

P004 Engineering EGFP to produce a novel system for the detection of protein-protein interactions
Emma Barnard, David J. Timson, Neil V. McFerran and John Nelson
Queen's University Belfast, UK

The budding yeast (*Saccharomyces cerevisiae*) genome is small and fully sequenced. A wealth of information regarding yeast biochemistry is available, making yeast an ideal candidate for proteomic analysis.

In order to investigate interactions within the yeast proteome a split enhanced green fluorescent protein (EGFP) complementation assay was designed based on a similar ubiquitin complementation strategy. EGFP, a 238 amino acid protein, can be easily detected. A pair of compatible plasmids was designed to facilitate the use of split EGFP fragments in detecting interactions between yeast proteins. Vectors containing N- and C-terminal EGFP fragments and different yeast selectable markers have been engineered. Linear DNA fragments containing the hapto EGFP fragment and a marker have been produced and homologous recombination used to introduce these constructs into specific sites within the genome of a suitably deficient host. The system has been validated using the known interaction between phosphofructokinase subunits pfk1p and pfk2p. Linear DNA fragments encoding C and N fragments of EGFP were sequentially transformed into yeast at the 3' ends of the *PFK1* and *PFK2* genes respectively to yield active pfk fusion proteins whose interaction could be detected by fluorescence microscopy. After further verification the system shall be made available to the wider research community as a tool for investigating interactions within the yeast proteome.