Enhancing solubility of protein targets: innovations in expression strategies using eProtein Discovery™

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Technology applications

- Unprecedented drug targets
- Difficult to express proteins
- AI/ML protein engineering
- De novo protein design
- Developability assessment
- Amino acid labelling

Introduction

Solubility fusion tags can help with protein solubility and increase expression yield. However, they often interfere with binding and protein function and therefore require removal for downstream assays. The eProtein Discovery™ is a benchtop automated protein expression and purification screening platform that incorporates the evaluation of solubility tags on expression yield and the effect of removal. Integrating cell-free protein synthesis (CFPS) and digital microfluidics on a cartridge, the system performs expression screening of 24 constructs with 8 expression conditions with in situ detagging and quantifies soluble protein yield afterwards. A cartridge screen identifies the ideal conditions to scale up in tubes the next day to get µg to low mg of proteins to progress protein projects.

Objective: Evaluation of solubility tags and their effects on soluble protein expression, as well as the impact of tag removal, is a necessary and high-value capability.

Methods

- **Step 1:** Pick and design solubility tags
- **Step 2:** Automated protein expression and purification screen on cartridge
- **Step 3:** Scale up on the bench

Figure 1. The eProtein Discovery workflow. In Step 1 when you Design & Prep you choose the solubility tag to include. In Step 2 you can add in the 3C Protease (3CP) to detag the solubility tags.

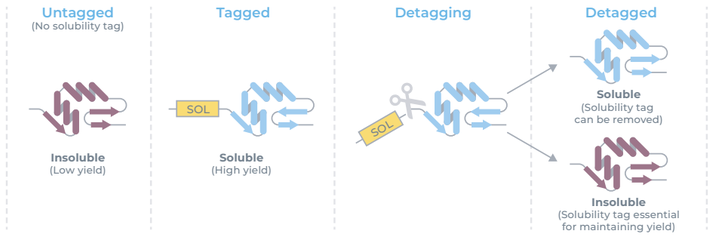


Figure 2. The concept of tagging and detagging. Yield advantage with solubility tags and the impact (or consequence) of removal.

Results

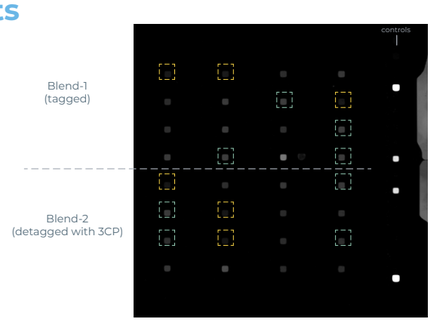
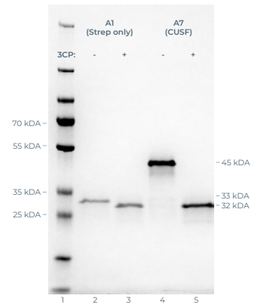


Figure 3. eProtein Discovery Cartridge determination of protein purification yield.



Lane	Details
1	Molecular weight ladder
2	Purified CAT (strep-only tag)
3	Purified CAT (strep-only tag, detagged)
4	Purified CAT (CUSF solubility tag)
5	Purified CAT (CUSF detagged)

Figure 4. Validating detagging on SDS-PAGE gel analysis.

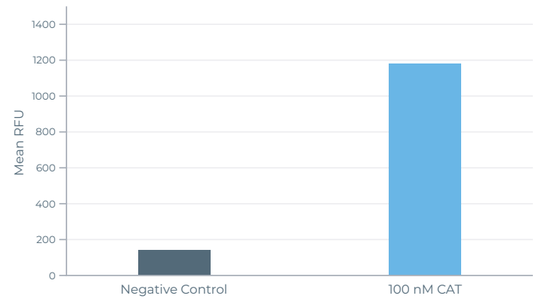


Figure 5. An acetyltransferase activity fluorometric assay with detagged CAT.

Conclusions

- Expression and *in situ* detagging of solubility-tagged fusion proteins results in higher purified yields than simply expressing and purifying the untagged version of the protein
- We exemplified the advantage of using the eProtein Discovery system to screen and predict expression yield, and evaluate the impact of our detagging strategy using the important drug target candidate chloramphenicol acetyltransferase
- Demonstration of a simple and user-friendly way to increase the obtainability of active proteins for drug discovery pipelines

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