Expression of Toxic Proteins in *E. coli*

Contents

1. Definition of Toxic Protein

Toxic proteins defined here as proteins that cause cell death or severe cultivation and maintenance defects during the growth phase when their genes were introduced into *E. coli* strain.

2. Mechanisms of Protein Toxicity

The over-expressed recombinant protein will perform certain functions in the host cell if the soluble protein is expressed with proper folding. The function of the expressed recombinant protein may be detrimental to the proliferation and differentiation of the host cell. The phenotype of the host cells is slow growth rate and low cell density. In some cases, the recombinant protein causes death of the host cell.

3. Percentage of Protein Toxicity

Protein toxicity is a commonly observed phenomenon. It is estimated that about 80% soluble recombinant proteins have a certain degree of toxicity to their hosts. About 10% of these are highly toxic to host cells. The completely insoluble or dysfunctional proteins will not be toxic to the host cell, though they create metabolic burden for the host cell. Some low solubility or partially functional proteins may still be toxic to the host cell.

4. Phenotypes of Protein Toxicity

Protein toxicity adversely affects the cloning and expression. Protein toxicity is the most important reason for DNA cloning or subcloning problems. Most expression problems are also the result of protein toxicity.

4.1 Toxic Protein Cloning Problems

- No colony in cloning
- Fewer colonies than those in regular cloning experiments
- Small percentage of positive clones
- Wrong orientation
- Mutations leading to defective products or no expression

4.2 Transformation Efficiency Problems

- The transformation efficiency of the vector containing a toxic protein is lower or significantly lower than the control vectors or the vectors containing non-toxic proteins.
- Transformation efficiency in a normal cell strain is significantly lower than that in a detoxification strain.

4.3 Host Cell Growth Rate and Cell Density Problems

- Cell grows significantly slower and cannot reach its normal density in a given medium before induction.
- Cell grows significantly slower and cannot reach its normal density after induction.
- Growth rate and cell density are low in normal strain. In contrast, the growth rate and cell density are close to normal in a detoxification cell strain.

4.4 Toxic Protein Expression Problems

- No expression
- Low yield
- Defective proteins

- Inconsistent expression

5. Strategies of Toxic Protein Cloning and Expression

Once the toxicity of a protein is determined, different strategies should be used.

5.1 Suppress Basal Expression

- **Use a more tightly regulated promoter**, e.g. the arabinose promoter (P_{BAD}).
- **Use a lower copy number plasmid** to better regulate expression.
- **Use a strong terminator upstream of inducible promoter** to prevent transcription read-through from upstream real and cryptic promoters.
- **Use a tightly regulated vector** which contains multiple lac operators and transcription repressors.
- **Constitutive expression of phage T7 lysozyme** from a compatible pLysS or pLysE plasmid. T7 lysozyme, a natural inhibitor of T7 RNA polymerase, can reduce basal activity from an inducible gene for T7 RNA polymerase and allow relatively toxic genes to be established in the same cell under control of a T7 promoter.
- **Use detoxification strains.** Some host strains, e.g. C41 (DE3) and C43 (DE3), appear to be useful in some toxic proteins especially some membrane proteins.
- **Addition of 1% glucose to culture medium** to repress induction of the lac promoter by lactose, which is present in most rich media (such as LB, 2xYT).

5.2 Optimization for Toxic Protein Expression

- **Induction conditions**

Induction time

Induction time should be closely monitored to produce a reasonable amount of protein while the host cells are not devastated (protein degradation or cell death).

Inducer concentration

Cells induced using very low IPTG concentrations have more metabolic control over the toxic effect than those induced with standard IPTG concentrations (0.1- 1.0 mM). IPTG concentrations in the range 0 -10 μM are commonly used for induction of toxic proteins.

Induction temperature

Protein toxicity may be reduced or eliminated at a lower temperature. Lower temperature also results in increased protein solubility. Longer induction time should always be associated with lower temperature because of lower protein synthesis rate. Temperatures of 25 and 15 degree Celsius are commonly used for induction of toxic proteins.

