

## E0076

**Production of Eukaryotic Proteins for High-Throughput Structure Determination** G. N. Phillips, Jr.  
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CESG has implemented pipeline procedures for protein production of targets selected from the *A. thaliana* and other eukaryotic genomes. CESG uses ligation-independent cloning (Gateway) to produce entry clones and specialized destination clones for *E. coli*-based protein production. The destination clone includes an N-terminal (His)<sub>x</sub>-MBP followed by a TEV cleavage site. Expression testing of this construct in *E. coli* shows that ~60% of cloned *Arabidopsis* genes are expressed as a soluble fusion protein. A variant of the self-inducing medium developed by Studier is used for the production of labeled proteins (Se, <sup>15</sup>N, or <sup>13</sup>C;<sup>15</sup>N). Proteins are isolated and purified by semi-automated chromatography. In ~1200 trials starting with a correctly sequenced entry clone, purified protein was produced for X-ray/NMR studies with a success frequency of ~25%. With Ehime University and Cell-Free Sciences (Yokohama), CESG is also developing a wheat germ cell-free expression technology for semi-automated protein production. CESG's initial success rate (96 trials) in going from a sequenced clone to soluble protein has been 50% for (His)<sub>6</sub>-tagged proteins and 49% for GST-fusions including recovery of the target protein following protease cleavage versus 58% for the same set in *E. coli*. Supported by NIH GM64598.