NICK BERROW

Protein Expression Core Facility

The Protein Expression Core Facility Unit was founded to carry out High Through-Put (HTP) activities in which many variations of an experiment (*eg*, cloning and expression screening of truncations or mutants of a protein) can be performed in parallel. The capacity to simultaneously perform many experimental variations on a single theme can significantly decrease the time taken to solve a particular cloning- or protein-related problem, thereby bringing experiments to more rapid 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 cost-effective and can significantly reduce project and laboratory costs. Many of the protocols are automated, with the Facility making full use of liquid handling robotics for small-scale HTP plate handling and automated purification systems for larger scale protein purifications. The Facility also offers many high quality reagents for cloning and expression, competent bacterio-phage-resistant *E. coli* strains, specialised expression media and recombinant enzymes at prices substantially lower than commercial ones. We also offer custom cloning and vector modification services.

Figure 1. *A selection of instruments purchased for the Facility. Caliper LabChipGX for HTP protein, DNA and RNA analysis and documentation, Äkta Xpress systems for automated large scale protein purifications and Theonyx liquid handling robot for HTP expression screening, plasmid purification, etc.*

The Facility (founded in the autumn of 2007) moved into its current laboratory (Laboratory PBB12) when refurbishment was completed in spring 2008. Since then, the instruments required for its full operation have been purchased and staff have been recruited to ensure that the Facility can offer a full portfolio of services (Mª Carmen Romero and Raquel Garcia joined in October and December of 2008, respectively). Throughout 2008 the Facility has been implementing the first phase (mainly HTP cloning and expression screening in *E. coli*) of the planned services, which are now available as standard services (documented below).

The Steering Committee for the Facility approved an initial group of seven project applications of various sizes and complexity arising from our first call for projects, and many of these have been completed, or are nearing completion.

The Facility has already begun to deliver purified proteins to IRB Barcelona researchers and we hope to be able to deliver many more in the coming year. In addition, it has also completed many smaller scale cloning projects to help IRB Barcelona researchers and others from the local academic community.

The end of 2008 also saw the completion of equipment purchasing and installation for the tissue culture room. The Facility is now fully equipped for expression screening in mammalian

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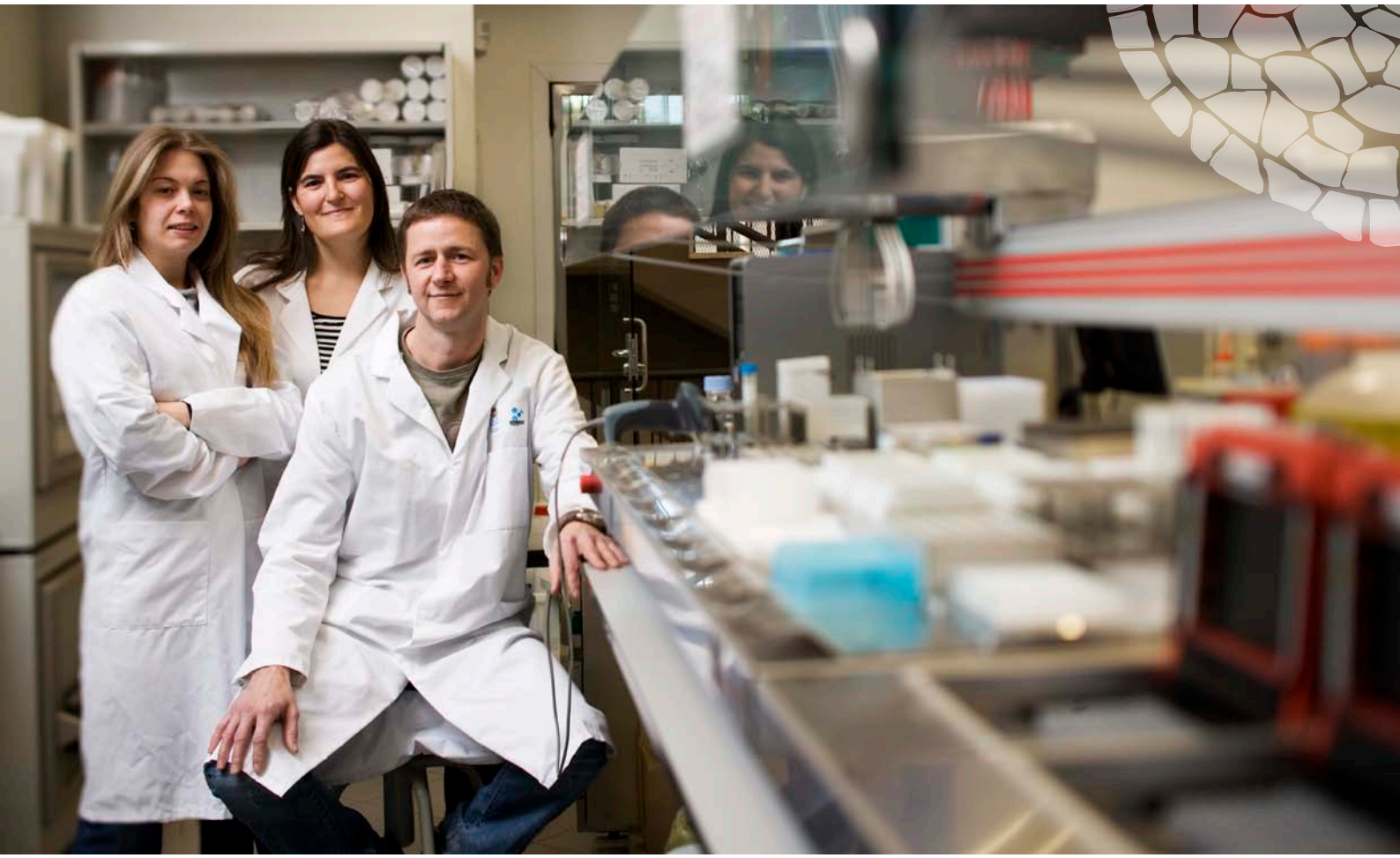
Research Technician Mª Carmen Romero



