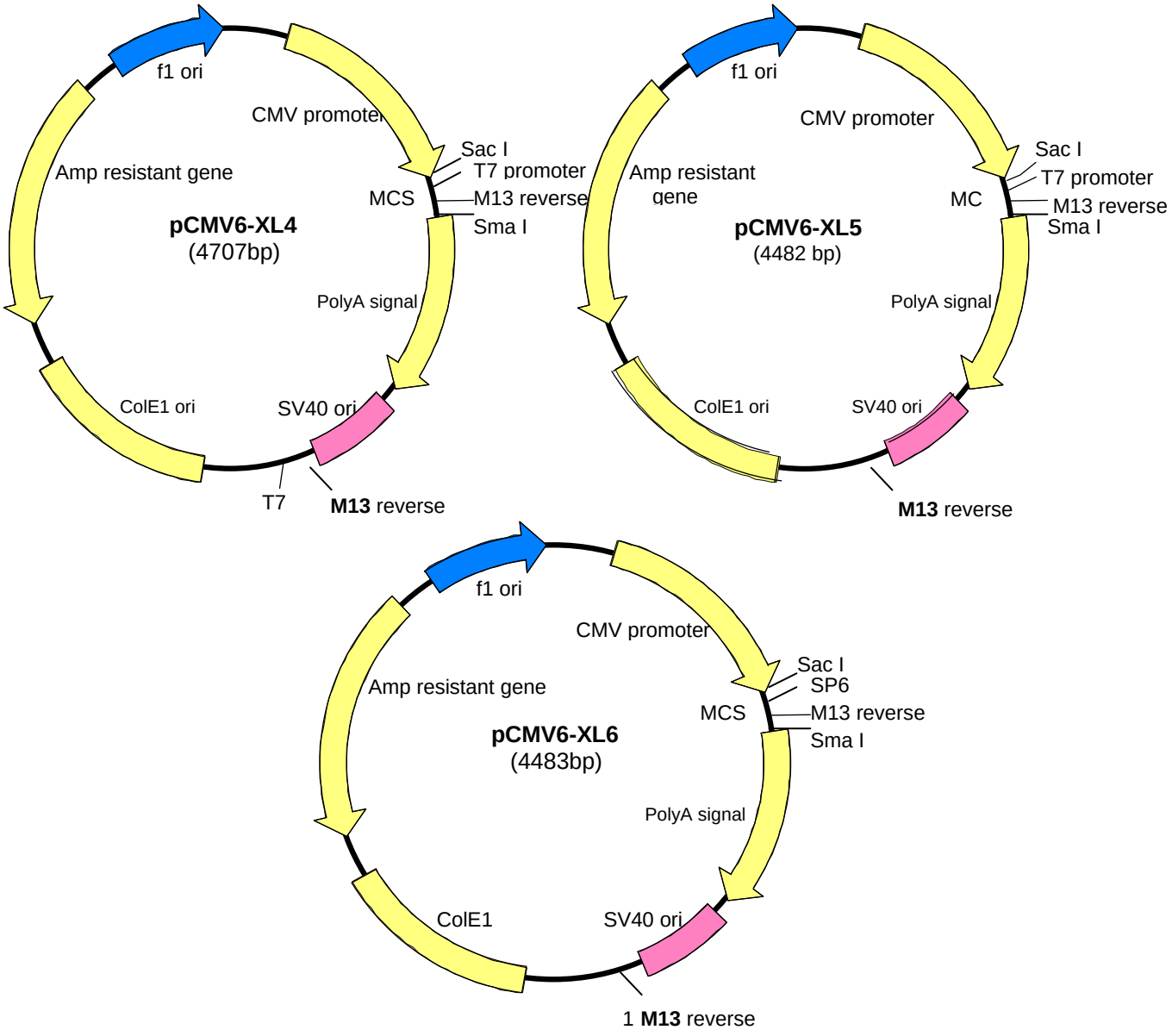
pCMV6 Mammalian Expression Vectors– Application Guide

Table of Contents

Package Contents and Storage Conditions

- Each tube contains 5ug lyophilized DNA
- Shipped in ambient temperature. Store in –20^{o}C upon arrival.

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



Description of the vectors

The pCMV6 series of vectors were used in the construction of all OriGene Rapid-Screen libraries and therefore are the vectors present in all TrueClones. Therefore, these "empty" vectors are the most appropriate negative control plasmids for use in any over-expression assay of TrueClones. All three vectors contain the same polylinker (*Sac* I to *Sma* I). 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. The **ampicillin resistant gene** confers the selection of the plasmid in E. coli.

