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.

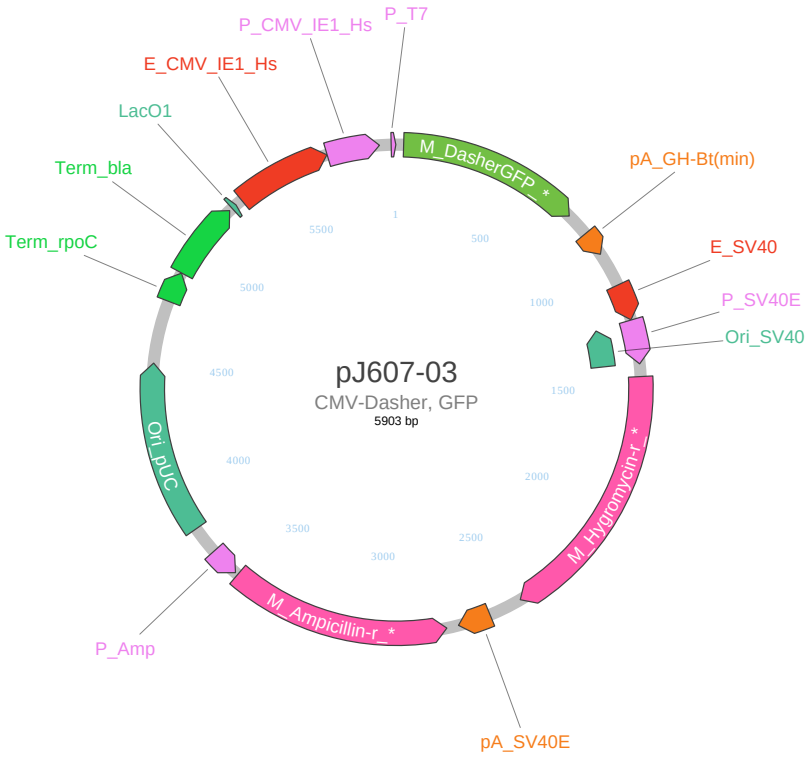
Mammalian Transient Expression Vectors

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
pJ607-03	2µg	−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.

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 (*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)
DasherGFP	IP-Free© green fluorescent reporter protein that is used as a selectable marker for expression monitoring of your protein. Ex/Em: 505/525 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)
Hygromycin-r	Hygromycin B is an aminoglycoside antibiotic and inhibits protein synthesis by interfering with translocation and causing mistranslation at the 70S ribosome. It is effective on most bacteria, fungi and higher eukaryotes. Resistance to hygromycin is conferred by hph gene from *E.coli*. Hygromycin B is normally used at a concentration of 50-200 µg/ml in mammalian cells and 100 µg/ml in bacteria. (www.sciencedirect.com/science/article/pii/0378111983902238)
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)
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)