Expresso® Cloning and Expression Systems: Expressioneering™ Technology streamlines recombinant protein expression.

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The Expresso® Cloning and Expression Systems use Expressioneering™ Technology to dramatically increase the speed and efficiency of target gene cloning and soluble protein expression in *E. coli.* With Expressioneering, PCR products are cloned instantly and directionally into pre-processed expression vectors without sample clean-up or enzyme treatment. The Expresso Rhamnose System is a single-host system ideal for high throughput, allowing cloning in an effortless afternoon, and recombinant protein expression the next day.

Lucigen has developed the Expresso Cloning and Expression Systems to meet the increasing demand for fast and efficient cloning and expression methods for recombinant protein production. The Expresso kits use an *in vivo* recombinational cloning strategy called Expressioneering Technology (Fig.1). Expressioneering resembles ligase-independent cloning (LIC), in that it depends on the addition of short sequences at both ends of a target gene by PCR. These added sequences provide complementarity to the ends of the vector flanking the cloning site, enabling recombination *in vivo*. However, Expressioneering eliminates the requirement inherent to LIC methodology for generation of single-stranded DNA ends using the exonuclease activity of T4 DNA polymerase *in vitro*.

To clone by Expressioneering, an aliquot (typically 1 µl) of unpurified PCR product is mixed with the pre-processed Expresso Vector and immediately transformed into the Chemically Competent cells provided. Recombination between the vector and PCR product within the host cells precisely fuses the target gene to the vector in the proper orientation. The design and preparation of the Expresso System Vectors ensure minimal background transformation with non-recombinant clones. For most genes, more than 90% of colonies will have the target gene correctly inserted into the vector.

Elimination of the requirement for PCR product cleanup and enzyme treatment not only saves the cost of enzymes and multiple incubation and sample handling steps, but also simplifies the design of expression clones. Seamless fusion to the vector eliminates undesirable amino acids encoded by restriction sites or site-specific recombination sites. Endpoints of the target protein can be selected at will. Additional sequences, such as short fusion tags or protease cleavage sites, can be introduced via primer design as desired.

Expresso kits are available with pre-processed vectors containing either of two inducible promoters (T7 or Rhamnose),

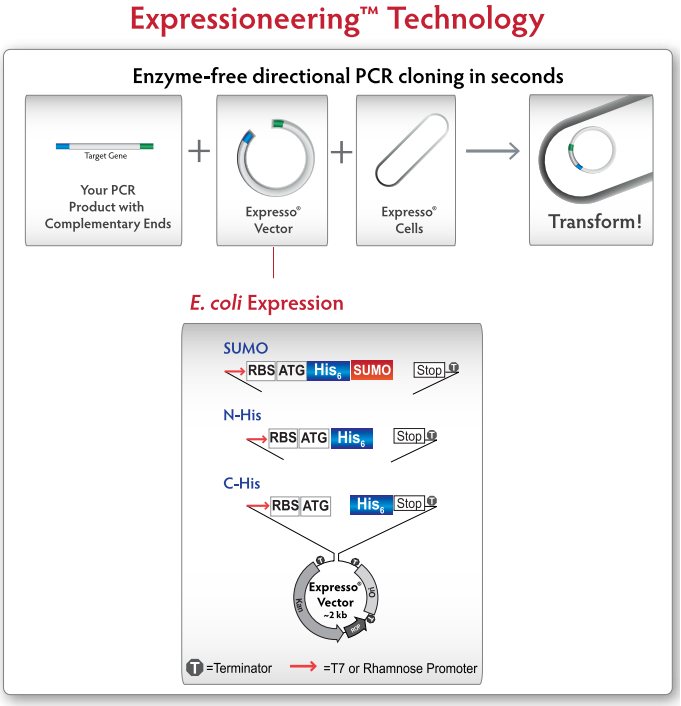

Fig 1. Expressioneering Technology for efficient, high-speed cloning and protein expression. PCR amplified product of the target gene containing 18-bp overlap with the vector ends is mixed with the appropriate Expresso vector and *E. cloni®* 10G cells. Heat shock transformation followed by selection of transformants on kanamycin agar plates yields more than 90% correct recombinant clones.

and in several different configurations for expression of target proteins with convenient fusion tags for enhanced expression and purification (Fig. 1). Currently available Expresso System vectors include sequences encoding either amino- or carboxyl-terminal 6x His tags, enabling convenient target protein purification by immobilized metal-affinity chromatography (IMAC). The Expresso SUMO vectors allow fusion to an amino-terminal, cleavable 6xHis-SUMO tag for enhanced soluble expression of difficult target proteins.

