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Library-Based Construct Screening for Soluble Protein Expression: Influenza Polymerase as a Case Study. Darren Hart. *High Throughput Protein Technologies, EMBL, Grenoble, France.*
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The ESPRIT construct screening technology has been developed at EMBL to identify soluble constructs of "difficult-to-express" protein targets that resist the classical approach of bioinformatics and PCR cloning. It employs the principles of directed evolution whereby a diverse random library of DNA constructs is generated and screened to identify rare clones of interest (soluble expressers) [1]. All unidirectional truncations of the target gene, both 5' and 3', are synthesized by exonuclease degradation to generate potential expression constructs. Additionally, a "scanning" version identifies internal domains. In each experiment, 30,000 individual constructs are assayed in parallel for yield and solubility using a highly automated colony array format.
Results will be presented on the influenza polymerase that has, prior to this study, proved intractable due to the absence of homologues required for multiple sequence alignments. Well-behaving proteins were produced encompassing previously unsuspected domains [2, 3, 4]. These were then characterized structurally and functionally, providing insights into the mechanisms of RNA replication, nuclear translocation and host-species determination.

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Keywords: protein expression; random libraries; directed evolution

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Promoting Crystallization by Increasing and Enhancing Intermolecular Interactions. Alexander McPherson. *Dept. of Molecular Biology and Biochemistry University of California, Irvine, California, USA.*
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One approach to promoting the crystallization of proteins is to try and enhance the number and strength of intermolecular contacts between macromolecules, or to eliminate intermolecular interactions, or interactions with solvent, that might inhibit crystallization. Site specific mutations of various sorts have been employed to accomplish this, as have truncations by genetic or proteolytic means. A host of impressive successes suggest this to be a profitable strategy for further development. There are, however, two significant problems attending this approach. First, and foremost, because the structure of the target macromolecule is unknown, there is no good basis for the design of mutants or truncations. Second, the approach requires that the protein be produced by recombinant DNA technology and an efficient expression system be available, which is frequently not the case.

We have attempted to address these issues by initiating experiments based on two ideas. The first is that a wide variety of conventional small molecules might be systematically introduced into mother liquors during crystallization screening. By incorporation into the crystal lattice, the additional intermolecular interactions that the small molecules provide might enhance crystal nucleation and growth. A second approach that we are pursuing is the chemical modification of various amino acid side chains using traditional protein chemistry. We believe that in some cases chemically modified proteins might be induced to crystallize, or crystallize better than the native protein. We are currently exploring a range of chemical modifications with the objective of deducing which of those might be practical and useful for promoting crystallization.

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Production of Recombinant Viral Proteins for Structural and Functional Studies. Bruno Coutard. *AFMB UMR6098 CNRS/Université Aix-Marseille I & II, Marseilles, France.*
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RNA viruses infecting human and animal can have a devastating impact on health and economy, as exemplified by the SARS-CoV or H5N1 viruses. New potential drug targets against these RNA viruses can be identified through comprehensive structural characterization of the replicative machinery. Since these proteins are naturally produced in the virus host as large poly-proteins and since the virus production can require the use of high bio-safety level facilities, this strategy implies the production of recombinant proteic domains into easy to handle expression systems. This presentation will describe the tools that were devised within the VIZIER consortium (http://www.vizier-europe.org/) to produce viral proteins for crystallographic and functional studies, and that could be, for most of them, applied for any kind of soluble protein. The first part will be devoted to the "multi-constructs" strategy enabling the design of single domains to be crystallized. We will then present how both expression level and solubility level can be optimized using respectively *in silico* directed evolution and Design Of Experiments (DOE) for the bacterial cultures. Following the purification stage, we will show how a pre-crystallization screening (buffer and binders screening) can provide functional information and improve the protein crystallization. Finally, several examples, highlighting its advantages and limits, will illustrate the overall strategy.

Keywords: viral replication enzymes; e. coli; recombinant protein