

Versatile medium-throughput strategies for recombinant expression screening in structural biology

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The increasing complexity of biological targets subject to structural characterization constantly demands highly versatile approaches of recombinantly expression and purification.

When targeting macromolecular complexes, the difficulties associated to the initial recombinant screening phase are further amplified by the need to identify suitable constructs to co-express or reconstitute in vitro the molecular interactions essential for complex stabilization. Large industry-scale high-throughput platforms offer extremely efficient and automated methods to obtain libraries of protein constructs for expression and purification scouting, whereas several academic research labs still rely on more conservative "one construct, one recombinant host, one target" approaches. Frequently, the choice depends on investments in automation, as the costs associated to creation of high-throughput facilities are not affordable to all research groups. Aiming at minimizing time and costs associated to the initial screening for recombinant expression; we optimized cloning and expression strategies to increase the throughput without the need of automation.

Our system consists of two major components: 1) a large library of expression vectors, based on a limited number of commercial backbones with customized expression cassettes allowing rapid switch of combinations of expression hosts, affinity tags and protein fusions to enhance target stability and solubility, coupled to standardized sub-cloning sites for easy genes transfer from one expression vector to another; 2) digital tools to facilitate design of DNA constructs for expression scouting.

In this talk, we will present the basic concepts behind the construction and functioning of our system, and we will briefly showcase it successful usage for the identification of optimal expression constructs for various ongoing structural biology projects in our lab, including extracellular enzymes, cytosolic macromolecular complexes, and membrane proteins.

Keywords: [protein expression](#), [macromolecular complexes](#), [membrane proteins](#)