

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.

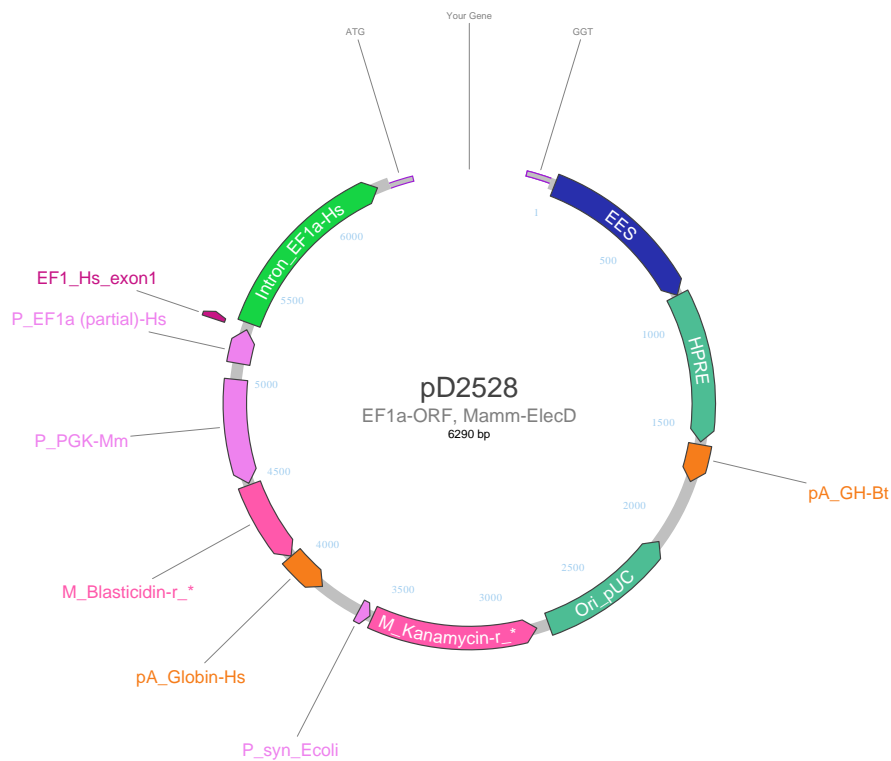
Mammalian Stable Expression Vectors

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
pD2528	10Rx	-20°C

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: $0.25-1.0 \times 10^6$, 24 well plate: $0.5-2.0 \times 10^5$, 96 well plate: $1-4 \times 10^4$)
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

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

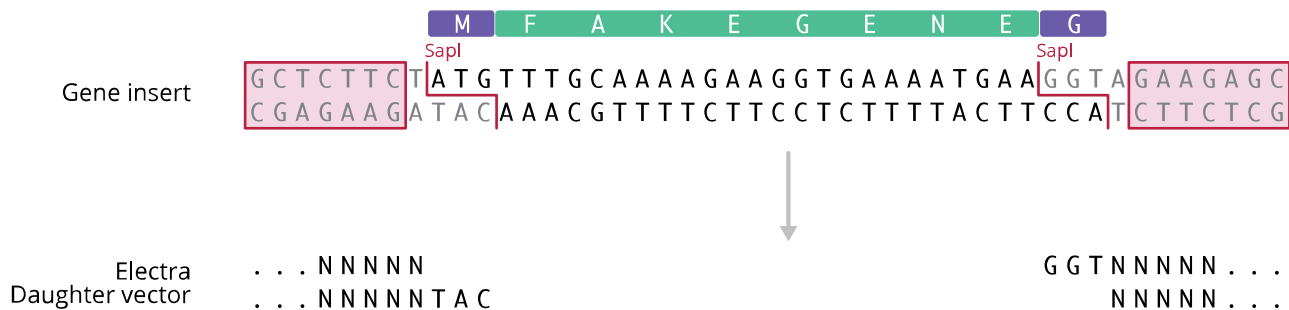
Component	Volume (μ l)
MOTHER DNA / Positive control (20 ng)	1
DAUGHTER Vector (20 ng)	1
Electra Buffer (10x)	2
Electra Enzyme (20x)	1
Water	15
Total	20

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 Sapl. 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 Sapl recognition sites, since the Electra cloning process utilizes the typells enzyme Sapl.

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

Feature list descriptions

Blasticidin-r	Blasticidin is a peptidyl nucleoside antibiotic that inhibits protein synthesis by interfering with the peptide bond formation in the ribosomal machinery. Resistance to blasticidin is conferred by the blasticidin resistance gene from <i>Bacillus cereus</i> (<i>bsr</i>) which codes for blasticidin S-deaminase. Cell death induced by blasticidin is rapid and cell lines carrying a blasticidin resistance gene can be obtained in less than a week. Typically, mammalian cells are sensitive to blasticidin concentrations of 1-10 µg/ml, and bacteria to 25-100 µg/ml. (www.ncbi.nlm.nih.gov/pubmed/1720391)
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)
Intron_EF1a-Hs	The human elongation factor one alpha (EF1 alpha) first intron comprises enhancer elements and has been shown to enhance expression of heterologous proteins in mammalian cells. (www.ncbi.nlm.nih.gov/pubmed/11738725)
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)
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_EF1a (partial)-Hs,P_EF1a_(min)-Hs	EF1 a promoter is a constitutive mammalian promoter used to drive ectopic gene expression in various in vitro and in vivo contexts. The EF-1 alpha promoter, which offers a broad host range, is derived from the human EF1A1 gene that expresses the alpha subunit of eukaryotic elongation factor 1. EF-1 alpha promoters allow robust, constitutive, long-term expression of your gene of interest in cell types in which CMV promoters are often silenced, such as hematopoietic and stem cells. The EF1 a promoter is shown to be superior to the CMV promoter in undifferentiated mouse, monkey and human ESCs. (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 <i>E.coli</i> . It has been shown to be stronger than the tac promoter in <i>E.coli</i> (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)

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.