriptional promoter (See appendix) which supports coupled transcripton-translation of the cDNA using an appropriate cell-free system. If the cDNA clone is in pCMV6-XL4, the insert must be liberated from the plamid backbone before attempting *in vitro* transcription (See appendix). OriGene's TrueClones may be used to generate hydribization probes, or antibodies via DNA immunization, and to search for polymorphisms or alternatively spliced isoforms.

Production and Quality Assurance

The human full-length TrueClones™ were isolated directly from human cDNA libraries. The libraries were constructed using the pCMV6-XL4, pCMV6-XL5, or pCMV6-XL6 mammalian expression vectors and the cDNA inserts were cloned unidirectionally using a linker-based strategy; EcoRI on the 5` and Xho I on the 3` end of the insert. The linker contributes an adapter sequence between the EcoRI site and the start of the insert cDNA and should not be considered part of the endogenous cDNA insert sequence (vector-GAATTCGGCACGAGG-cDNA insert). The inserts were cloned into the EcoRI and Sal I sites of the pCMV6-XL expression vector, destroying the Sal I site on the 3` end of the insert. The cDNAs can be removed from the plasmid using Not I (Not I is present twice in the vector, once before the EcoR I site and once downstream of the Sal I site).

If your TrueClone was provided in pCMV6-Neo, it most likely has the standard EcoRI and Sal I cloning sites and can be released from the vector using Not I.

If your TrueClone was provided in pCMV6-AC, it most likely does not have the standard EcoRI and Sal I cloning sites. It cannot be released from the vector using Not I. Please contact OriGene technical support for details on the cloning sites of these products.

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 and without PCR amplification, thus having 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 that has been similarly confirmed for its 5' and 3' sequences, as well as for its approximate insert size.

Nucleotide sequences of pCMV6-XL4, XL5, XL6, Neo and AC are available for download from the following URLs

- http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-XL4.doc
- http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-XL5.doc
- http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-XL6.doc
- http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-Neo.doc
- http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-AC.doc

METHODS

Protocol for Plasmid DNA Recovery and Sequencing

Carefully open the tube and add 100 ul dH_2O to produce a concentration of 100 ng/ul. NOTE: Dissolving the plamid DNA in a lower volume is not recommended as an increased EDTA concentration may affect some downstream applications.
Close the tube and let it sit for 10 minutes at room temperature, or 4^{o} C overnight. Vortex the tube briefly and then do a quick spin to concentrate the liquid at the bottom.
The DNA is ready for immediate use in:

- Transfection to mammalian cells
- Restriction enzyme digestion
- Protein expression in cell-free systems
- PCR amplification
- Probe labeling
- *E.coli* transformation for DNA amplification

Protocol for Primer Re-dissolution and Sequencing

Carefully open the tube and add 10 ul of dH_2O to generate a 10 uM stock.
Close the tube and let it sit for 10 minutes at room temperature, or 4^{o} C overnight. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. The primer stock is now ready to be added to a DNA sequencing reaction (1 ul=10pmol). DNA sequencing from the 5` end of the cDNA insert should be performed with VP1.5 (5`-GGACTTTCCAAAATGTCG-3`) whose priming site is located ~120 bp upstream of the polylinker. DNA seuquencing from the 3` end of the cDNA insert should be performed with XL39 (5`-ATTAGGACAAGGCTGGTGGG-3`)whose priming site is located ~70 bp downstream of the polylinker. Do not use other common sequencing primers such as M13rev or T7 as these are not always unique in OriGene vectors.

Introduction of cDNA OverExpression Plasmids into Mammalian Cells via Transient Transfection

Step 1. Plate 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.

The exact number will depend on the size of the cells (see Table I for examples). Grow the cells overnight at 37°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	1.5 - 6 x 10^6 cells	12 mL
6 well plate	0.3 - 1 x 10^6 cells	2 mL / well
12 well plate	0.15 - 0.5 x 10^6 cells	1 mL / well
24 well plate	0.6 - 2 x 10^5 cells	500 uL / well
96 well plate	1 - 4 x 10^4 cells	100 uL / 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).

