

Protein Expression Core Facility



The Protein Expression Core Facility is currently completing its instrument purchasing and is expected to be able to begin operations shortly in order to provide a wide range of services to support IRB Barcelona researchers. Traditionally, researchers tackle a particular problem with a protein in an iterative process of trial and re-design that can potentially be time-consuming and costly. In contrast, the Facility will concentrate on delivering High Throughput (HTP) activities where many variations of an experiment (eg truncations or mutations of a protein) will be run in parallel. The capacity to perform up to ninety-six experimental variations on a theme, in parallel, can significantly decrease the time taken to solve a particular protein-related problem, thus bringing experiments to faster conclusions and, more importantly, leading to rapid publication of data. In addition to the time savings offered by HTP methods, they are also generally considered economical and can significantly reduce project and laboratory costs. In the future, the Facility will offer many high quality reagents eg aliquots of competent bacterio-phage resistant *E. coli* strains for cloning and expression, specialized expression media and cloning reagents for use by individual researchers.

The Protein Expression Core Facility commenced purchasing specialised equipment and laboratory instruments in September 2007. All the equipment required for implementation of the first 'phase' (HTP cloning and expression screening) of the Facility was ordered and a number of instruments were operational by the end of the year. A variety of custom-prepared reagents were also purchased and delivered. In addition, an advisory board was created to help direct the diverse research activities in order to ensure maximum benefit to users of the Facility.

Services for IRB Barcelona researchers

The Protein Expression Core Facility plans to offer the following services to IRB Barcelona researchers:

Custom HTP cloning to generate expression vectors

The Facility will provide some of the latest cloning technologies to simplify often complex cloning (DNA manipulation) procedures, thereby allowing them to be performed more easily, reliably and efficiently. These methods will allow the Facility to generate a micro-titre plate of 96 expression-ready clones within 1-2 weeks of receiving template and primers. The plate may be comprised of:

- constructs (defined regions plus fusion 'tags') of many different 'target' proteins from a single

organism under investigation (ie, a structural genomics approach)

- or, alternatively, multiple constructs of fewer, more complex proteins, where expression may have previously proven difficult and the researcher wishes to explore more expression options.

Constructs may contain defined N- or C-terminal deletions of the proteins in combination with a choice of 'fusion' proteins (eg, Maltose Binding Protein, MBP, Glutathione-S-Transferase, GST, or Small Ubiquitin-like Modifier, SUMO) to aid the solubility and yield of the proteins of interest. The In-Fusion™ cloning technique and the pOPIN suite of vectors (originally developed at the Oxford Protein Production Facility) will be used to generate expression constructs. A His₆ tag is included in all constructs to simplify parallel expression screening and protein purification.

Expression screening in *E. coli*

A micro-titre plate of 96 (Core Facility or user-derived) expression clones can be screened in *E. coli* in approximately one week. The screen generally consists of the use of two expression strains, with expression in each strain being tested using both IPTG and auto-induction methods. Additional (DE3) *E. coli* strains can be incorporated into the screening process if required.



Core Facility Members | Manager: Nick Berrow | **Research Technicians:** The Protein Expression Core Facility will be recruiting two technicians in 2008.

Expression screening in mammalian cells

A micro-titre plate of 96 (Core Facility or user-derived) expression clones can be screened in *HEK293* cells in 1-2 weeks. The introduction of this process is planned for the third quarter of 2008.

Other services to be introduced during 2008 and 2009 include: recombinant baculovirus generation, expression screening in *Sf9* cells, custom vector production and vectors for expression screening in *P. pastoris* or *K. lactis*.

Collaborations

Continued development of pOPIN vector suite
Ray Owens, Oxford Protein Production Facility (Oxford, UK)

Other future collaborations include:

Adaptation of HTP cloning and screening pipeline for use with membrane proteins
Manuel Palacín, IRB Barcelona (Barcelona, Spain)

*Adaptation of HTP cloning and screening pipeline for use with *P. pastoris* expression system*
Francisco José Fernández, IRB Barcelona (Barcelona, Spain)

The Facility also plans to offer many high quality reagents eg aliquots of competent bacterio-phage resistant *E. coli* strains for cloning and expression, specialized expression media (including auto-induction media and seleno-methionine) and cloning reagents for use by individual researchers. The purchase of these items through the Facility should also result in considerable cost savings for researchers compared to normal distributor prices.

The Protein Expression Core Facility is currently advising many groups within IRB Barcelona on cloning and expression techniques and hopes to build many more collaborations within the Institute, the Barcelona Science Park, the University of Barcelona and the Universitat Autònoma de Barcelona in the near future.

