APPLICATION OF *IN VITRO* PROTEIN EXPRESSION TO HUMAN PROTEOMICS

Peter T. Beernink¹, Simone S. Krupka¹, Victoria Lao¹, George Martin²,
and Matthew A. Coleman¹

¹Molecular & Structural Biology Division, Lawrence Livermore National Laboratory, 7000 East Ave., Livermore, CA 94550; ²Roche Diagnostics Corp., 2929 7th St., Ste. 100, Berkeley, CA 94710

*ptb@llnl.gov

INTRODUCTION. A critical step in many proteomics projects is the identification of genes that express proteins in sufficient quantities for subsequent studies. LLNL houses a collection of >200,000 human cDNAs, known as the IMAGE Consortium (<http://image.llnl.gov>)[1], including some full-length enriched cDNA libraries. We have developed an integrated system that exploits coupled *in vitro* transcription/translation (IVT) to identify human IMAGE cDNAs that express proteins of the predicted mass. Moreover, this expression screening can be conducted without plasmid DNA preparation or subcloning. IVT-produced proteins are used directly in functional assays or the cDNAs are transported to a bacterial expression system for large-scale protein production.

METHODS. IVT reactions are performed using the RTS-500 Rapid Translation System (Roche Molecular Biochemicals) in conjunction with a biotin-lysine-tRNA conjugate, FluoroTectTM Green_{Lys} (Promega). IVT products are visualized on a UV transilluminator or quantitated on a Packard fluorescence plate reader ($\lambda_{\text{ex}} = 485 \text{ nm}$; $\lambda_{\text{em}} = 510 \text{ nm}$). For micro- and far-western blots, 100 nl of IVT-produced protein are spotted on a glass slide. Detection is accomplished using adapted protocols and a rhodamine-conjugated secondary antibody. Crystallization experiments are conducted by the LLNL TB Crystallization Center (<http://www-structure.llnl.gov/TB>) using CrysTool software (2) interfaced with Packard MultiProbeII liquid handling robots.

RESULTS. We have performed preliminary studies to optimize steps in small-scale IVT screening. We have compared *Escherichia. coli* extracts from several different vendors. Using the extract of choice, we have obtained similar protein yields using plasmid and PCR template DNA. We have scaled reactions down to 12 μl volumes, which results in significant cost savings in both RTS extracts and FluoroTectTM Green_{Lys}. Finally, we have optimized the ratio of labeled to unlabeled lysine in the reactions. The yields from *in vitro* and *in vivo* protein expression correlate well, which validates the use of IVT for expression screening. For large-scale expression, the cDNAs are subcloned into a modified version of pETBlue-2 (Novagen) for high-level expression of C-terminally His-tagged proteins.

Figure 1. See text for details.

DISCUSSION. Our IVT system identifies genes, such as those in the IMAGE cDNA collection, that express full-length proteins at significant levels. Our expression screen requires no plasmid preparation or subcloning. Portability to a bacterial expression system allows large-scale production of affinity-tagged proteins for structural studies.

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