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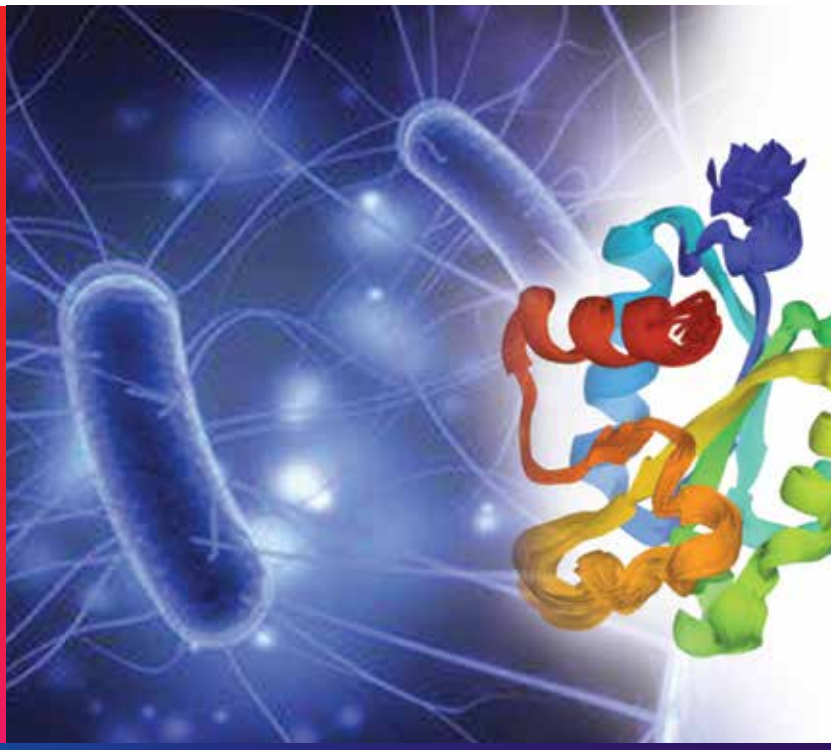
Case Study

Expression, *in vivo* Maturation and Tag-less Purification of a Recombinant Protein



The existing workflow must be redesigned to a cost - effective, scalable process in which, the untagged protein is recombinantly expressed in *E. coli* and undergoes *in vivo* maturation. The first step in achieving this is the optimization of expression parameters to enrich the mature form of the protein in the soluble cytoplasmic fraction. Following this, the scalable purification strategy designed, needs to be addressing the quality and quantity specifications of the final protein. This case study demonstrates the experience and expertise of scientists at Aragen that helped the client in re-shaping their strategy to reach a critical milestone.

The project

The client was looking at obtaining >50 mg of a mammalian secretory protein which is known to be difficult to express as a soluble form in *E. coli.* Aragen was approached to recombinantly produce this protein in its precursor form in *E. coli,* facilitate *in vivo* maturation by co-expression of a processing enzyme, and produce the tag-less protein with >95% purity. This project was a crucial step for the client to initiate a new program in their drug discovery pipeline.

About the client

The client is a biotechnology company focussed on protein engineering to create multifunctional, precision therapies for various indications.

Why Aragen?

- A solid track record of purifying 200+ functionally active recombinant proteins and generating 100+ cell lines as stable or transient expression systems for drug candidate screenings.
- A highly experienced scientific team working with a wide variety of proteins such as antibodies, engineered antibodies, Fc-fusion and other chimeric proteins, enzymes such as CYPs and kinases, oligomeric proteins, receptors, interleukins, protein complexes, synthetic proteins and many more targets.
- Advanced laboratories for recombinant protein generation in microbial, insect and mammalian expression systems along with three well equipped protein purification labs to cater process upstream deliverables from microbial, insect and mammalian cell cultures respectively.
- Experience of working on a variety of biologics and building their effective delivery roadmaps.
- A robust IT infrastructure ensured complete data protection and IP security.

Aragen's approach

The initial trial protocol provided by the client was further modified and optimized to achieve required quality and quantity of the final product. As a first step, the scientific team at Aragen re-worked the expression protocol to maximize the yield of the soluble protein. This was followed by redesigning a purification protocol to address the specifications in a scalable format with reduced turn-around time.

Abstract

The expression and purification protocol were redesigned at Aragen to address the target quantity and purity of the deliverable. In a series of small – scale experiments (< 1 L cultures), optimal temperature for expression was determined which enhanced maximal soluble expression of the precursor protein and provided optimal *in vivo* reaction condition for the co-expressed processing enzyme to convert the soluble precursor protein into its mature form. A 4-step purification methodology was developed to obtain the final product with the desired purity in the shortest timeframe. Optimized parameters were tested for scalability (up to 10 L per batch) and reproducibility. More than 80 mg of the protein, meeting desired specifications, was produced, and delivered to the client.

Process Development

Step 1: Expression (<1 L scale)

- Screening for optimal temperature of expression and Isopropyl β-D-1-thiogalactopyranoside (IPTG) concentration for maximal soluble expression of the precursor protein.
- Screening for optimal temperature of expression which is also suitable for efficient processing enzyme activity to turn over the precursor protein to the mature form.
- Screening for optimal growth media.

Step 2: Purification

- Screening for optimal parameters for the first step of purification to reduce impurities.
- Screening for optimal first step chromatography parameters: column, elution conditions.
- Screening for optimal second step chromatography parameters: column, elution conditions.
- Polishing step chromatography.

Step 3: Characterization and QC

- Confirmed identity using mass spectrometry, peptide mass fingerprinting and Western blot analysis.
- Purity analysis using reducing and non - reducing SDS PAGE, analytical size exclusion chromatography and analytical reverse phase chromatography.

Step 4: Large – scale expression and purification (> 10 L scale)

- Expression and purification protocol developed in small – scale was scalable for > 10 L scale cultures.
- Product qualified using the QC parameters described in Step 3.
- Batch – to – batch reproducibility of yield and quality of recombinant protein.

Project outcome

A robust, scalable, and reproducible process was designed for expression and purification of a recombinant protein in bacterial (*E. coli*) system. Expression parameters were thoroughly optimized to enrich the mature form of the protein in the soluble cytoplasmic fraction. A scalable purification strategy was designed to addresses the quality and quantity specifications of the final recombinant protein. More than 80 mg of the protein, meeting desired specifications, was produced, and delivered to the client.