Library-based construct screening for soluble protein expression from Influenza ORFs TARENDEAU F., MERESSE, P., MAS P. & HART D.J. EMBL, 6 rue Jules Horowitz, Grenoble, France

We are developing empirical strategies for the determination of soluble constructs for protein production in E. coli using the principle of directed evolution whereby a diverse random library of genetic constructs is generated and then the rare clones of interest (soluble expressers) identified by high throughput screening. By removing the need for rational construct design, clones can be obtained from targets that resist the classical approach of bioinformatic analysis and PCR cloning. The ESPRIT (Expression of Soluble Proteins by Random Incremental Truncation) screening platform has been developed at EMBL and will be presented. All unidirectional truncations of the target gene, both 5' and 3', are synthesised resulting in a combinatorial library of expression constructs. Additionally, we

have developed a "scanning" method to identify internal domains. 30,000 individual constructs are assayed in parallel for yield and solubility using a novel high density, protein array format. Several results will be presented including the high-level soluble expression, biochemical characterisation and structural determination of a previously unsuspected domain from influenza polymerase. The results throw light on one mechanism of mutation-induced birdto-human transmission of influenza.