- **High concentration of antibiotics**

Use of elevated levels of antibiotics (up to 200 mg/ml), which helps prevent losing the expression vector from the cells.

- **Plating inoculation**

Cultures are inoculated by scraping off agar plates, which is a more stringent method to select plasmid-containing cells than in liquid culture media.

5.3 Other Approaches for Toxic Protein Expression

Other technologies may also be used in combination with above technologies to improve cloning efficiency and protein yield.

- **Fusion expression**

Large fusion partners such as GST or Trx will reduce protein toxicity in some cases. Small tags such as his-tag will not alter toxicity property significantly. In rare cases fusion partner can increase protein toxicity.

- **Express individual domains**

Individual domains are almost always less toxic than the corresponding full-length proteins. Sometimes individual domains may lose toxicity completely and therefore are not functional.

- **Periplasmic expression**

Secretion of the target protein to the periplasm (or the medium) allows for the accumulation of proteins that are toxic in the cytoplasm.

- **Express in inclusion bodies**

It is a good strategy to express toxic protein in inclusion bodies. In aggregates, the proteins are not toxic to the cell and they can be obtained by in vitro denaturation and refolding.

6. Vectors Suitable for Expression of Toxic Proteins

Table1.Vectors Suitable for Expression of Toxic Proteins

Vector	Features
pLysS	Constitutive expression of lysozyme, a natural inhibitor of T7 RNAP
pLysE	Expression of more lysozyme than pLysS
pBAD series	Tight regulation of gene expression
pT7SC series	Blocking read-through transcription by upstream terminators

pETcoco-1	Arabinose-based control of copy number

7. *E. coli* Strains Suitable for Expression of Toxic Proteins

Table2. *E. coli* Strains Suitable for Expression of Toxic Proteins

***E. coli* Strain**	**Features**
BL21-AI	T7 RNAP gene under the control of P_{BAD} promoter
PB4144	The strain carries a temperature-sensitive allele of supF suppressor
CopyCutter™ and EPI400™	Regulation of the copy number of ColE1-type plasmids using a chromosomal copy of the pcnB gene under the control of an inducible promoter
C41(DE3) and C43(DE3)	Empirically-selected strains with lower basal expression and slower induction rates compared to the original BL21(DE3)
ABLE C	The strain reduces 4-fold the copy number of ColE1-derived plasmids
ABLE K	The strain reduces 10-fold the copy number of ColE1-derived plasmids

8. Conclusion

We reviewed strategies for the expression of toxic proteins. The set of solutions summarized in this review highlight two important points: the need to characterize the level of toxicity of problematic genes and the need to tune the tightness of the inducible expression system to match the basal expression level that *E. coli* tolerates. By combining many rational strategies, the desirable tightness can be achieved.

References:

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Protein Expression Service Highlights

Guaranteed quantity and purity: Get your desired quantity and purity or your money back.

Flexible solutions: Multiple approaches for assured success.

Guaranteed soluble proteins: You only get billed if solubility is achieved.

Maximum activity: We prioritize supernatant proteins.

Protein Expression

Bacterial Expression System	
Guaranteed *E.coli* Protein Expression	$1,550 for 3-5mg ~85% purity soluble protein No protein, no billing
SupernateIN™ Protein Expression	Protein with maximum biological activity High soluble expression level
Tag-free Protein Production	Close to native protein Soluble protein with over 95% purity
Mammalian Expression System	
Transient Transfection	High yield: >200mg/L High transfection efficiency
Stable Cell Line Establishment	Scalable and reproducible production 30% below industry average
Recombinant Antibody Production	High yield: up to 1g/L expression level High-specificity: bind to corresponding antigen specifically with high-affinity
Other Protein Services	
Endotoxin/RNase Removal	Endotoxin level below 1 EU/μg >90% RNase removal from protein
Fermentation	Fed-batch fermentation Protein production up to 2000 L
Biomolecular Interaction Analysis	Label-free and real-time analysis K_a, K_d and K_D for small molecule and protein binding interactions

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