ATUM

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.

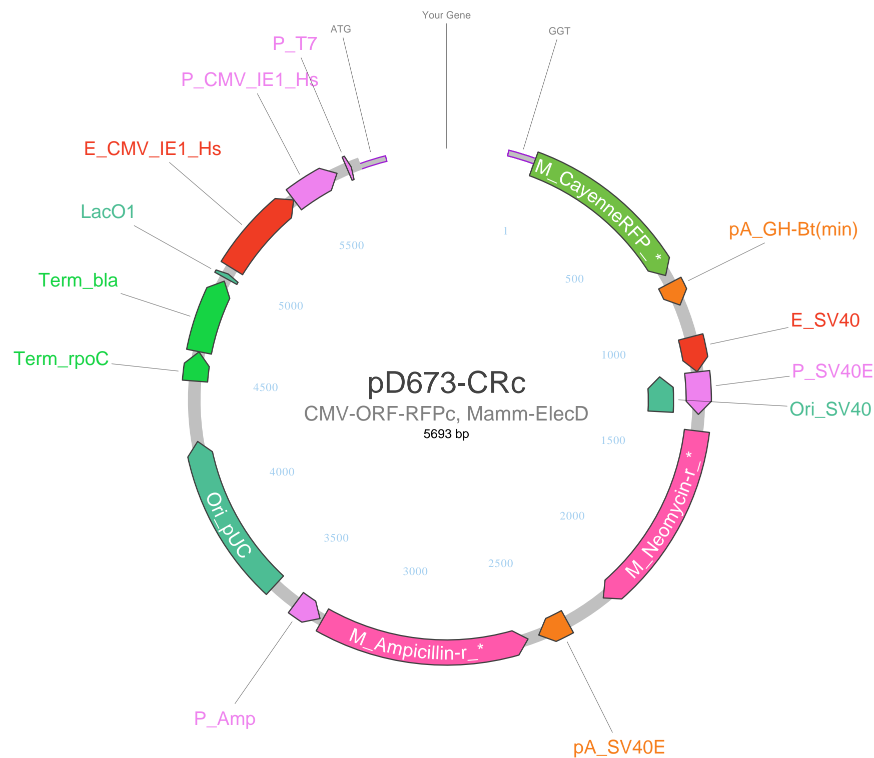
Mammalian Transient Expression Vectors

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

Mammalian Transient Expression Vectors: CMV Promoter

Vectors are available for fusion of your ORF to fluorescent proteins or localization signals. Vectors are also available with IRES or CHYSEL sequences for translational coupling of your ORF and a fluorescent protein to provide expression monitoring. Some vectors contain mammalian selectable markers for generating stable cell lines, though this is much more efficient with our Stable Expression Vectors.

Plasmid Map



Name	Qty	Storage
pD673-CRc	10Rx	-20℃

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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

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.

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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

Feature	Description
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 (*bla*) is a beta lactamase which is secreted into the periplasmic space where it catalyzes hydrolysis of the beta-lactam ring of ampicillin. *E.coli* 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)
CayenneRFP	IP-Free© red fluorescent reporter protein that is used as a selectable marker for expression monitoring of your protein. Ex/Em: 554/590 nm.
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)
E_SV40	The enhancer element from simian virus 40 (SV40) plays a critical role in overcoming inefficient transcriptional activities of promoters, thereby enhancing transcription. (www.sciencedirect.com/science/article/pii/1044577393800037)
LacO1	LacO is a regulatory gene of the lac operon. If lactose is missing from the growth medium, the repressor binds very tightly to a short DNA sequence just downstream of the promoter near the beginning of lacZ called the lac operator. The repressor binding to the operator interferes with binding of RNAP to the promoter, and therefore transcription occurs only at very low levels. When cells are grown in the presence of lactose, however, a lactose metabolite called allolactose, which is a combination of glucose and galactose, binds to the repressor, causing a change in its shape. Thus altered, the repressor is unable to bind to the operator, allowing RNAP to transcribe and thereby leading to higher levels of the encoded proteins. Silencing of the promoter prior to IPTG induction is achieved using symmetrical lac operators (Proc Natl Acad Sci USA 1983. 80:6785. Sadler et al) spaced around the promoter to maximize cooperativity (EMBO J 1994. 13:3348. Oehler et al). This operator pair ensures significantly tighter repression than regular lac operators. Overlapping T5 promoter/lac operator has been described (Proc Natl Acad Sci USA 1988. 85:8973. Lanzer and Bujard). (www.ncbi.nlm.nih.gov/pubmed/6316325)
Neomycin-r	Neomycin resistance is conferred by either one of two aminoglycoside phosphotransferase genes. A neo gene is included as a selectable marker and confers resistance to Neomycin and Kanamycin in prokaryotes, geneticin (G418) is needed for eukaryotes. Typically 10 µg/ml neomycin is used for bacterial cultures. (www.web.mnstate.edu/provost/Neomycin%20resistance%20background%20and%20data.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 *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_SV40E	The SV40 early promoter is a weak promoter from Simian Virus 40 (SV40) compared to the CMV promoter. The SV40 promoter works well in most cells but performs best in cell lines containing the stably integrated SV40 large T antigen, such as the African green monkey kidney COS cell lines. (www.ncbi.nlm.nih.gov/pubmed/6313230)
pA_GH-Bt(min)	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_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)