Screening and quantification methods for high-throughput production of unlabeled and labeled soluble *Arabidopsis thaliana* proteins in *Escherichia coli*

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Analytical procedures for detecting and assigning the results of expression trials are critical inputs to the operation, reproducibility, and stability of protein production operations in the high-throughput structural genomics environment. Here we present the details of denaturing gel electrophoretic evaluations of a high-throughput process for production of unlabeled, selenomethionine-, and ^{15}N-labeled *Arabidopsis* proteins in *Escherichia coli* B834 pLacIRare. All recombinant proteins produced in this *E. coli* expression host were evaluated for total protein expression and the partition between soluble and insoluble fractions using a simple, three category evaluation scheme. The total protein content was normalized as the sum of soluble protein fraction plus the insoluble fraction re-dissolved to a normalized volume. Pre-treatment of the host cells with lysozyme followed by sonication in a multi-well device gave reproducible, optimal breakage of the expression cells obtained from a variety of growth conditions and containing a wide variety of expression targets. The scoring scheme for denaturing electrophoresis gels was based on the pattern and the intensity of both constitutive and recombinant protein markers present in the soluble fraction. UW CESG has found that two highly soluble *E. coli* proteins of ~45 kDa and ~27 kDa provide a useful correlation between the completeness of cell lysis and the fractionation of the recombinant target protein between soluble and insoluble fractions. Two other integral membrane *E. coli* proteins of 37 and 40 kDa provide reliable markers for the insoluble fraction. Examples of how this scoring system can be used to evaluate expression results in a high-throughput environment will be presented (funded by NIGMS GM645980).