

## Enzyme-free directional cloning of PCR amplicons in seconds.

- **Ready-to-use vectors and competent cells - NO ligation step!**
- **High efficiency:** >90% recombinants.
- **Tightly-controlled expression** of 6xHis-tagged proteins.
- Systems available with or without **SUMO solubility tag**.
- **Save time!** Avoid days of vector and cell preparation time.

The Expresso T7 Cloning and Protein Expression Systems are designed for fast, easy, and efficient directional cloning and expression of PCR-amplified genes. The Systems are complete with pre-processed pETite™ T7 cloning vectors, and two competent cell lines, supplied in single transformation vials. High efficiency HI-Control™ 10G Chemically Competent Cells enable stable cloning and HI-Control BL21(DE3) Competent Cells provide tightly controlled protein expression, thus helping you avoid expression problems seen with leaky T7 promoter-based systems.

For proteins that are difficult to express in soluble form, the **new pETite N-His SUMO Vector** allows expression of target proteins with an amino-terminal 6xHis-SUMO fusion tag. SUMO (small ubiquitin-like modifier) is a relatively small (100-residue) polypeptide that has been shown to enhance the soluble expression of many proteins that are otherwise difficult to produce in *E. coli*.

### Five-second directional cloning of PCR-amplified genes.

The Expresso T7 Cloning and Protein Expression System uses an **enzyme-free recombinational cloning strategy**. The target gene is amplified by PCR using primers that add ~18 base-pairs of vector-complementary sequence to both ends of the gene. Unlike other ligase-free cloning methods, no further cleanup or enzymatic treatment of the PCR product is necessary. Simply mix 1 µl of the PCR reaction with the supplied pre-processed pETite™ T7 expression vector, and transform immediately into the HI-Control™ 10G Chemically Competent Cells provided (Figure 1).

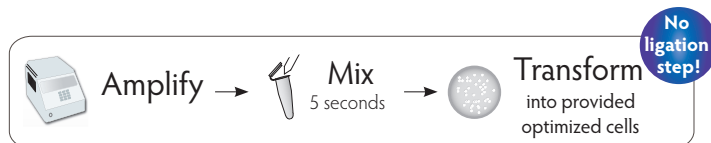


Figure 1. Expresso™ T7 Cloning and Expression Systems

### Features of the pETite T7 vectors include:

- Pre-processed, linearized vectors are supplied ready for cloning.
- Patented CloneSmart® technology increases cloning efficiency.
- Strong T7 promoter for high-level expression.
- Choice of N-terminal 6xHis, C-terminal 6xHis or N-terminal 6xHis SUMO fusion tags for fast protein purification.
- pETite T7 SUMO vector expresses a cleavable SUMO solubility tag.
- Small size (2.2kb-2.5kb) for easier downstream manipulation.

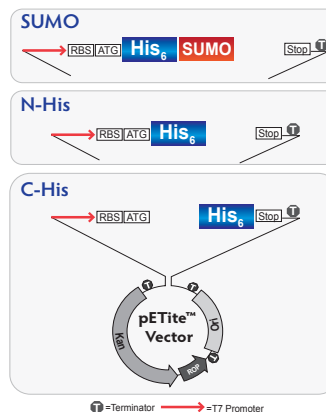
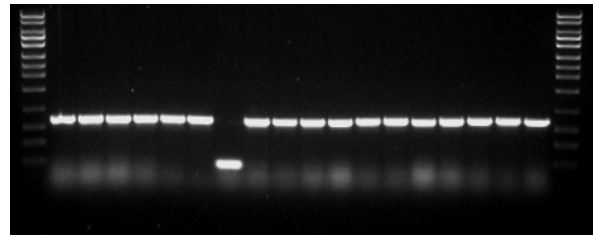


Figure 2. Enzyme-free cloning with pETite Vectors

The pETite Vectors include a choice of N-terminal or C-terminal 6xHis tags, or an N-terminal 6xHis-SUMO tag for enhanced soluble expression. Kan: kanamycin resistance gene; Ori: origin of replication; ROP: repressor of primer (control of copy #); RBS: Ribosome binding site. Translational start (ATG) and stop codons are included in the vectors.

## High cloning efficiency with HI-Control™ 10G Chemically Competent Cells.

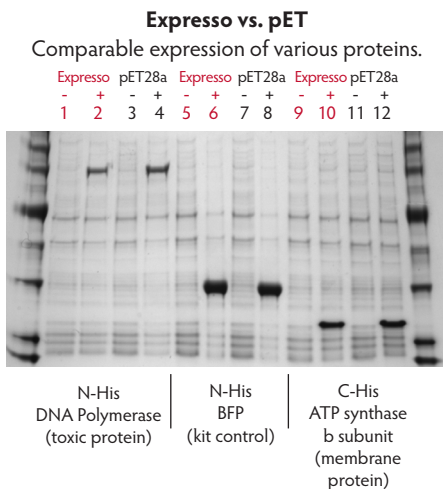
The high transformation efficiency of HI-Control 10G Chemically Competent Cells ensures recovery of clones with precise junctions and the correct orientation. For most genes, > 90% of colonies will have the target gene inserted in the correct orientation. (Figure 3).