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.

*A low toxicity serum compatible agent must be used. We recommend using TurboFectin 8.0 (OriGene) but other transfection reagents such as Fugene6 (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/transfection.mspx) and Roche's web site (https://www.roche-applied-science.com/sis/transfection/index.jsp).

Table II. Recommended starting transfection conditions for TurboFectin 8.0

Vessel Type	TurboFectin: DNA	OptiMEM	cDNA Expression Plamid
10 cm dish	3:1	1.5 mL	5 - 25 ug
6 well plate	3:1	250 uL	1 - 5 ug
12 well plate	3:1	100 uL	0.5 - 2.5 ug
24 well plate	3:1	50 uL	0.25 - 1.25 ug

Vessel Type	TurboFectin: DNA	OptiMEM	cDNA Expression Plamid
96 well plate	3:1	10 uL	0.05 - 0.25 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_2 incubator before testing for effects of overexpression (usually a minimum of 24-48 hours).

Creating a stable cell line expressing a TrueClone (Only for TrueClones in pCMV-Neo or pCMV6-AC Vectors)

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 neomycin (G418). The optimal G418 concentration varies among different cell lines and should be determined by performing a kill curve. 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 96-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.

Plasmid DNA Amplification in *E. coli* (OPTIONAL)

Step 1. Resuspension of lyophilized DNA constructs
Add 100 uL of sterile water into a tube containing 10 ug of DNA expression plasmid. Vortex the tubes gently or pipet up and down to resuspend the lyophilized DNA. This resuspension produces a DNA solution with an approximate concentration of 100 ng/uL, which should be stored at -20°C.

Step 2. 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 10^8 CFU/ug DNA. We routinely use NEB DH5α. For toxic clones we routinely use EpiCentre CopyCutter cells.

Transformation with chemically competent cells
Briefly thaw on ice a tube of competent cells. Aliquot into pre-chilled 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 uL 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-amp 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 100 ug/mL ampicillin (LB-amp). Incubate overnight at 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 uL of each expression plasmid to an aliquot of com-

petent cells, stir gently with the pipet tip, and transfer the mixture to a prechilled electroporation cuvette. Incubate cuvette on ice for 30 minutes. Perform the electroporation with settings optimized for your electroporator. 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-amp 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 100 ug/mL ampicillin (LB-amp). Incubate overnight at 37°C with agitation.

Step 3. Creating a glycerol stock
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-amp. The culture should be incubated overnight at 37°C with agitation before proceeding to step 4.

Step 4. 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.

FREQUENTLY ASKED QUESTIONS

What is a TrueClone?
OriGene's TrueClones are isolated from our full-length cDNA libraries and contain the coding sequence as well as the untranslated regions (UTRs) of the mRNA transcript appropriate to the library from which they were isolated. The majority of TrueClones are in the pCMV6-XL4/XL5/XL6 vectors for transient overexpression in mammalian cells. Any of our TrueClones can be provided in a vector for stable overexpression (pCMV6-Neo) for a nominal fee.

What is the difference between a TrueClone and a TrueORF?
TrueORFs are much more "engineered" than TrueClones. TrueORFs are just the cod-

ing sequence (Open Reading Frame aka ORF) in our pCMV6-Entry vector, designed to put C-terminal Myc-DDK tags on the expressed protein. TrueORFs do not contain any UTR sequences (these would interfere with making the fusion protein) but can be used for transient or stable overexpression in mammalian cells. The Open Reading Frame in any TrueORF can be easily shuttled to another destination vector adding various tags or functions.

