Small-scale, high-throughput screen of eukaryotic protein production

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E. coli-based Protein Production Pipeline

The CEGS high-throughput (HT) protein production pipeline (Acel et al., 2003) (Figure 1) uses an economical small-scale screening (0.4 mL cultures) trials to predict the outcome of large-scale production (LSP) cell-based growths (Figure 2), and to lower protein production costs. All protein targets are first produced as MBP-fusion proteins in a single vector (pVP61K in the methionine auxotrophic E. coli strain, B343-pRARE2 (matE) (Studier, 2005 & Frederick et al., 2007), using seleno-methionine-mediated purification using a generic immobilized metal-chelating chromatography (IMAC) scheme. In small-scale screening the production of MBP-target protein fusions are analyzed by SDS-PAGE to evaluate protein expression, solubility, and TEV protease cleavage (Figure 1). Well-behaved fusion targets are judged to be "amenable" for structure and/or activity analysis by committing valuable resources for downstream processes. Over a 2-year period, this approach has successfully predicted proteins that are suitable for large-scale production and purification with ~80% accuracy. In summary, the CEGS small-scale purification approach saves considerable resources and labor, and reduces expenditure of resources on proteins that will ultimately fail in protein purification.

Optimization of Protein Production: Improved Growth Media Composition and Vector Design

Several improved methodologies are being researched by CEGS to improve protein production efficiency and increase the output of protein structures by the pipeline. One of these methodologies is to aim for the ability of the small-scale to accurately predict events further down pipeline into the protein purification section. To achieve these goals, CEGS has developed new techniques for expressing and purifying sufficient target protein directly from the small-scale (see accompanying CEGS poster) for initial biophysical analysis, and macro-scale protein production for NMR and X-ray analysis. Here we show how we have combined two basic improvements at several steps of our screening pipeline. The two relevant underlying methodologies are (1) factorial evolution of auto-induction medium to improve growth and expression, and (2) vector expression engineering to better match performance in small- and large-scale cell growths.

Factorial evolution of the auto-induction medium revealed new combinations of carbon sources that were better matched to CEGS pipeline activities (Figure 5, 6 and 7) (Frederick et al., 2007). Furthermore, by altering the promoter for LacI (provided by LuQiType plasmids, such as the pQ020 (Glalign) derivatives used in the CEGS), we obtained close correlation between small- and large-scale expression trials, while also increasing total protein expression (Figures 7 and 8).

References


Acknowledgments

This project was supported by the National Institute of General Medical Sciences through The Protein Structure Initiative NIGMS grant number U54 GM074901.

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Protein Structure Initiative

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