Cell-Based Protein Production

PRINT

The LARGE-SCALE PROTEIN PRODUCTION Team, headed by Brian Fox, PhD, is responsible for large-scale protein production and labeling for the project.

- Bioinformatics
- Cell-Free
- Cloning
- Crystallography
- Expression Testing
- NMR
- Protein Production
- Protein Purification
- Quality Assurance
- Sesame

Goals for CESG Protein Production Team

- Provide high-throughput, large-scale production of unlabeled and labeled *Arabidopsis* proteins in *E. coli* of high analytical quality.
- Process optimization of economic and high-quality protein production.
- Production of project resource materials for the pipeline.

Overview of Protein Production at CESG

The major goal of the Protein Production Team is to reliably produce sufficient mass of bacterial cells expressing recombinant proteins to facilitate subsequent protein purification efforts. By working closely with the Small-Scale Expression, Protein Purification, and X-ray Crystallography teams, deliverable goals of 15 g of recombinant cell paste with favorable scoring for total expression, solubility of expressed protein, and percentage of proeolysis from the fusion construct using tobacco etch virus protease were established.

The Team produces SeMet-labeled proteins by heterologous expression in *Escherichia coli* grown using the auto-induction approach introduced by Studier [Protein production by auto induction in high density shaking cultures and Recipes and stock solutions described in protein production by auto induction in high density shaking cultures]. As compared to other methods for growth and induction of recombinant cultures, we have found the auto-induction method to be reliable and scalable for all types of expression monitoring used at CESG. Moreover, for large-scale cell growth, the auto-induction minimizes the amount of labor required for culture monitoring. As described below, our implementation of the large-scale cell growth pipeline is sufficiently robust that all proteins studied by CESG are labeled with selenomethionine (SeMet) as the first-pass production effort.

Pipeline Production of SeMet-Labeled Proteins in Auto-Induction Medium. We have developed a cell-based protein production pipeline that incorporates the auto-induction strategy introduced by Studier for first-pass production of SeMet-labeled proteins in *Escherichia coli* B834. The entire production cycle from receipt of freshly transformed expression host through the growth, induction, and expression was designed to take ~72 h, and capacity for up to 36 growths per week has been demonstrated with existing labor and equipment. The medium used for culture scale-up was adjusted to provide rapid and reproducible growth, while the production cell growth was optimized for timing, cell mass yield, total protein expression, and percentage of SeMet incorporation. At 250 rpm shaking in a standard refrigerated shaker, an average final optical density at 600 nm of greater than 6 and a yield of ~14 g of cell paste were obtained over ~500 growths. Cell growth with shaking at 350 rpm gave an ~1.6 fold increase in OD600 and a corresponding increase in the mass of cell paste with similar characteristics for the expressed fusion proteins (total expression, solubility, and proteolysis of the fusion protein to release the target). Increased agitation indicates a potential pathway to improvements in process yield. However, it was not possible to fully load the shaker at this higher agitation rate due to overheating before the 24 h growth cycle could be completed. Engineering efforts to improve the shaker and rack design are under way. Results of these and other features of our SeMet protein production pipeline will be discussed.

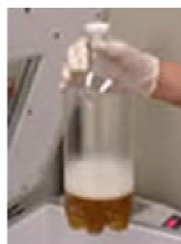
Scoring of Protein Expression.CESG uses three-tier scoring to evaluate expression gels: (1) total protein expression, (2) the level of soluble expression, and (3) the percentage of total soluble fusion protein that can be proteolyzed by tobacco

etch virus (TEV) protease. This scoring is based on visual comparison with the stained intensity in standard lanes. Similar assessments are made for the lanes containing the soluble fraction, the pellet fraction, and the TEV protease-treated soluble fraction. MALDI-TOF mass spectrometry was used to identify endogenous *E. coli* proteins useful as control markers for cell fractionation. By including evaluation of these internal control proteins to assure consistency in cell lysis, the CESG scoring system has been successfully standardized across the expression of several hundreds of targets.