Is it better to purchase a TrueClone or a TrueORF?
It really depends on the purpose of your experiments. For example, if you simply need to overexpress the protein under transient conditions, move the cDNA to your own vector, or use the clone as a template for PCR, then the TrueClone is a good choice. If you don't have a good antibody against your protein, need to easily purify your protein, or you need to make stable cell lines, the TrueORF is a good choice.

How do I search for my cDNA clone?
The most precise search of our website is by using the NCBI reference accession number of the mRNA transcript (e.g. NM_000123). This will bring up all products related to this reference sequence including TrueClone, ORF clone, antigen standard, and HuSH silencing kit. If you don't know the NCBI reference accession number, use the gene symbol to search. This will often generate many more hits than expected because it is a simple text search that may return many splice variants and occasionally unwanted transcripts. If you get too many positives, we recommend that you search Entrez Gene from NCBI to find the reference accession number and then repeat the search of OriGene's website with the accession number. Go to http://www.ncbi.nlm.nih.gov/ and select gene from the dropdown menu on the left of the search box.

How do I know the cloning sites and/or sequence of my TrueClone?
The majority of TrueClones were inserted into the pCMV6-XL4/XL5/XL6 vectors using a linker-based strategy. The linkers were ligated to the EcoRI (5`) and Sal I (3`) sites of the corresponding pCMV6-XL vector. For the 3` site, a Xho I linker was fused to the Sal I site, destroying both Xho I and Sal I in the process. These sites were not used for TrueClones in the pCMV6-AC vector (see pCMV6-AC vector map on the OriGene website). If you need information on the cloning sites for an AC-vector, please contact our Technical Support Team.

Are the cloning sites for pCMV6-AC EcoRI and Sal I?
No. Inserts were subcloned into the pCMV6-AC vector using various combinations of restriction enzymes appropriate to each specific cDNA. Please contact OriGene technical support for information on your specific construct.

Are OriGene's clones fully sequenced?
For all TrueClones, OriGene posts the available sequence data on our website in each specific clone data table (sometimes, it is necessary to access a separate "Details and Pricing" page). 5' or 3' Read Nucleotide Sequence refers to an unedited sequence primed with a vector primer from the corresponding insert end. Se-

quence errors are likely to be present and therefore these reads should not be used for base-by-base analysis. Should the Open Reading Frame be covered by edited sequence, it is indicated by a link called "Edited Nucelotide Sequence". All TrueORF clones contain the insert sequence that you should expect to be correct. Always note that the exact sequence of an OriGene clone may differ from the NCBI reference with respect to biological polymorphisms.

I received three tubes with my clone, what are they?
OriGene provides the full-length cDNA clone plus the VP1.5 (forward) and XL39 (reverse) vector sequencing primers. Should you need to amplify the plasmid DNA, we recommend that you end sequence with the VP1.5 (5') primer.

Can I use VP1.5 and XL39 for PCR amplication of my insert?
VP1.5, 5' GGACTTTCCAAAATGTCG 3' Tm=51C and XL39, 5' ATTAGGACAAGGCTG-GTGGG 3' Tm=60C usually do not work well for amplification of the insert due to the large difference in their Tms. VP1.5 and XL39 are provided so that if you amplify the plasmid DNA, you can confirm your DNA prep by sequencing.

My VP1.5 sequence read matches the reference but my XL39 shows no BLAST similarity to anything?
Because most of OriGene's TrueClones contain a polyA tail, the XL39 primer can fail to read through this region accurately. In these instances, we recommend sequencing with a gene-specific forward primer to get a good 3` read. It is also possible that OriGene's clone has a longer UTR than was present in the reference.

I cannot detect any biological activity after transfecting my clone. What do I do?
Lack of activity can occur for a wide variety of causes. First, be sure that the preparation of DNA that you are working with is of the expected concentration and is not a purified contaminant (from your lab or from OriGene). Secondly, check to see if your protein is being expressed in your cell type by Western blot. If it is not, next check for expression of the mRNA transcript by RT-PCR. These are very different problems with very different solutions. Once you know the source of the problem, please contact our technical support scientists for assistance at 888-267-4436 (USA) or techsupport@origene.com.