**Figure 3. High Cloning Efficiency:**  
Pre-processed pETite C-His vector was mixed with 1 µl of unpurified PCR product and transformed into HI-Control 10G Chemically Competent Cells. Colony PCR was performed on 18 randomly chosen colonies; 17 of 18 contained insert of correct size.

## HI-Control BL21(DE3) Cells control leaky protein expression

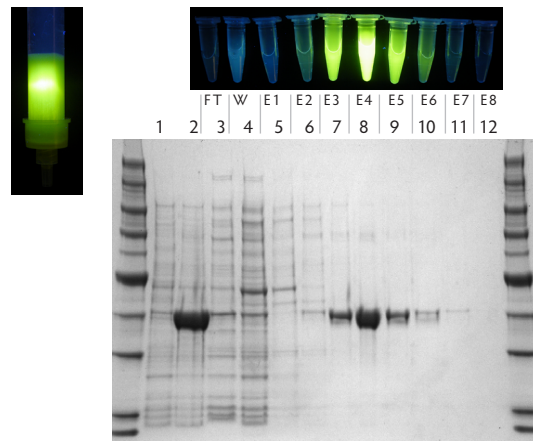
HI-Control BL21(DE3) Cells contain high levels of lac repressor to maintain tight control over expression of T7 RNA polymerase. Tighter control means better tolerance of potentially toxic gene products (Figure 4).



**Figure 4. Comparable expression of various proteins.**

Genes encoding a DNA polymerase, a blue fluorescent protein (BFP), or ATP synthase b subunit were cloned into pET28a or pETite vectors with N-terminal or C-terminal 6x His tags (as indicated). pET28a clones were transformed into standard BL21(DE3) cells, and pETite clones were transformed into HI-Control BL21(DE3) cells for expression. Cultures were grown in LB at 37° to an OD<sub>600</sub> of 0.5 to 0.7 (odd-numbered lanes) and induced for 3 hours with 1 mM IPTG (even-numbered lanes). Cells were pelleted and lysed directly in SDS-PAGE loading buffer, and 0.05 OD equivalents were analyzed by gel electrophoresis. The gel was stained with Coomassie blue.

## Expression and purification of active soluble fluorescent protein.



**Figure 5. Purification of Histidine-tagged proteins:**

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> of 0.6 (lane 1), then induced with 1 mM IPTG for 4 hours (lane 2). Cells were harvested and lysed by sonication in 300 mM NaCl, 50 mM Tris-HCl pH 8.0. Cleared lysate was loaded onto an Ni-NTA Sepharose® column. Column flow-through (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).

## Ordering Information :

The Expresso T7 Cloning and Expression System contains pre-processed pETite™ N-His and/or pETite C-His Vector DNA, HI-Control™ 10G Chemically Competent Cells for cloning, and HI-Control BL21(DE3) Chemically Competent Cells for protein expression. Also included are N-His and/or C-His Positive Control Insert DNAs, and transformation positive control pUC DNA.

The Expresso T7 SUMO Cloning and Expression System contains pre-processed pETite™ N-His SUMO Vector DNA, HI-Control™ 10G Chemically Competent Cells for cloning, and HI Control BL21(DE3) Chemically Competent Cells for protein expression. Also included are SUMO Positive Control C Insert DNA, transformation positive control pUC DNA, SUMO Express Protease, SUMO Cleavage Control Protein, and forward and reverse PCR primers to confirm clones.

HI-Control Cells, SUMO Express Protease, and SUMO Cleavage Control Protein are also available separately.

Description	Size	Cat. No.	Price
Expresso™ T7 Cloning and Expression System, N-His	5 rxn	49001-1	\$179
	10 rxn	49001-2	\$329
Expresso T7 Cloning and Expression System, C-His	5 rxn	49002-1	\$179
	10 rxn	49002-2	\$329
Expresso T7 Cloning and Expression System, N/C-His Combo, 5 of each of N-His and C-His	10 rxn	49000-1	\$329
Expresso T7 SUMO Cloning and Expression System	5 rxns	49003-1	\$249
	10 rxns	49003-2	\$445

Visit [lucigen.com](http://lucigen.com) to see how Expresso T7 SUMO can help you express soluble proteins with a cleavable solubility tag.

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