Mammalian Expression Vectors

ATUM has mammalian expression vectors suitable for transient or stable expression. These vectors are available with features including various promoters, markers, and fusions.

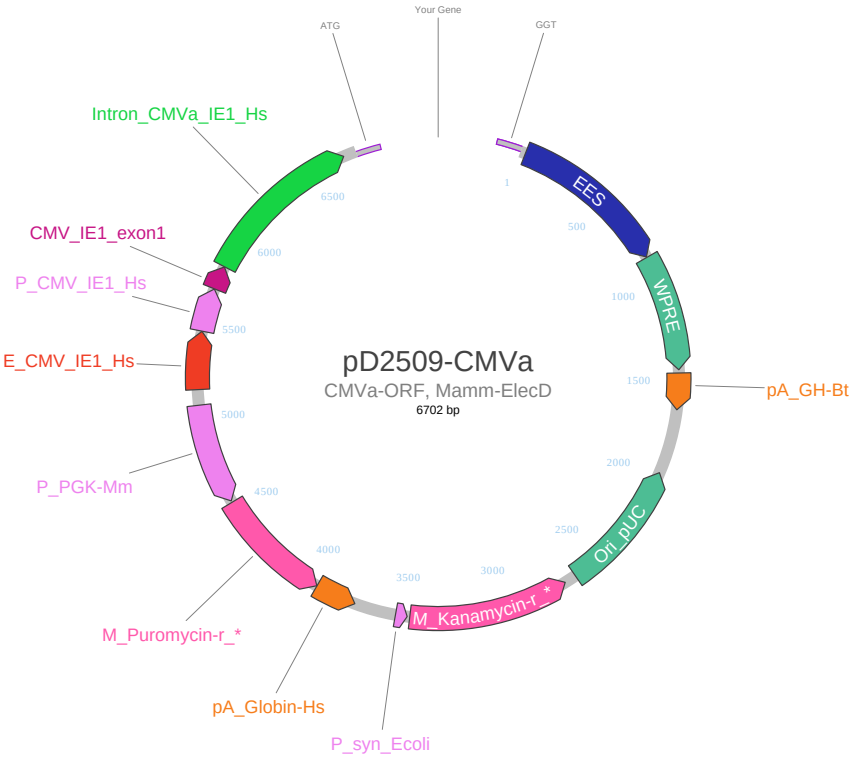
Mammalian Stable Expression Vectors

These vectors can produce high levels of transient expression, and they also contain sequences to enhance expression after integration into the genome of transfected cells. These vectors contain mammalian selectable markers to allow selection of stable integrants. 80-90% of stable transformants express the gene cloned into the ORF slot.

Leap-In Integration Vectors

These vectors are available with a variety of mammalian promoters and different selection markers. Vectors are also available with IRES or CHYSEL sequences for translational coupling of an ORF with a fluorescent protein for expression monitoring.

Plasmid Map



Name	Qty	Storage
pD2509-CMVa	10Rx	−20℃

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

Standard Transfection Protocol

1. 24 hours before transfection seed cells (in antibiotic free media) to be 70-90% Confluent at time of transfection. (6 well plate: 0.25-1.0x10^6, 24 well plate: 0.5-2.0x10^5, 96 well plate: 1-4x10^4)
2. *Dilute Lipofectamine in DMEM or Opti-MEM (LifeTech)
3. *Dilute DNA (endotoxin free is optimal) in DMEM or Opti-MEM (LifeTech)
4. Add diluted DNA mixture to diluted Lipofectamine mixture.
5. Incubate for 10 minutes.
6. Add DNA-Lipid complex to cells
7. Visualize cells using microscope and analyze.

*At ATUM we typically use a 1:1 ratio of DNA:Lipofectamine

Puromycin Selection

1. Change media 48-72 hours post-transfection to complete growth media + selection drug (2-5µg/mL for puromycin selection)
2. Change media 48 hours post selection.
3. Harvest cells 1 day later.
4. Plate 1-3 cells per well in 96 well plates.
5. Select stable expressing clones.

Mammalian Expression Vector Controls

ATUM's mammalian expression vectors are available with Protein Paintbox genes to serve as controls. In addition, any mammalian-optimized Paintbox Protein gene in an Electra MOTHER vector can be cloned into any Electra DAUGHTER vector.

Electra Cloning System

Electra is a simple one-tube universal cloning process that can be performed in a 5 minute bench-top reaction with the fidelity of a restriction-based cloning system. A gene from one MOTHER vector is compatible with all DAUGHTER vectors, allowing rapid testing of many different sequence contexts simultaneously.

