

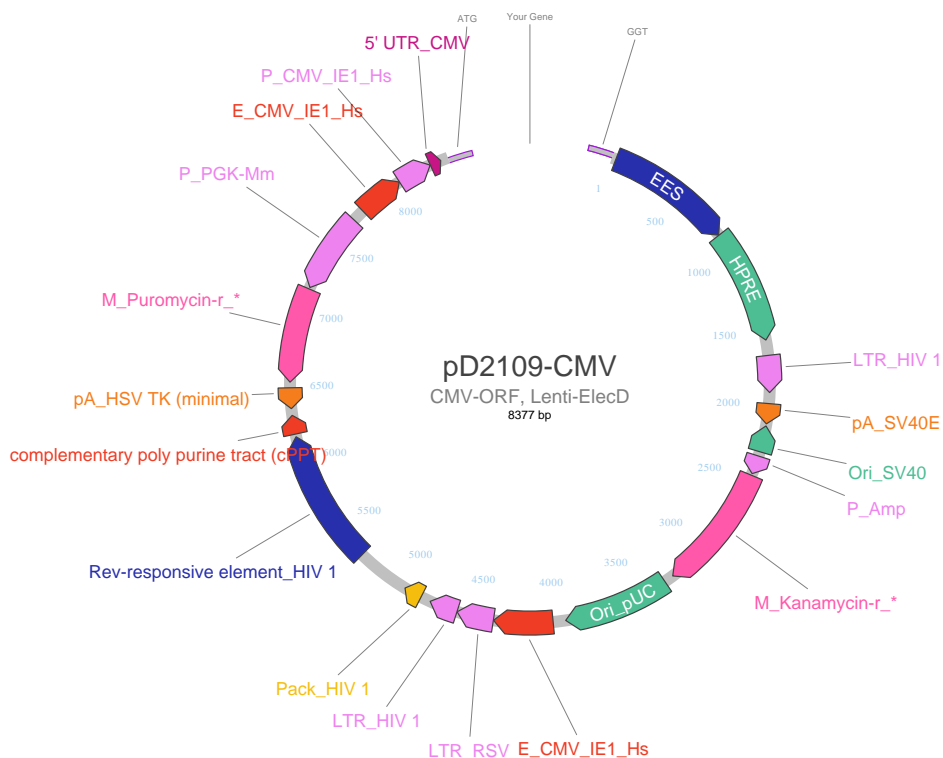
Mammalian Expression Vectors

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

Lentiviral Integration Vectors

These vectors are configured to maximize packaging efficiency and expression of the integrated construct. They are available with a choice of mammalian selectable markers and mammalian promoters.

Plasmid Map



Name	Qty	Storage
pD2109-CMV	10Rx	-20°C

Packaging Protocol

1. Plate 293T cells two days before packaging in a 10cm dish in 10mLs of DMEM supplemented with 10% heat inactivated fetal bovine serum so that cells are 70-90% confluent at time of transfection.
2. Prepare DNA/Lipofectamine Lenti complexes. DNA will include Lentiviral Expression Plasmid and Packaging Plasmids. (At ATUM we dilute our plasmid and lipofectmaine in Opti-MEM from LifeTech)
3. Add the DNA/Lipofectamine complex to each 10cm dish and gently swirl to distribute complex.
4. Incubate cells overnight (8-14 hours) in a CO2 incubator at 37°C.
5. Replace culture media (recommended within 14 hours of transfection).

48 hours post transfection harvest lentivirus: Collect culture media in sterile capped tubes. Centrifuge tubes at 500 x g for 10 minutes. After, filter the supernatant through 0.45µm PES (polyethersulfone) low protein-binding filters. (Peak virus production can occur 24-48 hours post transfection. Lentiviral stocks should be made and stored at 80°C. Expect loss of titer with each thaw and freeze cycle.)

Transduction Protocol

1. Plate cells to be 70-80% confluent at time of infection 24 hours prior to infection.
2. Dilute virus suspension in complete media with polybrene at a final concentration of 5-8 μ g/ml.
3. Remove the old culture medium and replace with diluted viral supernatant.
4. Incubate overnight.
5. Replace old medium with fresh complete medium.
6. Continue incubating for 48 hours in cell specific medium.
7. Analyze cells. (To elect stable cells, replace old medium with fresh complete growth medium containing appropriate selection drug every 72 hours until drug-resistant colonies become visible (generally 7-14 days post selection).

Puromycin Selection

1. Change media 48-72 hours post-transfection to complete growth media + selection drug (2-5 μ g/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.

Bacterial Propagation

E. coli lines transformed with the Lentiviral Vector should be grown at 30°C or lower to maintain plasmid stability.

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 μ l in a 20 μ l reaction)