Methods Being Investigated and Publications

+ Economical Approaches to the Production of [Se-Met]-, [U-^{15}N]- and [U^{13}C,U^{15}N]-Labeled Proteins from *Escherichia coli* Cells

Protocols are presented for growing culture media for stable isotope labeling in disposable, non-autoclaved two-liter beverage bottles, for utilization of self-induction media for [Se-Met]-labeling, [U-^{15}N]- and [U^{13}C,U^{15}N]-labeling, and for improving yields with self-induction media through supplementation with (5% v/v) Celtone liquid (Spectra Stable Isotopes, Columbia, MD).



- STACK OF PET BOTTLES USED FOR PROTEIN PRODUCTION (LEFT)
- POLYETHYLENE TEREPHTHALATE (PET) PET BOTTLES IN PIPELINE FOR PROTEIN PRODUCTION (MIDDLE)
- TERRIFIC BROTH E. COLI CULTURE IN PET BOTTLE (RIGHT)

Q. Zhao, R. Frederick, K. Seder, S. Thao, H. Sreenath, F. Peterson, B.F. Volkman, J.L. Markley, B.G. Fox. (2004) **Production in two-liter beverage bottles of proteins for NMR structure determination labeled with either ^{15}N- or ^{13}C-^{15}N.** *JSFG* 5(1-2):87-93. |15263847|

Sreenath, H.K., Bingman, C.A., Buchan, B.W., Seder, K.D., Burns, B.T., Geetha, H.V., Jeon, W.B., Vojtik, F.C., Aceti, D.J., Frederick, R.O., Phillips, G.N., Jr., Fox, B.G. (2005) **Protocols for production of selenomethionine-labeled proteins in 2-liter polyethylene terephthalate bottles using auto-induction medium.** *Protein Expr Purif* 40(2):256-67. |15766867|

Tyler, R.C., Sreenath, H., Aceti, D.J., Bingman, C.A., Singh, S., Markley, J.L., Fox, B.G. (2005) **Auto-induction medium for the production of [U -^{15}N]- and [U -13C, U -^{15}N]-labeled proteins for NMR screening and structure determination.** *Protein Expr Purif* 40(2):268-78. |15766868|

Frederick, R.O., Bergeman, L., Blommel, P.G., Bailey, L.J., Song, J., Meske, L., Bingman, C.A., Riters, M.. Dillon, N., Kunert, J., Yoon, J., Lim, A.-Y., Cassidy, M., Bunge, J., Aceti, D.J., Primm, J.P., Markley, J.L., Phillips, G.N., Jr., Fox, B.G. (2007) **Small-scale, semi-automated purification of eukaryotic proteins for structure determination.** *JSFG* 8(4):153-66. |17985212|

+ Factorial Evolved Auto-Induction Medium

The auto-induction method of protein expression in *E. coli* is based on diauxic growth resulting from dynamic function of lac operon regulatory elements (lacO and LacI) in mixtures of glucose, glycerol, and lactose. The results show that successful execution of auto-induction is strongly dependent on the plasmid promoter and repressor construction, on the oxygenation state of the culture, and on the composition of the auto-induction medium. Thus expression hosts expressing high levels of LacI during aerobic growth exhibit reduced ability to effectively complete the auto-induction process. Manipulation of the promoter to decrease the expression of LacI altered the preference for lactose consumption in a manner that led to increased protein expression and partially relieved the sensitivity of the auto-induction process to the oxygenation state of the culture. Factorial design methods were used to optimize the chemically defined growth medium used for expression of two model proteins, Photinus luciferase and enhanced green fluorescent protein, including variations for production of both unlabeled and selenomethionine-labeled samples. The optimization included studies of the expression from T7 and T7-lacI promoter plasmids and from T5 phage promoter plasmids expressing two levels of LacI. Upon the basis of the analysis of over 500 independent expression results, combinations of optimized expression media and expression plasmids that gave protein yields of greater than 1000 ug/mL of expression culture were identified.

Blommel, P.G., Becker, K.J., Duvnjak, P., Fox, B.G. (2007) **Enhanced bacterial protein expression during auto-induction obtained by alteration of lac repressor dosage and medium composition.** *Biotechnol Prog* 23(3):585-98. |17506520|

Large-Scale Protein Production Team

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