S007 Optimising expression of mammalian proteins for interaction studies

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A complete toolbox of open reading frames (ORFs) would be useful for several experimental approaches for mapping protein – protein interactions. Although good progress has been made in the assembly of large ORF collections, the expression and purification of full-length proteins in heterologous systems has been more problematic. One solution to this problem is the expression of single or tandem domains of large multi-domain containing proteins, which are often easier to express than their full-length parents. This requires knowledge of the structural domain boundaries which can be different to domain boundaries mapped by sequence alignment. A method for identifying protein fragments capable of soluble expression in *E. coli* will be described by random gene fragmentation and genetic selection. A domain mapping method will also be described to enable the expression of receptor ectodomains secreted from HEK293 cells. Two applications of the availability of protein family libraries cloned into expression vectors will be described. The first is the creation of a murine immunoglobulin superfamily (IgSF) receptor protein array and its use in mapping receptor – receptor interactions. The second describes the generation of single chain antibodies (scFv) specific for SH2 (Src Homology 2) domains and their use in mapping signal transduction networks by immunoprecipitation.