

Mammalian Expression Vectors

DNA2.0 has mammalian expression vectors suitable for transient or stable expression. These vectors are available with features including various promoters, markers, and fusions.

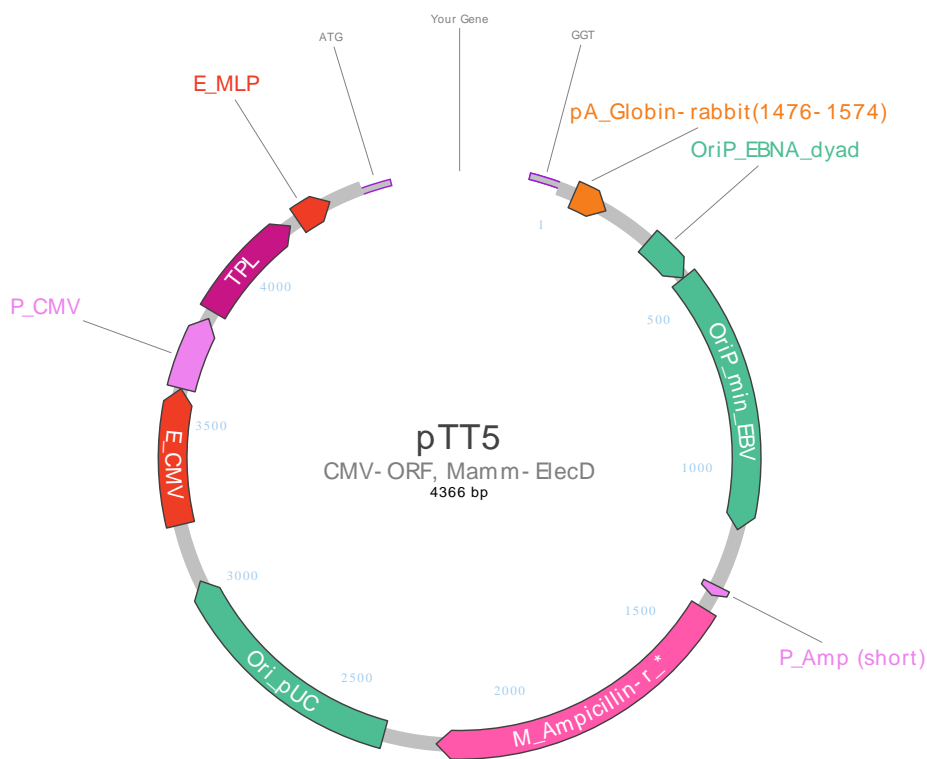
IP-Free© Mammalian Transient Expression Vectors

These vectors are primarily designed for high level protein expression in transiently transfected cells.

IP-Free© Mammalian Transient Expression Vectors: Vector GPS

Vector GPS was used to develop a panel of transient expression vectors containing various combinations of enhancers, promoters, introns, polyadenylation sequences and viral plasmid amplification systems. Custom combinations are also available. These vectors do not contain mammalian selectable markers to minimize size and maximize transfection efficiency.

Plasmid Map



Name	Qty	Storage
pTT5	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 DNA2.0 we typically use a 1:1 ratio of DNA:Lipofectamine

Mammalian Expression Vector Controls

DNA2.0'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

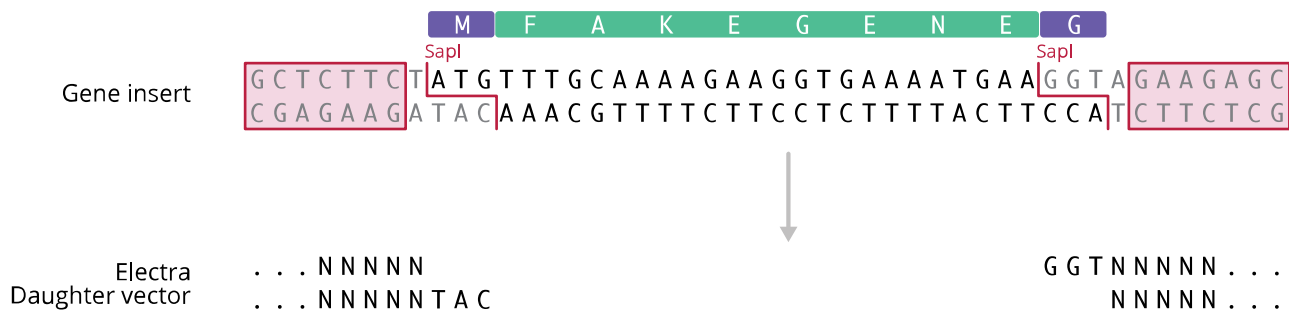
Component	Volume (μ l)
MOTHER DNA / Positive control (20 ng)	1
DAUGHTER Vector (20 ng)	1
Electra Buffer (10x)	2
Electra Enzyme (20x)	1
Water	15
Total	20

1. Combine components and incubate at 25-37°C for 5-20 minutes.
2. Transform 1-2 μ l into chemically competent E. coli.
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'-TAGGTACGA A C TCGATTGACGGCTCTTCTACC....(ORF Reverse Complement)....-3'

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

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

License

The Electra Vector System® is available for both R&D and commercial applications, and is IP-Free© with no licensing restrictions.

Feature list descriptions

Ampicillin-r	A semi-synthetic penicillin derived from 6-amino-penicillanic acid causes cell death by inhibiting cell wall biosynthesis. The gene coding for ampicillin resistance (<i>bla</i>) is a beta lactamase which is secreted into the periplasmic space where it catalyzes hydrolysis of the beta-lactam ring of ampicillin. <i>E.coli</i> transformed with plasmid containing the ampicillin resistance gene can grow on media containing 50-100 µg/ml ampicillin. (www.jac.oxfordjournals.org/content/43/5/699.full)
E_CMV	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)
E_MLP	An enhancer element from the adenovirus major late promoter (MLP) that enhances protein expression. (www.nar.oxfordjournals.org/content/30/2/e9/F2.expansion)
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)
TPL	The tripartite leader sequence (TPL) from Adenovirus is a 200 nucleotide 5' noncoding sequence carried on a majority of late viral mRNAs. While the leader sequence has no effect on translation of mRNA early in the infection, it significantly enhances efficiency of mRNA translation late in the infection. (http://www.pnas.org/content/81/12/3655.full.pdf)