

## ABSTRACT

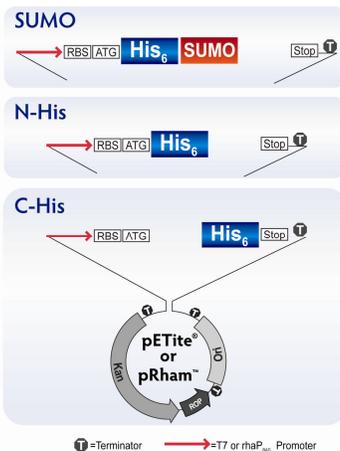
Lucigen has developed the Expresso Cloning and Expression Systems to simplify recombinant protein expression in *E. coli*. The Systems use a recombination-based PCR cloning strategy that is directional, eliminates the need for restriction digestion and ligation, and requires no PCR reaction clean-up or enzyme treatment. In the Expresso T7 System, pre-processed pETite® expression vectors allow one-step cloning of target gene PCR products under the control of a T7-lac promoter, with a choice of N-terminal or C-terminal 6x Histidine tag for purification. The new pETite N-His SUMO vector allows expression of SUMO-tagged proteins for enhanced solubility, and precise removal of the tag with SUMO protease. Cloning with the Expresso system is extremely simple, and suitable for high-throughput: PCR product containing 18 bp of complementarity to vector sequences at each end is simply mixed with the pETite vector preparation and transformed immediately into high-efficiency chemically-competent cells. There is no requirement for specific sequences at the vector/insertion junction, allowing complete freedom of fusion protein design. The small vector size (2.2 kb) facilitates downstream manipulation, such as site-directed mutagenesis. The new Expresso RhamnOS One-Strain Cloning and Expression System utilizes the versatile rhamnose-inducible rhaP<sub>BAD</sub> promoter. This promoter is transcribed by *E. coli* RNA polymerase, is tightly repressed in the absence of rhamnose, and can be induced to varying levels using different concentrations of rhamnose. This "tuning" capability can be exploited to maximize yield and soluble expression of difficult target proteins. The pRham™ vectors are configured in the same way as the pETite vectors, with a choice of N-or C-terminal His tags, or an amino-terminal N-His SUMO tag. With the RhamnOS System, clone construction and gene expression are carried out in a single host strain, allowing protein expression at least one day faster than with the two-host T7 system.

## Introduction

The Expresso Cloning and Expression Systems were developed to enable rapid, enzyme-free cloning and expression of proteins without sequence limitations. The original **Expresso T7 System** allows instant cloning of a target gene PCR product under the control of an inducible T7-lac promoter with a choice of amino- or carboxyl-terminal 6xHis tag, or with an amino-terminal 6xHis-SUMO tag to improve soluble expression. The new **Expresso RhamnOS One-Strain System** offers the same choice of tags, but features the rhamnose-inducible rhaP<sub>BAD</sub> promoter for tunable control of induction levels.

pETite and pRham vectors (Fig. 1) are small (2.2 - 2.6 kb) expression vectors built on Lucigen's transcription-free pSMART vector backbone. Their small size enables cloning of larger genes, and facilitates downstream manipulations such as site-directed mutagenesis.

Figure 1. pETite and pRham expression vectors.



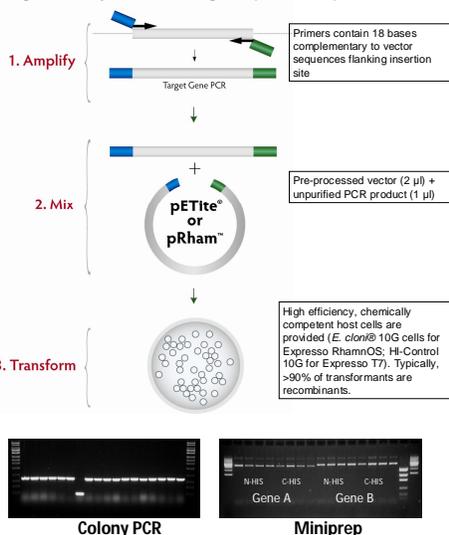
The pETite vectors have a T7-lac promoter for high-level expression of cloned genes. These vectors are designed for expression in Lucigen's Hi-Control™ BL21(DE3) cells, a host strain that expresses bacteriophage T7 RNA polymerase. Hi-Control BL21(DE3) cells contain an engineered lacI repressor gene to increase the level of repressor protein by ~200-fold. This level of repressor is sufficient to maintain control over basal expression of T7 RNAP in the host strain, and to ensure occupancy of the T7-lac promoter on the incoming expression vector. Hi-Control BL21(DE3) cells provide enhanced control over leaky target gene expression compared standard BL21(DE3).

The pRham vectors feature the rhaP<sub>BAD</sub> promoter, which is transcribed by *E. coli* RNA polymerase. This promoter is inducible by rhamnose in a concentration-dependent manner. In the absence of rhamnose, the promoter remains tightly shut off, and can be further controlled via catabolite repression in the presence of glucose. These features allow great flexibility in protein induction procedures, including tuning of expression levels for maximal yield of soluble protein, and auto-induction protocols requiring minimal intervention.

