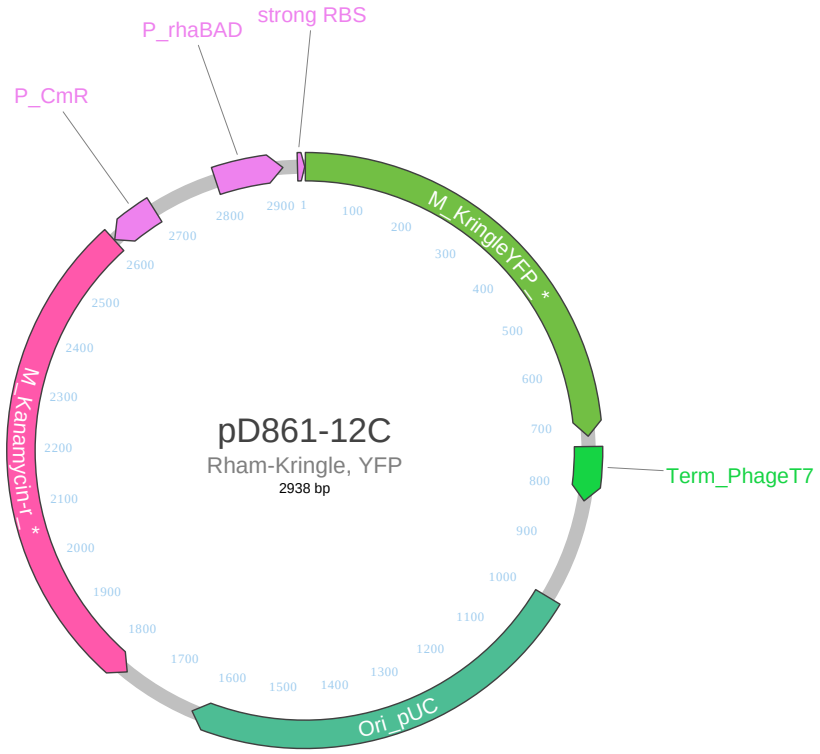
E. coli Inducible Expression Vectors

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 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
pD861-12C	2µg	−20℃

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.

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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.

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)
KringleYFP	Yellow fluorescent reporter protein that is used as a selectable marker for expression monitoring of your protein. Ex/Em: 520/542 nm.
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)
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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