Mammalian Cell Protein Over-Expression:

The polylinker sites are downstream of a CMV promoter * capable of driving heterologous gene expression in a variety of mammalian cell lines in culture. However, there are examples in the literature suggesting that post-transcriptional and/or regulation may affect the achievable protein expression levels in a given cell line. OriGene recommends the use of COS cells or HEK293 cells, however, the transfection conditions for the cell line as well as the assay conditions for the protein must first be optimized.

In vitro Transcription:

T7 RNA polymerase can be used for generating transcripts of the cDNA by *in vitro* transcription. However, the **pCMV-XL4** vector has a second but opposing T7 site (with a one-base substitution on the 3' end) located between **ColE1 ori** and **SV40 ori**. Inserts (cDNA) in this vector must first be released by digesting with *Sac* I and *Sma* I before the *in vitro* transcription reaction. The **pCMV-XL5** vector does not have a second T7 promoter site, and the **pCMV-XL6** vector has an SP6 promoter. Therefore, clones within the XL5 or XL6 vectors do not require prior insert excision.

The M13 primer sequence is represented twice within the pCMV6-XL4 and pCMV6-XL5 vector sequences with only a slight variation in bases between the two sites. Therefore, sequencing from the 3' end of cDNA inserts may not be performed using an M13 Reverse primer.

Polylinker Sequence of pCMV6-XL4 and pCMV6-XL5

```
                        Vector Primer v1.5 >
TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACG
AAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTATTGGGGCGGGGCAACTGCGTTTACCCGCCATCCGCACATGC

                           Sac 1                                  T7 Promoter              Not I    EcoRI
GTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGAATTTTGTAATACGACTCACTATAGGGCGGCCGCGAATT
CACCCTCCAGATATATTCGTCTCGAGCAAATCACTTGGCAGTCTTAAAACATTATGCTGAGTGATATCCCGCCGGCGCTTAA

 Bgl II  KpnI     EcoRV    Hind III        Xba I        Not I
CAGATCTGGTACCGATATCAAGCTTGTCGACTCTAGATTGCGGCCGCGGTCATAGCTGTTTCCTGAACAGATCCCGGGT
GTCTAGACCATGGCTATAGTTCGAACAGCTGAGATCTAACGCCGGCGCCAGTATCGACAAAGGACTTGTCTAGGGCCCA

GGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAA
CCGTAGGGACACTGGGGAGGGGTCACGGAGAGGACCGGGACCTTCAACGGTGAGGTCACGGGTGGTCGGAACAGGATTATTT
                                                                          < Vector Primer XL39
```

Polylinker Sequence of pCMV6-XL6

```
                        Vector Primer v1.5 >
TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACG
AAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCATTATTGGGGCGGGGCAACTGCGTTTACCCGCCATCCGCACATGC

                           Sac 1    SP6 Promoter                                    Not I    EcoRI
GTGGGAGGTCTATATAAGCAGAGCTCATTTAGGTGACACTATAGAATACAAGCTACTTGTTCTTTTTGCAGCGGCCGCGAATT
CACCCTCCAGATATATTCGTCTCGAGTAAATCCACTGTGATATCTTATGTTCGATGAACAAGAAAAACGTCGCCGGCGCTTAA

 Bgl II   KpnI    EcoRV   Hind III        Xba I         Not I
CAGATCTGGTACCGATATCAAGCTTGTCGACTCTAGATTGCGGCCGCGGTCATAGCTGTTTCCTGAACAGATCCCGGGT
GTCTAGACCATGGCTATAGTTCGAACAGCTGAGATCTAACGCCGGCGCCAGTATCGACAAAGGACTTGTCTAGGGCCCA

GGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAA
CCGTAGGGACACTGGGGAGGGGTCACGGAGAGGACCGGGACCTTCAACGGTGAGGTCACGGGTGGTCGGAACAGGATTATTT
                                                                    < Vector Primer XL39
```

Vector Primer v1.5	(forward seq primer)	5**'** GGACTT TCCAAA ATG TCG 3'
Primer XL39	(reverse seq primer)	5’ ATTAGGACAAGGCTGGTGGG 3’

Nucleotide Sequences of pCMV6-XL4, XL5 and XL6

All sequences are available electronically at the following URLs

1. http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-XL4.doc
2. http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-XL5.doc
3. http://www.origene.com/assets/Documents/NucleotideSequenceofpCMV6-XL6.doc

* 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.