Case study

Molecular biology

Increasing protein expression of a recombinant immunotoxin in a cell-free system

Company

A small biotechnology company was developing recombinant immunotoxins for cancer treatments. The customer wanted to rapidly screen candidates by reducing steps in the workflow.

Background

Targeted cancer therapies have the potential to deliver cancer treatments with fewer side effects, increasing therapeutic windows. Antibody–drug conjugates (ADCs) and recombinant immunotoxins (RITs) are a class of therapies that target cancer through a cancer-associated antigen and deliver a toxin moiety directly to the cancer cells. RITs typically use a cytotoxic portion of nonhuman origin, such as *Pseudomonas* exotoxin A (PE).

There are many obstacles to creating an effective RIT. All domains of the RIT, including the targeting region, linker, and toxin, can be immunogenic, limiting RIT efficacy. Off-target effects such as capillary leak syndrome and organ toxicities have also been observed with RITs [1]. To minimize these effects, an iterative approach to the design of the RIT must be taken.

In this study, the customer was creating an RIT targeting CD7, a membrane protein mainly expressed on T and NK cells. CD7 is associated with T cell acute lymphoblastic leukemia (T-ALL) and other forms of leukemia [2]. A single-chain variable fragment (scFv) domain targeting CD7 was linked to PE24 by a flexible glycine–serine (GS) linker. PE24 is the shortest version of PE known to effectively kill target cells [3].

Challenges

By design, RITs are cytotoxic to eukaryotic cells. As a result, common mammalian expression systems like CHO do not work. RITs are typically expressed in prokaryotic systems such as *E. coli* but usually are found in inclusion bodies [4]. Isolating protein from inclusion bodies is time-consuming and inefficient. Additionally, components of the RIT can be immunogenic.

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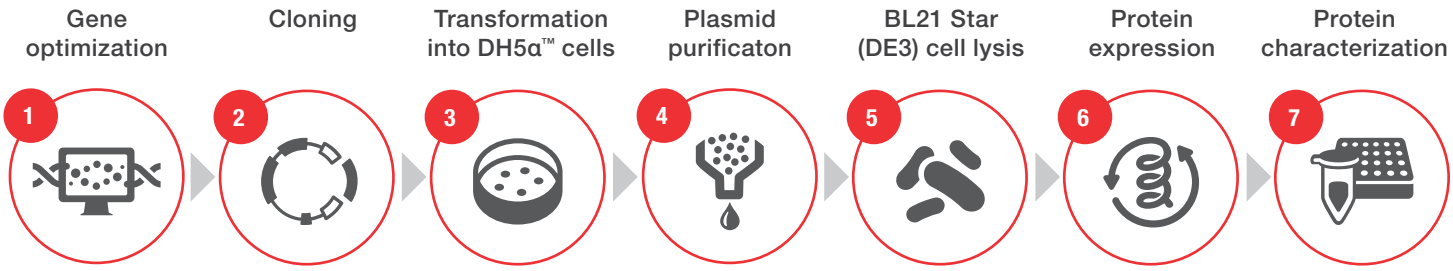

Figure 1. Workflow summary. Two types of competent cells were used in the procedure, one for cloning and one for protein expression.

Solutions

Three methods were used to improve functional protein expression and reduce experimental steps: (a) optimize the RIT gene sequence, (b) select competent cells with enhanced expression capabilities, and (c) employ a cell-free system.

Gene sequence optimization is a crucial step in enhancing protein expression in *E. coli*. By modifying the DNA sequence of the gene of interest to match preferred codon usage in *E. coli*, the customer could help ensure that the codons were efficiently recognized by the translational machinery of *E. coli*, to yield significant increases in expression. Gene optimization also includes removal of secondary structures in the mRNA that might impede translation, and adjustment of GC content to optimal levels for *E. coli* to lead to higher yields of the desired protein. By using the proprietary Invitrogen™ GeneArt™ GeneOptimizer™ algorithm, the customer could optimize the gene for expression with minimal effort. Further modifications were made to the sequence to remove known sites of immunogenicity.

For enhanced protein expression, Invitrogen™ BL21 Star™ (DE3) competent cells were selected. The BL21 (DE3) strain does not contain the lon protease and is also deficient in the outer membrane protease OmpT, resulting in reduced degradation of heterologous proteins. The BL21 Star (DE3) strain has the added feature of a mutation in the RNase E gene (*rne131*) that reduces levels of endogenous RNases and mRNA degradation, thereby increasing the stability of mRNA transcripts and increasing protein yield. To further improve protein folding and prevent inclusion bodies, BL21 Star (DE3) cells were transformed with plasmids that carry genes for chaperone expression.

To save time and steps with cell lysis and purification, cell extracts from the modified BL21 Star (DE3) strain were prepared [4]. A plasmid or PCR product corresponding to the RIT construct was added to the mixture. The overall workflow utilizing these methods is shown in Figure 1.

Summary

RITs show promise for the targeted treatment of cancer. Several drugs, such as moxetumomab (PE38-based anti-CD22 immunotoxin), have been approved by regulatory bodies and successfully used to treat patients, and more candidates are in clinical trials. Because of the eukaryotic cytotoxic properties of RITs, they can be difficult to express and can also be immunogenic. Using gene optimization approaches such as GeneArt GeneOptimizer software, increased expression of the target gene can be achieved with minimal effort for the desired expression system. Use of an *E. coli* strain can also play a critical role in eukaryotic gene expression. Modified strains such as BL21 Star (DE3) cells have enhanced protein expression properties. They can be further modified to prevent the protein from going into inclusion bodies. Using modified BL21 Star (DE3) lysates and optimized plasmids produces sufficient functional recombinant protein for screening without the need for cell lysis or purification.

Thermo Fisher Scientific offers a variety of tools including gene synthesis, plasmids, competent cells, restriction enzymes, and characterization instruments and reagents to support your research.

References

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3. Havaei SM et al. (2021) *Pseudomonas* exotoxin-based immunotoxins: over three decades of efforts on targeting cancer cells with the toxin. *Front Oncol* 11:781800. **doi.org/10.3389/fonc.2021.781800**
4. Krebs SK et al. (2022) Synthesis of an anti-CD7 recombinant immunotoxin based on PE24 in CHO and *E. coli* cell-free systems. *Int J Mol Sci* 23(22):13697. **doi.org/10.3390/ijms232213697**

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