IP-Free *E. coli* Expression Vectors with inducible promoters

IP-Free *E. coli* expression vectors are available with two different inducible promoters: an IPTG-inducible T5 promoter or a rhamnose-inducible rhaBAD promoter. All components are present on the vectors, so these vectors produce inducible expression in any strain of *E. coli.*

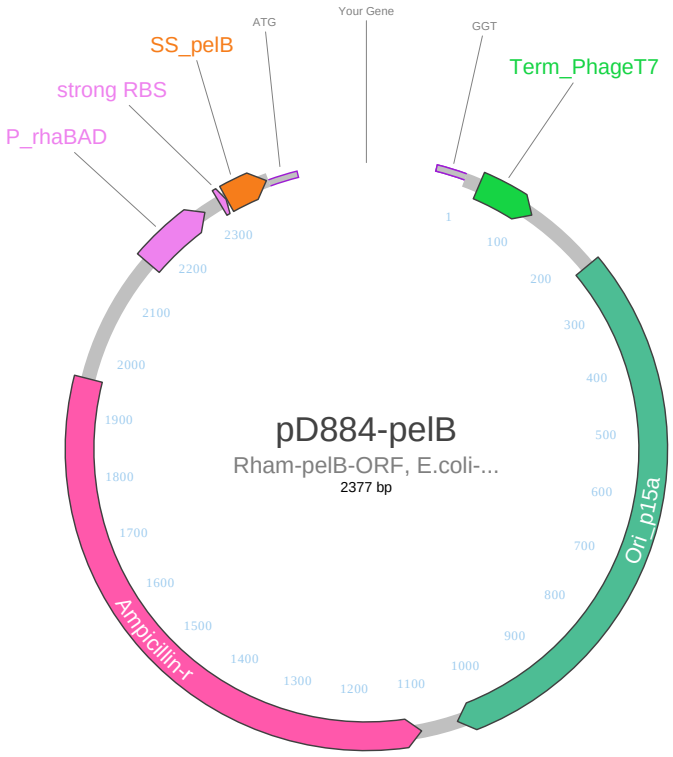
E. coli Secretion Signals

Producing recombinant proteins in the periplasm may simplify purification, avoid protease attack and improve correct protein folding. The optimal export mechanism is protein specific and unpredictable, so our *E. coli* expression vectors are available with different secretion signals to enable empirical selection of the translocation pathway best suited for a particular protein. Secretion signals offered include mal, gIII, ompA, pelB, phoA, ompC, ompT, dsbA, torT, sufI and torA, representing members of the post-translational (secB) and co-translational (SRP or TAT) pathways.

IP-Free *E. coli* Expression Vectors with rhamnose-inducible rhaBAD promoter

Expression Vectors with the rhamnose-inducible rhaBAD promoter are available with a choice of resistance markers and high, medium and low copy origins of replication. Different strength ribosome binding sites can be used to vary expression levels, a set of secretion signals can guide periplasmic expression.

Plasmid Map



Name	Qty	Storage
pD884-pelB	10Rx	−20℃

PrhaBAD Induction Protocol

The rhaBAD promoter is tightly regulated and tunable. Increasing rhamnose concentrations produce increasing protein expression levels within each cell. This is in contrast with IPTG-inducible systems in which increasing concentrations of inducer tends to increase the fraction of cells expressing protein rather than the mount 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 a further 4-8 hours. Titration of rhamnose concentrations allows optimal conditions to be identified. For example, in the case of expression into the periplasm, protein expression levels can be balanced with the secretion system.

Rhamnose Vector Controls

Vectors expressing KringleYFP are available as controls. In addition any *E. coli*-optimized Paintbox gene in an Electra MOTHER vector can be cloned into any Electra PrhaBAD 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.

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).

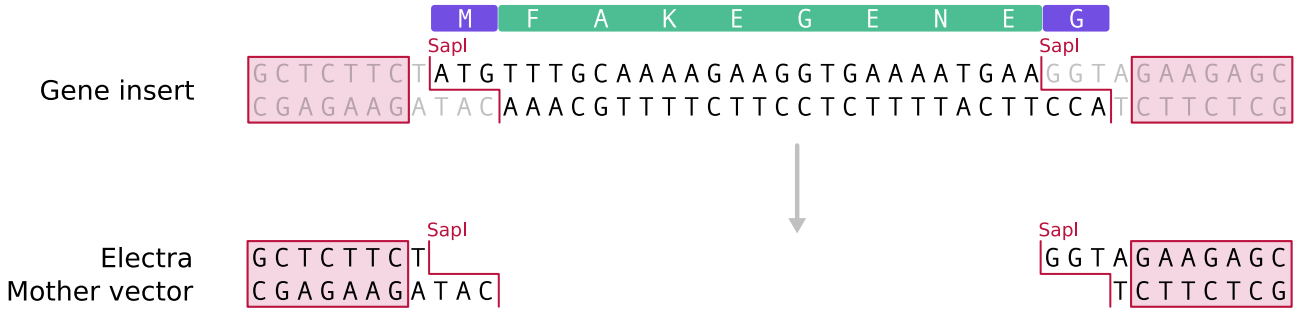
Positive Control

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.

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'-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.

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 (*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)
Ori_p15a	The origin of replication is a sequence in a genome at which replication is initiated. The p15a ori is a low copy ori producing 10-12 copies of plasmid per cell. (www.ncbi.nlm.nih.gov/pubmed/7557476)
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_pelB	A secretion signal which when attached to a protein directs the protein to the bacterial periplasm, where the sequence is removed by a signal peptidase. Specifically, pelB refers to pectate lyase B of *Erwinia carotovora*. (www.ncbi.nlm.nih.gov/pmc/articles/PMC213756/ www.aem.asm.org/content/73/3/906.full)
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)