How can I release my insert?
In most cases, you can use Not I to liberate the complete insert. There are two Not I sites outside of the cloning sites in all pCMV6-XL vectors. Although it is very rare for the 8-base cutter, Not I, to cut inside a mammalian gene, it is important to verify this before employing this strategy. TrueClone inserts in pCMV6-AC can not be released using this strategy (see earlier FAQ).

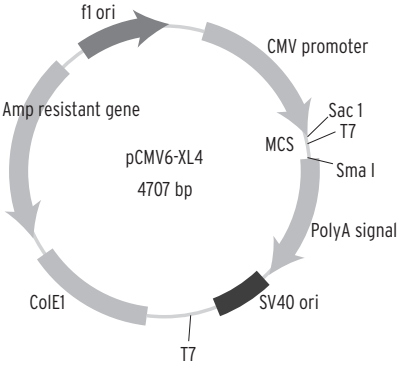
Can I use my TrueClone for *in vitro* transcription and translation?
Yes, we have tested our TrueClones for IVTT in rabbit reticulocyte lysate systems and have seen protein production. However, if your clone is in the XL4 vector, it is

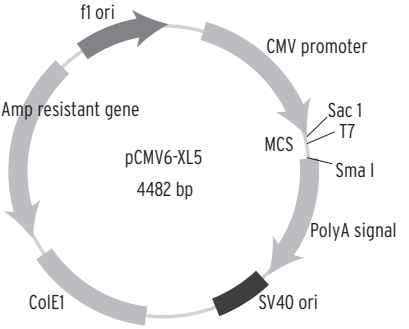
necessary to liberate the insert and the sense T7 promoter from the vector backbone because XL4 contains two opposing T7 promoters. We recommend digesting the insert and the sense T7 promoter away from the plasmid backbone using Sac I and Sma I. The fragment liberated by Sac I and Sma I can be used for IVTT, but it is important to make sure that these enzymes do not cut inside your insert before employing this approach.

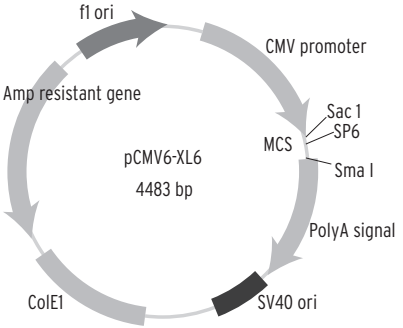
APPENDIX

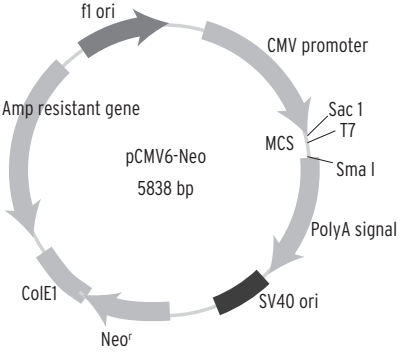
Physical Maps of pCMV6-XL4, XL5, XL6, Neo and AC

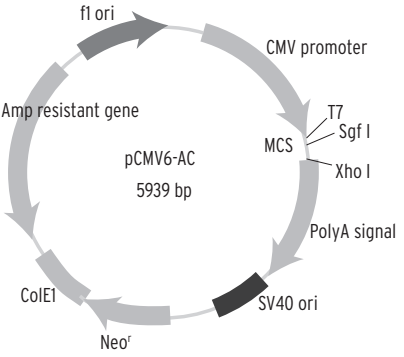
The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.