The Expresso T7 kits include pETite® vectors, which contain the bacteriophage T7 promoter under the control of lac operators. The T7 kits are provided with two types of competent cells: HI-Control™ 10G cells for stable clone construction, and HI-Control BL21(DE3) cells for expression from the T7 promoter. Confirmed clones constructed in HI-Control 10G Cells are transferred to HI-Control BL21(DE3) Cells for protein expression. The HI-Control strains feature a specially engineered lacI allele that expresses ~200-fold more lac repressor protein than native chromosomal lacI, providing enhanced control over leaky expression common in other T7 systems. The pETite T7 vectors do not carry the lacI gene, and at 2.2-2.5 kb are much smaller than typical pET vectors (~5.5 kb). The reduced vector size improves cloning efficiency, accomodates larger genes or operons, and simplifies downstream manipulations such as site-directed mutagenesis.

The Expresso Rhamnose System uses a single host strain for stable clone construction and protein expression. The Expresso Rhamnose kits include pRham™ vectors, which have the same design as the pETite vectors but feature the rhamnose-inducible $rhaP_{BAD}$ promoter. This highly versatile promoter, which is recognized by the *E. coli* RNA polymerase, is inducible to a variable degree in the presence of the sugar L-rhamnose in the range of 0.001% to 0.2% (Figure 2A). In the absence of rhamnose, the $rhaP_{BAD}$ promoter has very low activity, allowing stable clone construction. Expression from the $rhaP_{BAD}$ promoter is typically somewhat lower than from the T7 promoter. For some aggregation-prone proteins, this lower rate of expression may actually favor correct folding. We have obtained yields of recombinant protein as high as 100 mg/liter using the Expresso Rhamnose System.

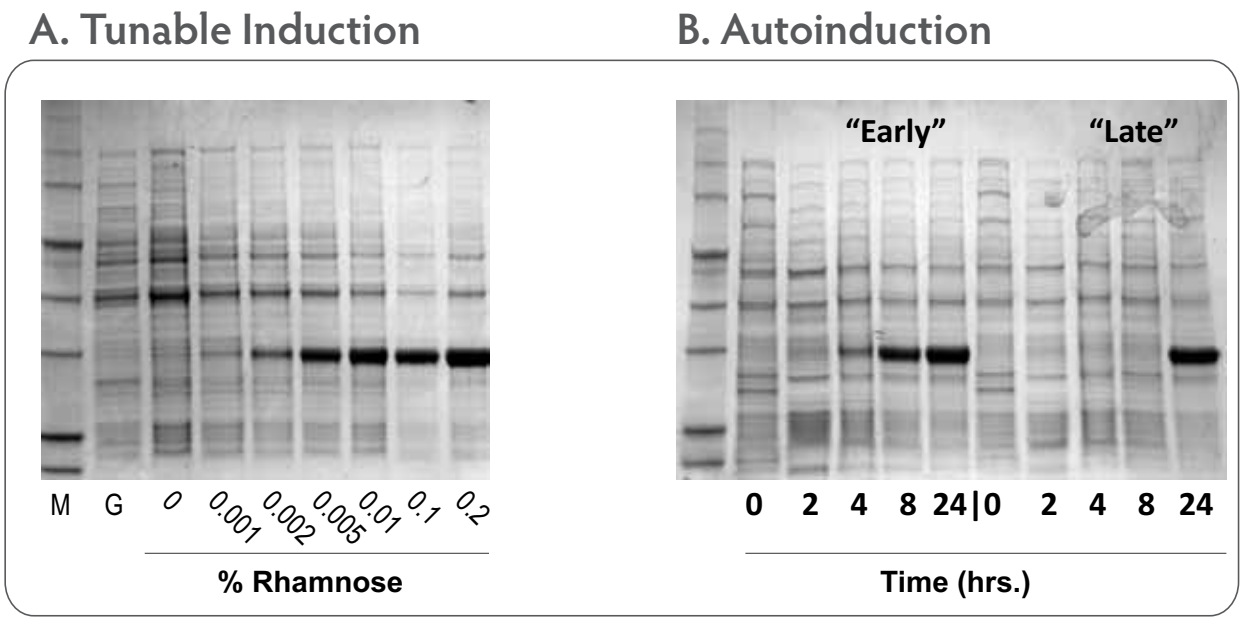

Figure 2. Flexible induction strategies with the Expresso Rhamnose System. (A) Tunable control of induction with increasing rhamnose concentration. M = Mol. Wt. markers; G = 2% glucose. **(B)** Autoinduction of protein expression using media containing 0.2% rhamnose and either 0.05% glucose (early) or 0.15% glucose (late). All cultures were grown in LB media at 37°C, with 30 µg/ml kanamycin and the indicated sugars. Samples containing equivalent OD_{600} units were lysed in SDS gel loading buffer. The induced protein in both panels is a blue fluorescent protein (BFP). The BFP gene is included as a control for cloning and expression in the Expresso kits.