Reagents

The Electra Reagents kit contains all necessary components to facilitate cloning a gene from a MOTHER into a DAUGHTER vector. The Electra reaction can also be used to clone a PCR product into either a MOTHER or a DAUGHTER vector.

Electra Buffer Mix is supplied at 10X final concentration (use 2 µl in a 20 µl reaction)

Electra Enzyme Mix is supplied at 20X final concentration (use 1 µl in a 20 µl reaction)

Cloning Protocol

Component	Volume (µl)
MOTHER DNA / Positive control (20 ng)	1
DAUGHTER Vector (20 ng)	1
Electra Buffer (10x)	2
Electra Enzyme (20x)	1
Water	15
Total	**20**

1. Combine components and incubate at 25-37°C for 5-20 minutes.

2. Transform 1-2 µl into chemically competent E. coli. (DH10B cells recommended)

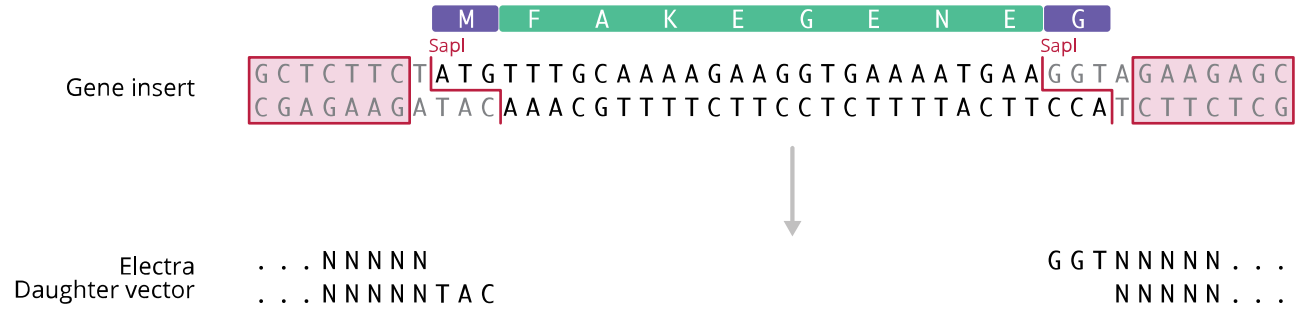
3. Recover cells for 45 minutes and then plate on appropriate antibiotic for the DAUGHTER.

3a. Optionally include streptomycin at 100 µg/ml (for selection against pMOTHER with rpsL); or plate on YEG with antibiotic plus p-chloro phenylalanine at 10mM (for selection against pMOTHER with pheS).

Positive Control

A positive control MOTHER vector carries a gene in which Ptet drives expression of green fluorescent protein (DasherGFP). A successful Electra reaction will produce green fluorescent colonies from the DAUGHTER vector.

Electra DAUGHTER Vectors



Electra DAUGHTER vectors are supplied as linearized DNA, with overhangs compatible with an ATG (encoding methionine) at the 5' end and GGT (encoding glycine) at the 3' end.

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

Genes in MOTHER vectors have adjacent restriction sites that produce overhangs compatible with an ATG at the 5' end and GGT at the 3' end upon digestion with SapI. Alternatively Electra ends can be added to any gene* by PCR. We recommend you add the following ends to your PCR primers:

5'-TACACGTACTTAGTCGCTGAAGCTCTTCTATG....(ORF)....-3'

5'-TAGGTACGAACTCGATTGACGGCTCTTCTACC....(ORF Reverse Complement)....-3'

*Your gene must not contain any internal SapI recognition sites, since the Electra cloning process utilizes the typeIIs enzyme SapI.

MOTHER vectors also contain a counter-selection gene. This can be used to eliminate any residual gene propagating in the MOTHER.

