pPB Protein Vectors

The pPB vectors are low-medium copy number vectors in which the gene expression is driven by the strong T7 promoter.

Below are some basic guidelines for using the pPB vectors for protein production:

1. The pPB vectors are designed to be used with *E. coli* strains that are DE3 lysogens i.e. the host *E. coli* cell has a source of T7 RNA polymerase.

2. Recombinant protein induction is usually done at OD_{600} of 0.6-1.2 using Isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.05 -1mM.

3. The ideal concentration of IPTG must be determined empirically for each recombinant protein/cell-line. Similarly, the length of time and temperature for induction provide other variables that need to be optimized on a case-to-case basis.

4. For toxic proteins, it is recommended to go for shorter induction time and also to try and suppress basal recombinant gene expression through (a) addition of glucose or use of pLysS plasmid. Please note that special cell-lines are also available in the market that cater to expression of toxic proteins.

5. Once grown for the desired length of time, harvest cells by centrifugation and either freeze the cells at -80 (as such or after re-suspending in the desired buffer) or proceed with the purification.

Please note that these are to serve as guidelines only and end users may be required to perform further optimization based on experimental conditions.

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