1. Introduction

The promoter of the E. coli trp operon has proven itself as a workhorse for the production of hundreds of proteins from small scale to pharmaceutical production levels (1). The trp promoter is strong, easily regulated, and well characterized. Transcription of a cloned gene from a trp promoter on a plasmid increases about 50-fold upon induction and the gene product can amount to 30% of the total cell protein (2–4).

Transcription from the trp promoter is controlled by the level of free tryptophan in the cell. When grown in rich media, the dimeric Trp aporepressor is bound to two molecules of L-tryptophan forming an active repressor complex that competes with RNA polymerase for binding to the trp promoter/operator. The inducer 3-β-indoleacrylic acid (IAA) binds 30-fold more tightly than L-tryptophan to the Trp aporepressor, and forms an inactive Trp pseudorepressor that doesn’t bind the trp operator (5,6). This allows RNA polymerase to bind the promoter and transcribe the gene of interest.

The method described here includes the design of a trp promoter expression vector, the promoter induction in culture, and assessment of the expression level of one’s protein of interest by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The design details of the expression vector can be critical to the levels of protein production achieved. Slight variations in the codons used especially at the amino terminal end of the coding sequence or large stretches of heterologous 3’-untranslated sequences, for example, can dramatically alter these levels. Therefore, it is best to incorporate as many of these details as early as possible to avoid excessive work in terms of plasmid construction. The actual steps involved in plasmid constructions are unique to the gene of interest, so they are not included in this review. Induction of the trp promoter in E. coli cultures with IAA is straightforward and
a protocol for analyzing the results by SDS-PAGE is also provided. Normally the expression levels are high enough to see the protein of interest after staining with Coomassie blue. Other detection methods can be used, such as Western blotting or ELISA using antibodies against the protein of interest or its fusion partner if appropriate (7). Detection of enzymatic activity can also be used if applicable, although the possibility exists that one’s protein may be present in insoluble inclusion bodies and require refolding (8–12). The use of DNA shuffling in combination with a selection or efficient screen can be used to improve production levels and protein solubility (13).

2. Materials
1. M9-casamino acids media for trp promoter inductions (per liter): 6 g Na₂HPO₄, 3 g KH₂PO₄, 0.5 g NaCl, 1 g NH₄Cl, adjust the pH to 7.4, autoclave, cool, then add 2 mL 1 M MgSO₄, 10 mL 20% (w/v) glucose, and 36 mL casamino acids (135 g/L).
2. 3-β-indoleacrylic acid can be dissolved in ethanol at a convenient concentration of 25 mg/mL and stored at 4°C.
3. TE: 10 mM Tris-HCl pH 7.6, 1 mM EDTA.
4. The reducing agents dithiothreitol (DTT) and β-mercaptoethanol (BME) can be made into 1 M solutions, and can be stored at 4°C for a few days. However, they will oxidize and become ineffective in reducing disulfide bonds. Freezing small aliquots or making fresh solutions is advisable.
5. For commercial SDS polyacrylamide gels there is usually a sample buffer available. Alternatively for lab cast gels the following sample buffer may be used: 60 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.025% (w/v) xylene cyanole, and 0.025% (w/v) bromphenol blue.
6. SDS polyacrylamide gels can be purchased from a number of commercial sources, such as Novex (San Diego, CA), or prepared in the lab as described (14).
7. Coomassie blue staining solution contains 25% (v/v) ethanol and 2.5 g Coomassie stain per liter. Acetic acid should be added to the staining solution prior to use (7% v/v). Destain contains 7% (v/v) acetic acid and 20% (v/v) ethanol.
8. L-tryptophan is conveniently made into a 5 mg/mL solution and should be filter sterilized and stored at 4°C.
9. 10% (w/v) SDS is stored at room temperature.

3. Methods
3.1. Vector Design
The basic vector design involves placing the coding region of interest immediately downstream of the E. coli trp operon promoter and Shine–Dalgarno (SD) sequence in a pBR322-based plasmid. This is often, but not always, sufficient to obtain high levels of protein. Some optimization may be required. The features and rationale for obtaining and maximizing expression are outlined below.
1. Plasmid pBR322 (15) is our preferred starting vector. It is more stable structurally and segregationally than higher copy number pUC-like plasmids.
2. The sequence of the trp promoter (16) is shown in Fig. 1. Vectors containing the trp promoter are available from many sources (17–21), can be easily synthesized or cloned by PCR from the E. coli chromosome with convenient restriction sites added to match your gene of interest. We usually place the trp promoter at the EcoRI site of pBR322 and direct it toward the tetracycline resistance (tet) gene (Fig. 2).
3. The coding sequence should begin with an ATG and be placed 5–12 bp from the SD. Variations in this distance and its composition can have large effects on translation initiation rates (22). The spacing in the natural trp operon is 9 bp, and this sequence and spacing are good starting points. Avoid multiple restriction sites in this region.
4. The mRNA translation initiation region (TIR) must be devoid of secondary structures that obscure the SD or ATG to allow efficient formation of the initiation complex. Even weak structures can have large effects on translation (23) and are hard to predict, so again avoid multiple restriction sites in this area. The TIR