The **pCMV-XL4** vector is 4.7 kb in size, and the **pCMV-XL5** and **pCMV-XL6 vectors** are 4.5 kb in size. All three vectors contain the same polylinker (Sac I to Sma I). The cDNA library inserts are directionally cloned between the EcoRI and Sal I sites. Note that the Sal I site is destroyed in the cloning process. The **CMV promoter**, which can be used to express the cloned cDNA, is followed by the hGH (human growth hormone) **polyA** signal located downstream of the insert. 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 **ampicillin resistance gene**.

Polylinker Sequence of pCMV6-XL4, XL5, XL6, Neo and AC

A) pCMV6-XL4, XL5 and Neo

```
                                  Vector Primer v1.5 >
TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACG
AAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTATTGGGGCGGGGCAACTGCGTTTACCCGCCATCCGCACATGC

                                                    T7 promoter >
                                  Sac 1             (Not for sequencing)      Not I   EcoRI
GTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGAATTTTGTAATACGACTCACTATAGGGCGGCCGCGAATT
CACCCTCCAGATATATTCGTCTCGAGCAAATCACTTGGCAGTCTTAAAACATTATGCTGAGTGATATCCCGCCGGCGCTTAA

                                  Xho I/Sal I Xba 1     Not I                       Sma I
C----TrueClone_Insert-----CTCGACTCTAGATTGCGGCCGCGGTCATAGCTGTTTCCTGAACATGTGATCCCGGGT
G----TrueClone_Insert-----GAGCTGAGATCTAACGCCGGCGCCAGTATCGACAAAGGACTTGTACACTAGGGCCCA

GGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAA
CCGTAGGGACACTGGGGAGGGGTCACGGAGAGGACCGGGACCTTCAACGGTGAGGTCACGGGTGGTCGGAACAGGATTATTT
                                                                  < Vector Primer XL39
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B) pCMV6-XL6

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                                  Vector Primer v1.5 >
TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACG
AAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTATTGGGGCGGGGCAACTGCGTTTACCCGCCATCCGCACATGC

                                  Sac 1   SP6 Promoter >                      Not I   EcoRI
GTGGGAGGTCTATATAAGCAGAGCTCATTTAGGTGACACTATAGAATACAAGCTACTTGTTCTTTTTGCAGCGGCCGCGAATT
CACCCTCCAGATATATTCGTCTCGAGTAAATCCACTGTGATATCTTATGTTCGATGAACAAGAAAAACGTCGCCGGCGCTTAA

                                  Xho I/Sal I Xba 1     Not I                       Sma I
C----TrueClone_Insert-----CTCGACTCTAGATTGCGGCCGCGGTCATAGCTGTTTCCTGAACATGTGATCCCGGGT
G----TrueClone_Insert-----GAGCTGAGATCTAACGCCGGCGCCAGTATCGACAAAGGACTTGTACACTAGGGCCCA

GGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAA
CCGTAGGGACACTGGGGAGGGGTCACGGAGAGGACCGGGACCTTCAACGGTGAGGTCACGGGTGGTCGGAACAGGATTATTT
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```
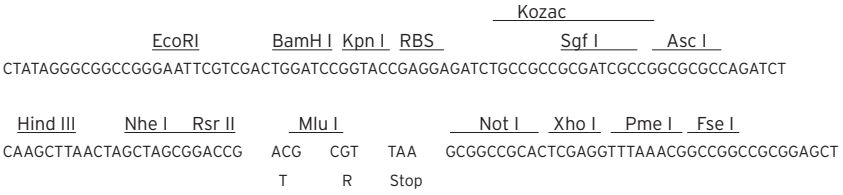

C) pCMV6-AC


Kozac
EcoRI
BamH I
Kpn I
RBS
Sgf I
Asc I
CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT
Hind III
Nhe I
Rsr II
Mlu I
Not I
Xho I
Pme I
Fse I
CAAGCTTAACTAGCTAGCGGACCG
ACG
CGT
TAA
GCGGCCGCACTCGAGGTTTAAACGGCCGGCCGCGGAGCT
T
R
Stop