Table 1. Expresso System features

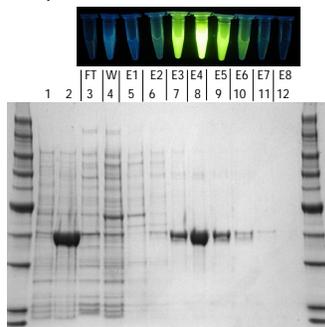
	Expresso T7	Expresso RhamnOS
Instant enzyme-free cloning of PCR products:	+	+
Choice of N-terminal or C-terminal 6xHis tag:	+	+
Available cleavable SUMO solubility tag:	+	+
Single host for cloning and expression:	-	+
Tunable induction from rhaP <sub>BAD</sub> promoter:	-	+
T7 promoter for maximal expression levels:	+	-

Figure 2. Enzyme-free cloning with pETite and pRham Vectors



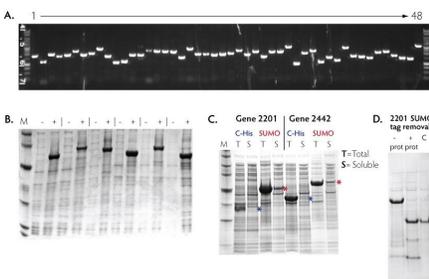
The high efficiency of the recombination-based cloning strategy means minimal screening is necessary to identify positive clones. Primers for clone validation by PCR and sequencing are provided in the Expresso kits. In the left panel above, colony PCR was performed on 18 candidate clones of a potentially toxic 0.55 kb gene in the pETite C-His Vector; all but one contained insert of the correct size. Sequencing verified that the remaining 17 clones were correct. In the right panel, 2 different genes were cloned into both the pETite N-His and pETite C-His vectors. Plasmid DNA was isolated by miniprep from four randomly-selected colonies of each transformation plate; all contained inserts of correct size.

Figure 3. Expression and purification of active soluble fluorescent protein



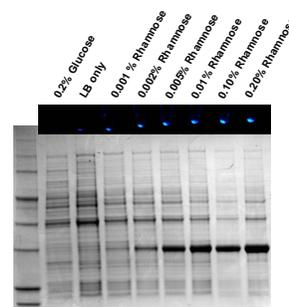
Hi-Control BL21(DE3) cells harboring pETite C-His vector containing a yellow fluorescent protein gene were grown at 37°C in LB media to an OD<sub>600</sub> 0.6 (lane 1), then induced for 4 hours with 1 mM IPTG (lane 2). Cells were harvested and lysed by sonication. Cleared lysate was loaded onto an Ni-NTA Sepharose® column. Column flowthrough (lane 3, FT) and wash (lane 4, W) fractions were collected. The bound YFP was eluted with wash buffer containing 300 mM imidazole (lanes 5-12, E1-E8)

Figure 4. Large-scale cloning and expression case study



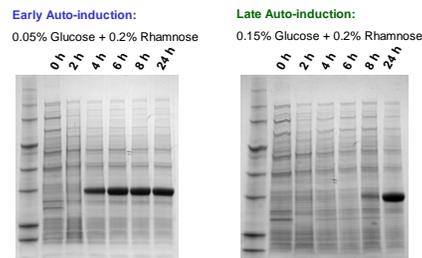
(A) PCR products from 48 putative hydrolase genes ranging from ~1 to > 3 kb. These PCR products were cloned into the pETite C-His vector. (B) Uninduced (-) and IPTG-induced (+) samples of Hi-Control BL21(DE3) Cells with 6 different genes cloned into the pETite C-His Vector. (C) Enhanced solubility of SUMO-tagged 2201 and 2442 gene products. Total cell extract and soluble fractions are shown. (D) Removal of 6xHis-SUMO tag from purified SUMO-2201 fusion protein by SUMO protease. -prot: uncleaved SUMO-2201 fusion protein after IMAC purification; +prot: SUMO protease-treated fusion protein; C: isolated 2201 protein after removal of 6xHis-SUMO fragment and SUMO protease by subtractive IMAC.

Figure 5. Tunable control of expression with RhamnOS System



A gene encoding a blue fluorescent protein was cloned into the pRham C-His vector. A culture was grown to an OD<sub>600</sub> of 0.8 in the absence of rhamnose, and then induced with rhamnose at the final concentration indicated. Samples containing equivalent OD<sub>600</sub> units were harvested and analyzed by SDS-PAGE. The capability of tuning the expression level may be beneficial for maximizing the yield of target protein, particularly in cases where the protein is toxic or insoluble when overexpressed to maximal levels.

Figure 6. Autoinduction with RhamnOS System



The rhaP<sub>BAD</sub> promoter is subject to catabolite repression. In the presence of the preferred carbon source glucose, the rhaP<sub>BAD</sub> promoter remains repressed even in the presence of rhamnose. This allows a convenient auto-induction strategy, in which media containing both 0.2% rhamnose and a limiting amount of glucose can be inoculated directly from a colony or from a saturated overnight culture. Upon depletion of glucose from the medium, the rhaP<sub>BAD</sub> promoter becomes active. The timing of induction can be adjusted by using different amounts of glucose. For potentially toxic gene products, late autoinduction may be advantageous for maximal yields.

## Conclusion

The Expresso cloning and expression systems have been designed for simple and rapid cloning and expression of target genes with a minimum of steps. PCR products are cloned directly into pre-processed pETite and pRham vectors using a recombinational cloning strategy that requires no restriction digests, ligase, or other enzymatic treatments. The Expresso T7 system allows maximal expression from the bacteriophage T7 promoter. The Expresso RhamnOS System uses a single host strain for both cloning and expression, and allows tunable control of induction. Both systems offer the option of fusion to a cleavable N-terminal SUMO tag for enhanced soluble protein expression.

Expresso System kits are complete with pre-processed vectors, chemically competent cells for clone construction and expression, primers for clone characterization, and control inserts. Expresso SUMO kits also include SUMOExpress Protease for precise removal of the SUMO tag. Expected availability of Expresso RhamnOS kits is mid-February 2011.