E. coli Inducible Expression Vectors

E. coli expression vectors are available with the following promoters: T5 or T7 (IPTG-inducible), rhaBAD (rhamnose-inducible), ara (arabinose and IPTG-inducible) and phoA (induced by phosphate starvation). These vectors express in any strain of *E. coli*, except T7 promoter vectors which require a strain that expresses the T7 RNA polymerase and ara promoter vectors which require a strain that expresses the repressor AraC.

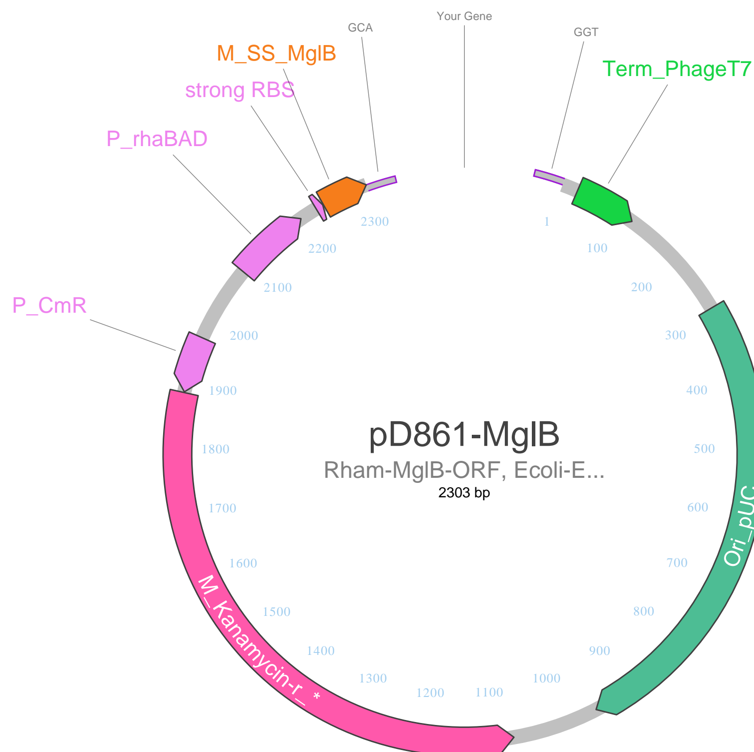
E. coli Secretion Signals

Recombinant protein production in the periplasm may simplify purification, avoid protease attack and improve correct protein folding. Different target proteins may prefer different secretion signals. Our *E. coli* expression vectors are available with a number of secretion signals to allow empirical selection of the best signal for a particular protein. The secretion signals offered include mal, gIII, ompA, pelB, phoA, ompC, ompT, dsbA, torT, sufI, torA, STII, EOX, lamb, MglB, SfmC, TolB and MmAp. These represent members of the post-translational (secB) and co-translational (SRP or TAT) pathways.

E. coli Expression Vectors with the Rhamnose-inducible rhaBAD Promoter

To vary expression levels, vectors with the rhaBAD promoter are available with different strength ribosome binding sites and a choice of high or low copy origins of replication. These vectors are also available with affinity tag fusions and with secretion signals to guide periplasmic expression. A choice of resistance markers is available.

Plasmid Map



Name	Qty	Storage
pD861-MglB	10Rx	-20℃

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

rhaBAD Induction Protocol

The rhaBAD promoter is tightly regulated and tunable. Protein expression levels within each cell can be increased by using higher rhamnose concentrations. This is in contrast to IPTG-inducible systems where a higher IPTG concentration increases the fraction of cells expressing protein rather than the amount produced by each cell. The rhaBAD promoter is compatible with any *E. coli* strain or other Gram-negative bacteria.

Grow cells overnight in LB plus antibiotic. Dilute into fresh LB with antibiotic, grow to mid-log (A600 0.6-0.8), induce by adding rhamnose to a final concentration between 25 µM and 4 mM, and grow for an additional 4-8 hours. Titration of rhamnose concentrations allows optimal conditions to be identified. This is particularly useful in the case of periplasmic expression because protein expression levels can be balanced with the secretion system.

Rhamnose Vector Controls

Vectors expressing KringleYFP are available as controls. Any *E. coli*-optimized Protein Paintbox gene in an Electra MOTHER vector can also be cloned into any Electra rhaBAD 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 Secretion DAUGHTER Vectors

Electra DAUGHTER vectors are supplied as linearized DNA, with overhangs compatible with an GCA (encoding alanine) at the 5' end and GGT (encoding glycine) at the 3' end.

Genes in MOTHER vectors have adjacent restriction sites that produce overhangs compatible with an GCA 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'-TACACGTACTTAGTCGCTGAAGCTCTTCTGCA....(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

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). *E.coli* 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 *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_rhaBAD	The rhamnose-inducible promoter rhaBAD is capable of high level recombinant protein expression in the presence of L-rhamnose and is tightly regulated by glucose in the absence of rhamnose. The rhaBAD promoter controls the genes rhaBAD organized in one operon. (www.wiley-vch.de/books/sample/3527327290_c01.pdf)
SS_MglB	A secretion signal that facilitates tranlocation of protein to the periplasm by the Sec pathway, where the sequence is removed by a signal peptidase. The secretion signal is derived from the methylgalactoside permease of *E.coli*. (http://www.ncbi.nlm.nih.gov/pubmed/6807987?dopt=Abstract)
strong RBS	A ribosome binding site (RBS) is a sequence on mRNA that is bound by the ribosome during protein translation. It can be either the 5' cap of a mRNA in eukaryotes, a region 6-7 nucleotides upstream of the start codon AUG in prokaryotes (called the Shine-Dalgarno sequence), or an internal ribosome entry site (IRES) in viruses. Prokaryotic ribosomes recognize RBSs primarily via base-pairing between the RBS and an unstructured end of the 16s rRNA molecule that forms part of the ribosome. Translation initiation rate of a particular mRNA can be regulated by sequence of the RBS, leading to varying strengths - strong, medium or weak. (www.msb.embopress.org/content/7/1/481.abstract)

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