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High-Throughput Methods for Gene Expression and Purification of Hyperthermophilic *Pyrococcus furiosus* Proteins. L-L. C. Kelley, B. Dillard, C. Shah, F. Sugar, H.-S Lee, F. Poole, F. E. Jenney, Jr., M.W.W. Adams, B.C. Wang, Southeast Collaboratory for Structural Genomics, Dept. of Biochemistry & Molecular Biology, Univ. of Georgia, Athens, GA 30602 USA.

We are developing protocols for high-throughput cloning, gene expression, and purification of recombinant proteins from the hyperthermophilic archaeon, *P. furiosus*. Our goal is to express all of the 2,200 open reading frames (ORFs) in this organism. Our methodology includes rapid, robotic-based screening protocols to investigate various conditions known to affect heterologous gene expression in *Escherichia coli*. We predict that about 25% of the ORFs can be expressed in this fashion. The remaining ORFs encode proteins that are multisubunit, membrane-associated and/or contain complex cofactors, and their expression will require specialized systems including other hosts. We have developed a pipeline for regular protein production. The pipeline can handle a minimum of 12 samples per week, with one person each handling the four purification steps, and a fifth person the quality control and final sample preparation. The methodology, capability and current results will be presented.

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