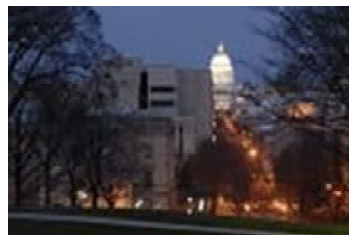


Methodology and Technology Development

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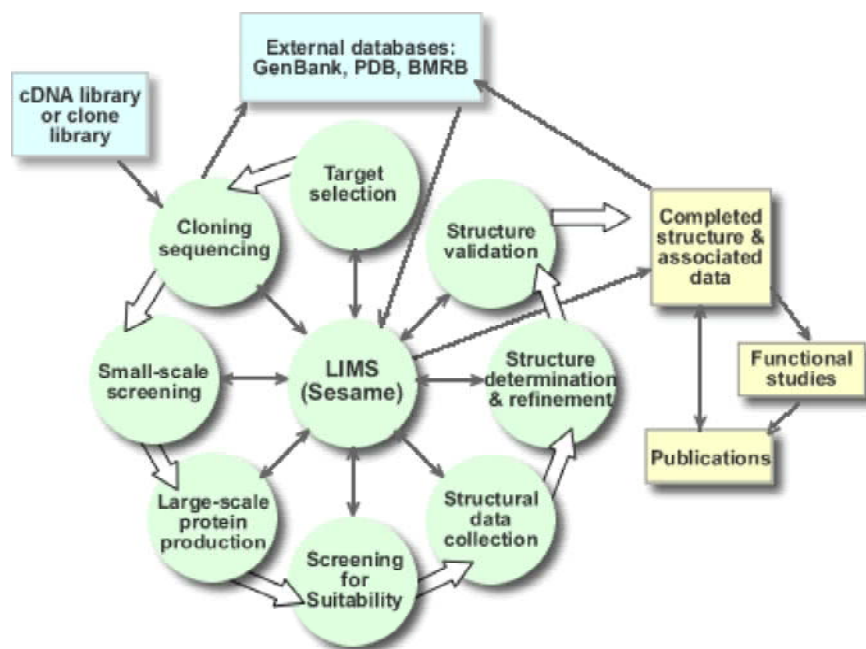
CESG performs research and development dedicated to providing eukaryotic targets for structure determination by NMR spectroscopy and X-ray crystallography. We have developed a multi-threaded platform that covers all steps from target selection to structure determination and data deposition. The platform supports single-step cloning leading to multiple vector possibilities, two complementary methods for producing protein (*E. coli* cell-based and wheat germ cell-free), and two complementary methods for structure determination (X-ray crystallography and NMR spectroscopy). CESG uses this platform as a test-bed to evaluate the performance of new technology as it is applied to challenging targets: soluble proteins, membrane proteins, and proteins containing membrane anchors or signal sequences from the genomes of humans and higher vertebrates. Targets progress through CESG's functional teams and can pass, or fail, at numerous points. All experimental data is recorded in [Sesame \(LIMS\)](#).

- [Bioinformatics](#)
- [Cell-Free](#)
- [Cloning](#)
- [Crystallography](#)
- [Expression Testing](#)
- [NMR](#)
- [Protein Production](#)
- [Protein Purification](#)
- [Quality Assurance](#)
- [Sesame](#)

Our extensive small-scale screening shows that 36% of all eukaryotic proteins are unsuitable for scale-up due to no expression and 11% are unsuitable because of low solubility. The remaining 53% of target proteins are expressed, soluble, and cleaved by TEV protease, and historically deemed suitable for scale-up. However, of this 53%, ~2/3 were eventually lost during purification for various reasons that will be reported. In order to address these different modes for loss of valuable protein targets, CESG is exploring several strategies and approaches, and wheat germ cell-free translation has been one effective way to increase the number of protein targets recovered.

In a cell-based approach, the modular CESG expression vector platform has been exploited to replace the MBP solubility tag with alternative solubility tags such as thioredoxin, trigger factor, and others. Research with these alternative tags is facilitated by side-by-side comparison with results obtained from CESG control workgroups composed of proteins markers for efficient function of each step in the protein production process.

In another cell-based approach, CESG bioinformatics and cloning methods were used to generate N- and C-terminal deletions of target proteins to improve their behavior. This approach has given new insight into the 3-dimensional structure of



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mammalian metallo-chaperones. The effectiveness of the domain engineering methods can be extended when coupled with the Promega Maxwell 16 Automation System to rapidly isolate and characterize purified proteins at the small-scale, and is often informed by evaluation of ^{15}N HSQC spectra.

Small-scale purification screening is also facilitated when coupled to bacterial expression in factorial evolved auto-induction medium, which can yield expressed protein levels as high as 1.5 mg/mL of culture fluid. In another approach, different affinity purification methods are being applied to recover proteins with intact cofactors and metal-binding sites. This approach has successfully yielded mouse and human Rieske-type ferredoxins, a novel fold not previously associated with higher eukaryotes.

For selected reports on the methodologies and technologies that CESG has developed:

[Technology Dissemination Reports](#)

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[Publications](#)

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