Feature list descriptions

E_CMV_IE1_Hs	The cytomegalovirus (CMV) enhancer element plays a critical role in overcoming inefficient transcriptional activities of promoters, thereby enhancing transcription. The hCMV IE1 enhancer/promoter is one of the strongest enhancer/promoters known and is active in a wide range of cell types. (www.link.springer.com/article/10.1007%2Fs11248-008-9235-y)
EES	DNA2.0's proprietary expression enhancement element
Intron_CMVa_IE1_Hs	The Intron A region from the hCMV IE1 enhancer/promoter has been shown to contain elements that enhance expression of heterologous proteins in mammalian cells. (www.ncbi.nlm.nih.gov/pmc/articles/PMC328492/)
Kanamycin-r	An effective bacteriocidal agent that inhibits ribosomal translocation thereby causing miscoding. The gene coding for kanamycin resistance is Neomycin phosphotransferase II (NPT II/Neo). *E.coli* transformed with plasmid containing the kanamycin resistance gene can grow on media containing 25 µg/ml kanamycin. Kanamycin is a white to off-white powder that is soluble in water (50mg/ml). (www.en.wikipedia.org/wiki/Kanamycin)
Ori_pUC	The origin of replication is a sequence in a genome at which replication is initiated. The pUC ori is a mutated form of origin derived from *E. coli* plasmid pBR322 which allows production of greater than 500 copies of plasmid per cell. (www.en.wikipedia.org/wiki/Origin_of_replication)
P_CMV_IE1_Hs	The CMV promoter is a constitutive mammalian promoter and mediates strong expression in various cellular systems. We have seen strong expression in HEK 293 and CHO cells. CMV mediates strong Cas9 transient expression compared to CAG or CBh promoters. CMV promoter mediated only transient expression in hESCs. CMV promoters have been reported to be prone to 'silencing' in some cell lines. (www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0010611)
P_PGK-Mm	The mouse phosphoglycerate kinase I promoter (PGK) is a weak constitutive promoter compared to CMV, EF1 alpha and SV40. (www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0010611)
P_syn_Ecoli	A synthetic promoter (Psyn) whose sequence is based on the consensus of a number of naturally occurring promoters and displays strong activity in *E.coli*. It has been shown to be stronger than the tac promoter in E.coli (www.sciencedirect.com/science/article/pii/037811199490197X)
pA_GH-Bt	The bovine growth hormone polyadenylation (bgh-PolyA) signal is a specialized termination sequence for protein expression in eukaryotic cells. (www.ncbi.nlm.nih.gov/pubmed/17407167)
pA_Globin-Hs	The human beta-globin polyadenylation signal is a strong signal that is required for transcription termination. (www.sciencedirect.com/science/article/pii/0092867487902923)
Puromycin-r	Resistance to puromycin is conferred by the Pac gene encoding a puromycin N-acetyl-transferase (PAC) that was found in a Streptomyces producer strain. It is used as a selective agent in cell culture systems and is toxic to prokaryotic and eukaryotic cells. It is poorly active on *E.coli*. Puromycin is soluble in water (50 mg/ml), is a colorless solution at 10 mg/ml and is stable for one year in solution when stored at -20°C. The recommended dose as a selection agent in cell cultures is within a range of 1-10 µg/ml, although it can be toxic to eukaryotic cells at concentrations as low as 1 µg/ml. Puromycin acts quickly and can kill up to 99% of nonresistant cells within 2 days. (www.en.wikipedia.org/wiki/Puromycin)
WPRE	A *cis*-acting element, the Woodchuck hepatitis post-transcriptional regulatory element (WPRE), contributes to higher transgene expression. (www.ncbi.nlm.nih.gov/pubmed/17597793)

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

Licenses

pD1300, 1400, 2100, 2500, 2600 & ATUM Proprietary Mammalian Expression Vectors - RESEARCH USE ONLY

Any product containing pD1300, pD1400, pD2100, pD2500, pD2600, pD3500, or pD3600-series Vectors (the "Licensed Vectors") (including Electra vectors, vector configurations for expression of multiple genes and other customized configurations of the Licensed Vectors, and ProteinPaintbox genes or CUSTOMER genes cloned into the Licensed Vectors) is subject to a limited, non-transferable license pursuant to which CUSTOMER acknowledges and agrees that the Licensed Vector may be used for internal research purposes only and may not be used for commercial purposes. For clarity, use for commercial purposes includes any use in manufacturing a product or service that is provided to a third party for consideration. In addition, CUSTOMER acknowledges and agrees that CUSTOMER and any Authorized Transferee (as defined in Section 11) may not (a) modify the Licensed Vectors in any way, including but not limited to replacing any protein-encoding sequence with any other protein-encoding sequence; (b) reverse-engineer, deconstruct, or disassemble the Licensed Vectors; (c) create any variant or derivative vector of the Licensed Vectors; or (d) transfer, disclose, or otherwise provide access to the Licensed Vectors (including sequences of same) to any third party other than an Authorized Transferee, and provided that any transfer to an Authorized Transferee must comply with the Product Transfer terms of Section 11.