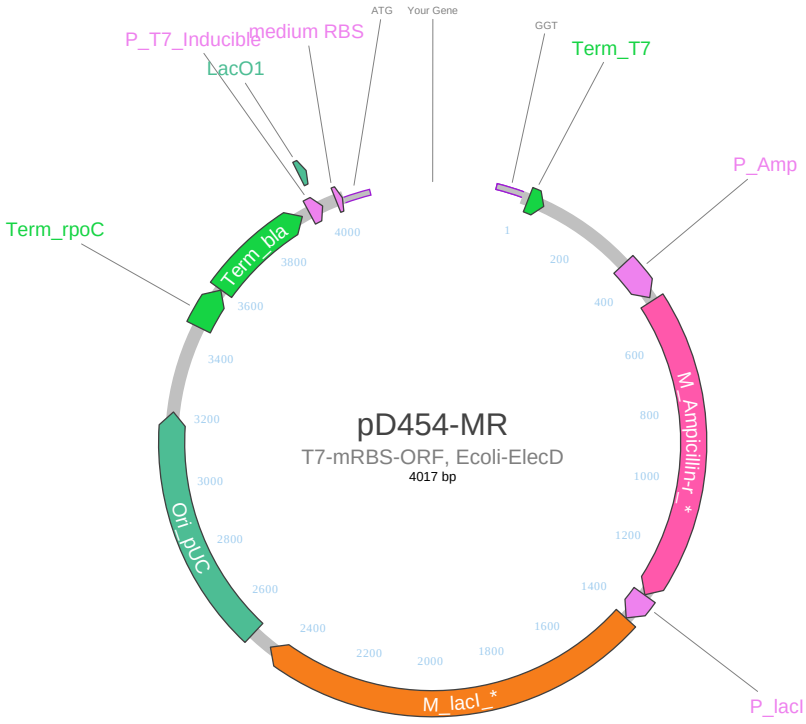
E. coli Inducible Expression Vectors

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 Expression Vectors with the IPTG-inducible T7 Promoter

To vary expression levels, vectors with the T7 promoter are available with different strength ribosome binding sites and a choice of high and low copy origins of replication. These vectors are also available with a choice of resistance markers.

Plasmid Map



Name	Qty	Storage
pD454-MR	10Rx	−20℃

T7 Induction Protocol

The IPTG-inducible T7 promoter works in *E. coli* strains BL21 (DE3) or T7 Express. The promoter is followed by lac operators that are recognized by the lac repressor, which is also carried on the plasmid. IPTG induces expression by binding to the repressor.

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 IPTG to 1 mM IPTG, and grow for an additional 4-8 hours.

T7 Vector Controls

Any *E. coli*-optimized Protein Paintbox gene in an Electra MOTHER vector can be cloned into any Electra T7 DAUGHTER vector.

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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)

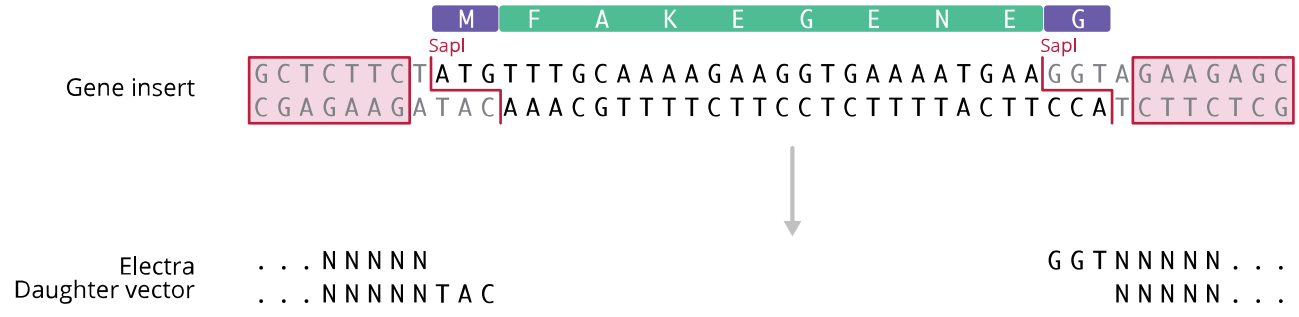
3. Recover cells for 45 minutes and then plate on appropriate antibiotic for the DAUGHTER.

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

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)
lacI,P_lacI	lacI is a regulatory gene of the lac operon that codes for the repressor that 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. (www.en.wikipedia.org/wiki/Lac_repressor)
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)
medium 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)
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_T7_Inducible	A phage T7 derived strong promoter which is recognized by T7 RNA polymerase. The promoter is controlled by a lac operator sequence that allows induction by addition of IPTG.

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