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

Cloning Protocol

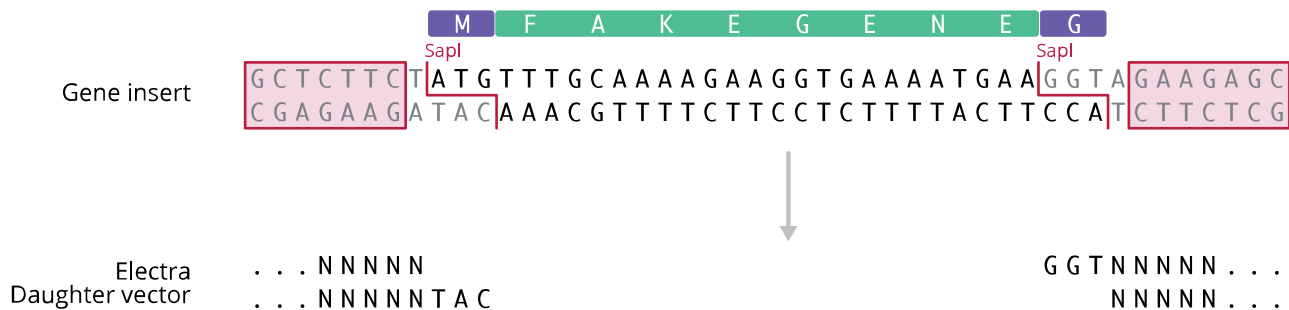
Component	Volume (μ l)
MOTHER DNA / Positive control (20 ng)	1
DAUGHTER Vector (20 ng)	1
Electra Buffer (10x)	2
Electra Enzyme (20x)	1
Water	15
Total	20

1. Combine components and incubate at 25-37°C for 5-20 minutes.
2. Transform 1-2 μ l 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 μ g/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

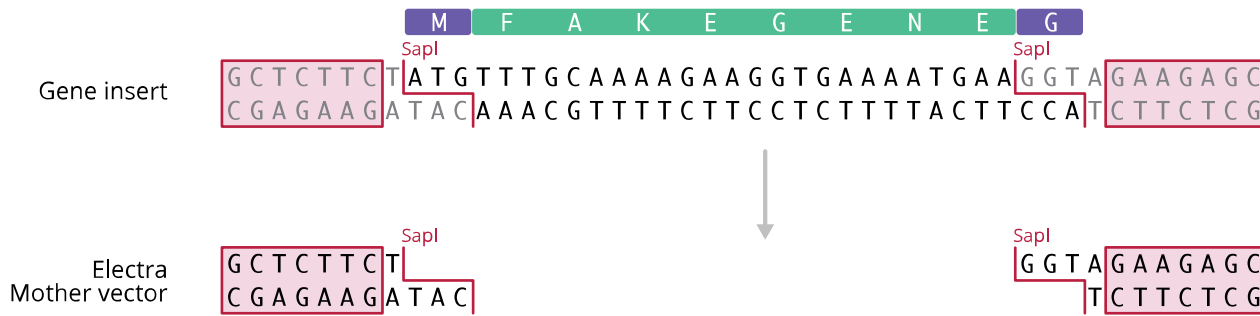
A positive control MOTHER vector carries a gene in which Ptet drives expression of green fluorescent protein (DasherGFP). A successful Electra reaction will produce green fluorescent colonies from the DAUGHTER vector.

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.

Electra MOTHER Vectors



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'-TACACGTA CTTAGTCGCTGAAGCTCTTCTATG....(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 typell's 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

complementary poly purine tract (cPPT)	A stretch of purine residues, the polypurine tract (PPT) is found in all retroviruses and is used to initiate plus-strand DNA synthesis. The sites of plus-strand and minus-strand initiation are important because they ultimately define the ends of the full-length proviral DNA, which are recognized by the viral integrase. Incorporation of a central polypurine tract (cPPT) into lentiviral vectors provides increased transduction efficiency and transgene expression. (www.jvi.asm.org/content/79/11/6859.full)
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
HPRE	A cis-acting sequence, the post-transcriptional regulatory element of hepatitis B virus (HPRE), which facilitates the cytoplasmic localization of intronless transcripts and contributes to higher gene expression in hepatitis B virus (HBV). (nar.oxfordjournals.org/content/26/21/4818.full)
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). <i>E.coli</i> 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)
LTR_RSV	Long terminal repeats (LTRs) are identical sequences of DNA that repeat hundreds or thousands of times. They are found at either end of retrotransposons or proviral DNA formed by reverse transcription of retroviral RNA. They are used by viruses to insert their genetic material into the host genomes. The Rous sarcoma virus (RSV) LTR contains a transcriptionally potent enhancer and promoter that functions in a variety of cell types. (www.pnas.org/content/79/22/6777.full.pdf)
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 <i>E. coli</i> 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)
pA_SV40E	The simian virus 40 early polyadenylation signal is an RNA element which promotes efficient polyadenylation resulting in high levels of steady-state mRNA. A poly(A) tail is added to an RNA at the end of transcription and protects the mRNA molecule from enzymatic degradation in the cytoplasm and aids in transcription termination, export of mRNA from the nucleus and translation. (www.ncbi.nlm.nih.gov/pubmed/2836265)
Pack_HIV 1	The RNA genome of the human immunodeficiency virus type-1 (HIV-1) contains a ~120 nucleotide Ψ-packaging signal that is recognized by the nucleocapsid (NC) domain of the Gag polyprotein during virus assembly. (www.sciencedirect.com/science/article/pii/S0022283600939792)
Puromycin-r	Resistance to puromycin is conferred by the Pac gene encoding a puromycin N-acetyl-transferase (PAC) that was found in a <i>Streptomyces</i> 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 <i>E.coli</i> . 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)
Rev-responsive element_HIV 1	The HIV-1 Rev response element (RRE) is a ~ 350 bp nucleotide, highly structured, cis-acting RNA element essential for viral replication. It was identified in the envelope gene (env) of the viral genome and is extremely well conserved across different HIV-1 isolates. (www.ncbi.nlm.nih.gov/pubmed/22258145)

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.