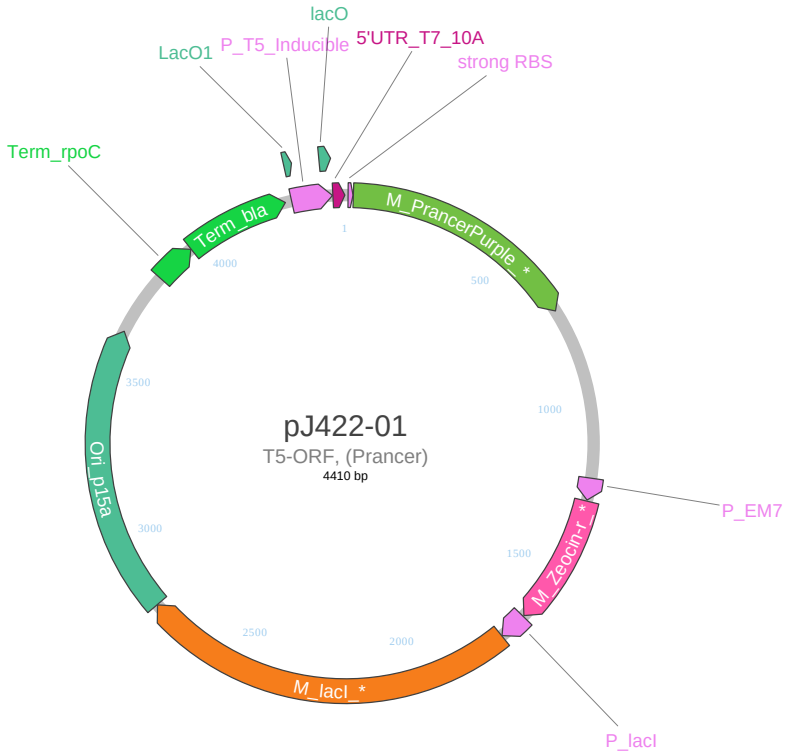
E. coli Inducible Expression Vectors

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 T5 Promoter

To vary expression levels, vectors with the T5 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 GFP or affinity tag fusions. A choice of resistance markers is available.

Plasmid Map



Name	Qty	Storage
pJ422-01	1µg	−20℃

T5 Induction Protocol

The IPTG-inducible T5 promoter works in any *E. coli* strain. The promoter is flanked by a pair of 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.

T5 Vector Controls

Fluorescent and chromogenic Protein Paintbox genes are available in T5 vectors to serve as controls. In addition, any *E. coli*-optimized Protein Paintbox gene in an Electra MOTHER vector can be cloned into any Electra T5 DAUGHTER vector.

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Feature list descriptions

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)
lacO,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_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_EM7	The EM7 promoter is a synthetic bacterial promoter derived from the T7 promoter that enables the constitutive expression of the antibiotic resistance gene in *E.coli*. (www.google.com/patents/US7244609)
P_T5_Inducible	A phage T5 derived promoter which is recognized by *E.coli* RNA polymerase. The promoter is controlled by two flanking lac operator sequences that allow induction by addition of IPTG. (www.wiley-vch.de/books/sample/3527327290_c01.pdf)
PrancerPurple	IP-Free© purple chromogenic reporter protein that is used as a selectable marker for expression monitoring of your protein.
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)
Zeocin-r	Resistance to zeocin is conferred by the product of the *Sh ble* gene. The *Sh ble* gene product binds the antibiotic so it can no longer cause cleavage of DNA. Zeocin is blue in color due to the presence of copper ion Cu^{2+}. The action of zeocin is effective on most aerobic cells. Typically 10-30 µg/ml is used in mammalian and yeast cells, and 25 µg/ml in bacteria. (www.en.wikipedia.org/wiki/Zeocin)