(HEK293) cells, the rapid production of recombinant baculoviruses and follow-up expression screening in insect (*Sf*9) cells.

Services offered to IRB Barcelona researchers

The services currently available include:

- **Custom HTP cloning to generate expression vectors.** The Facility has introduced 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 allow the Facility to generate a microtitre plate of 96 expression-ready clones within 1-2 weeks of receiving the template and primers. Vectors currently available include popular fusion proteins such as MBP, GST, SUMO, His-Tags (cleavable N- or C-terminal), GFP and Strep II tags to facilitate the solubility or yield of the proteins of interest and provide simple purification or detection strategies.

- **In-Fusion™**, a ligation and restriction enzyme-independent cloning technique, allows the precise production of user-defined constructs, including the production of mutant and chimaeric constructs.

- **Expression screening in *E. coli*.** A microtitre plate of 96 (Facility- or user-derived) expression clones can be screened in *E. coli* in approximately one week. The screen currently 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.

- **HTP plasmid mini-preparation-96 mini-preps** from *E. coli* pellets in less than 2 hours.

- **Custom protein expression and purification.** The introduction of common affinity 'tags' to proteins of interest during the cloning process enables the rapid purification of proteins at the milligram scale (dependent upon the particular protein being studied). Purity levels in excess of 95% are anticipated.

- **Expression screening** in mammalian, *eg*, HEK293, cells. A microtitre plate of 96 (Facility- or user-derived) expression clones can be screened in HEK293 cells in 1-2 weeks.

- **Other services** to be introduced early in 2009 include: recombinant baculo-virus generation and expression screening in insect (*Sf*9) cells and production of vectors for expression screening in *P. pastoris* or *K. lactis*.

The Facility also offers many high quality reagents for cloning, protein expression, protein labelling and also specialised *E. coli* competent cell strains (for expression or cloning) for purchase by individual researchers. In addition, we offer custom vector modification. Purchasing reagents through the Facility often leads to considerable cost savings for researchers.

The Facility has active projects with many IRB Barcelona teams, including the groups devoted to metabolic engineering and diabetes therapy, cell signalling, molecular pathology and therapy in heterogenic and multi-genic diseases, structural biology of proteins, nucleic acids and their complexes, experimental biology, and biomolecular NMR, in addition to local academic research groups. We hope to build many more collaborations within IRB Barcelona, the Barcelona Science Park, the University of Barcelona and the Autonomous University of Barcelona in the near future.

SCIENTIFIC OUTPUT

Collaborations

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)

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