

W0261

Evaluation of High Throughput Methods for Cloning and Expression of Soluble Domains. Collart, F.R., Moy, S.F., Dieckman, L, Landorf, E., Pepler, T., Maltsev, N, Stevens, F.J., Schiffer, M., Biosciences & Mathematics and Computer Science Div., Argonne National Laboratory, Argonne, IL 60439.

We have developed a high throughput domain-based cloning and expression approach for high molecular weight proteins and putative soluble domains of membrane proteins. The domain-based approach provides an alternative to full length expression studies and is often used in traditional benchtop approaches. This approach has been validated by the application of plate-based methods to over 200 secretory and membrane protein targets from *Bacillus subtilis*. For these targets, we observed an expression efficiency of 65% with approximately one third of the clones producing soluble protein. A similar approach was applied to high molecular weight proteins that are typically difficult to clone and express in a soluble form in *Escherichia coli*. We applied domain identification methods for assignment of putative domains for these large proteins (>800 aa) and automatically generated an iterative set of primes flanking the domain boundaries to optimize for expression of soluble domains. These domains targets are important biological molecules and constitute key enzymes and molecular receptors and many are involved in cell to cell signaling.