The use of a single host for both cloning and expression gives the Expresso Rhamnose System a major time advantage. This advantage can be maximized through a convenient autoinduction procedure, which uses media containing both rhamnose and a limiting concentration of glucose (Fig. 2B). When both sugars are present, glucose is metabolized preferentially, supporting culture growth while the rhamnose promoter remains repressed. Upon glucose depletion, cells begin to metabolize rhamnose and expression from the rhamnose promoter is activated. Timing of induction can be adjusted by varying the concentration of glucose. Colonies from the initial clone construction plate can be inoculated directly into autoinduction media, with no further intervention necessary until samples are harvested for evaluation of recombinant protein expression. Thus, cloning can be performed in an afternoon, and candidates can be evaluated for expression using autoinduction media the next day.

The Expresso SUMO kits are designed to enhance the soluble expression of target proteins that are otherwise poorly expressed or insoluble. SUMO (small ubiquitin-like modifier) is a 100 amino acid polypeptide derived from the yeast SMT3 gene that has been shown to improve the soluble yield of a variety of otherwise poorly soluble proteins. The SUMO tag can be removed from the target protein by SUMO Express protease, which cleaves precisely at the carboxyl terminus of SUMO, leaving no extraneous residues attached to the target protein. Figure 3 presents examples of two hydrolase enzymes that were produced in an insoluble form when expressed with a C-terminal 6X-His tag using the pETite C-His Vector, but with improved solubility using the pETite SUMO vector. Although tag removal was not necessary for hydrolase activity, the tag could be removed efficiently by SUMO Express protease.

The extreme simplicity of the Expressioneering Technology embodied in the Expresso Cloning and Expression Systems is well-suited to high-throughput cloning and expression studies. The Expresso Rhamnose System in particular is a single-host system with tunability and convenient autoinduction protocols, making it highly amenable to automation for high-throughput cloning and protein expression. The convenience of pre-processed vector, elimination of multiple enzyme treatment and clean-up steps, and improved control over leaky expression should also prove beneficial to researchers studying individual proteins.

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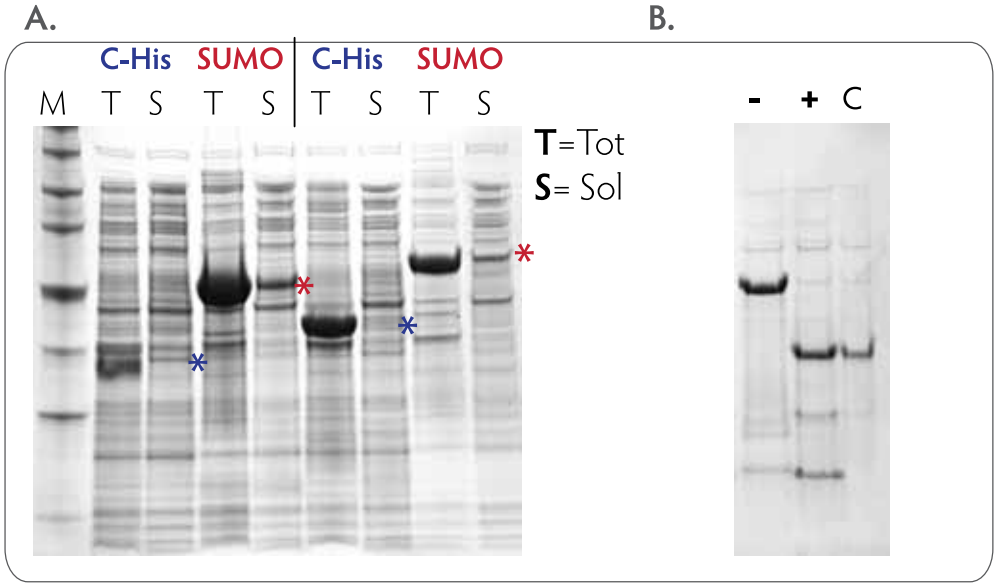

Figure 3. Enhanced soluble protein expression with cleavable SUMO tag. (A) Enhanced solubility of SUMO-tagged gene products. Total cell extract and soluble fractions are shown. (B) Removal of 6xHis-SUMO tag from purified SUMO- fusion protein by SUMO protease. Lanes are: –, uncleaved SUMO-fusion protein after IMAC purification; +, SUMO protease-treated fusion protein; C, isolated recombinant protein after removal of 6xHis-SUMO fragment and SUMO protease by subtractive IMAC.