

Nick Berrow



Protein Expression Core Facility

The Protein Expression Core Facility was founded to deliver 'High Through-Put' (HTP) cloning and expression screening 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. In addition, the facility has the expertise and equipment necessary to produce and purify milligram amounts of protein from prokaryotic and eukaryotic expression hosts (currently *E. coli*, Sf9 insect and HEK293T mammalian cells). Many of the protocols are automated, with the facility making full use of liquid handling robotics for HTP plate handling for small-scale (μg) expression screening and automated purification systems (Äkta Xpress) for larger (mg) scale protein purification. The facility also offers many high quality reagents for cloning and protein expression such as competent bacterio-phage-resistant *E. coli* strains, specialised expression media, and recombinant enzymes. It also offers custom cloning and vector modification services.

Since spring 2008, the facility has cloned almost 400 genes into expression constructs, most of which have been expression screened in at least one of our expression hosts. These expression constructs are also available for use either by the original investigator or for larger scale protein production and purification within the facility (in either *E. coli* or HEK293T cells). These include proteins from many different research projects with very different requirements; for example researchers may need seleno-methionine-labelled proteins for use in crystallisation and structure solution studies or secreted glycosylated proteins for use as tissue culture reagents.

The facility has already delivered purified proteins to IRB Barcelona researchers and this number is rapidly increasing. In addition, it has completed many smaller scale cloning projects to assist both IRB Barcelona researchers and others from the local academic community. During 2009 the baculoviral expression system was incorporated into the facility's services and we are in the process of optimising large-scale expression cultures of baculoviral-infected Sf9 cells, either in shaken flasks or in sterile 'wave' bags using an Appliflex Bioreactor.

The pPEU suite of In-Fusion-ready, multi-host, expression vectors was also developed during 2009, adding eleven new expression vectors to our vector list. Eight of these new vectors allow the production of fusion proteins with either enhanced Green Fluorescent Protein (eGFP) or Cherry fluorescent protein tags combined with either Hexa-Histidine or Strep-II tags. These vectors can be used in protein localisation studies (in conjunction with the Advanced Digital Microscopy Core Facility) or for the expression and purification of membrane proteins. The new pPEU1, pPEU10 and pPEU11 vectors can be used to produce GST C-His, N-His-thioredoxin or N-His Z-tag fusion proteins, respec-

tively. All pPEU vectors have been produced in such a way that they are compatible with the pOPIN expression vector suite (University of Oxford, UK).

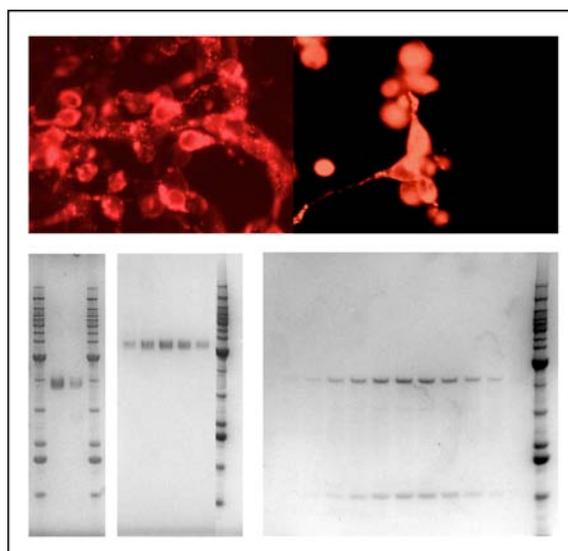
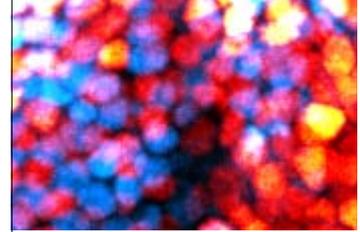


Figure 1. (Top panels) Using the pPEU vectors to study protein expression and localisation in HEK293T cells, two proteins fused to Cherry fluorescent protein are visualised by fluorescence microscopy. (Lower left panels) Purification of secreted and glycosylated proteins from HEK293T cells, SDS-PAGE gels of final products. (Lower right panel) Purifying protein dimers expressed in *E. coli* from bi-cistronic vectors. SDS-PAGE of purified products. Note that only the larger protein is his-tagged.



Services for IRB Barcelona researchers

Custom HTP Cloning to generate expression vectors

The In-Fusion™ ligation and restriction enzyme-independent cloning technique allows the precise production of user-defined constructs, including the production of mutant, chimaeric, and bi-cistronic (*E. coli* only) constructs. There are currently 18 pOPIN or pPEU vectors available.

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 two hours

Custom protein expression and purification at the milligram scale (depending on the particular protein being studied)

Purity in excess of 95% is anticipated for most proteins. The hosts currently available for large-scale expression cultures are *E. coli* and HEK293T cells; we are currently introducing large-scale insect cell culture to our list of services.

Production of seleno-methionine-labelled proteins for crystallographic structure determination in auxotrophic or prototrophic *E. coli* strains

Expression screening in mammalian (eg HEK293T) cells

A microtitre plate of 96 (facility- or user-derived) expression clones can be screened in cells in 1-2 weeks.

Research Group Members

Core Facility Manager:

Nick Berrow

Senior Research Officer:

Raquel Garcia

Technical Officer:

M^a Carmen Romero



Production of recombinant baculo-viruses either via the pOPIN or pPEU vector suites or from existing (eg pFastBac) constructs

Recombinant his-tagged 3C(PreScission) and SUMO proteases are available for the removal of fusion 'partners' from expressed proteins. We also hope to make the recombinant glycosidases PNGase and EndoF1 available for the removal of sugar moieties from recombinant proteins prior to crystallisation.

The facility also sources many high quality reagents, ranging from specialised *E. coli*-competent cell strains and reagents for protein expression to labelling and cloning reagents for use by individual researchers. Purchasing reagents through the facility generally produces considerable cost savings for researchers.

Scientific output

Collaborations

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

We collaborate with many different research groups to develop improved methods for the production, labelling and detection of proteins, and also improved cloning methods and expression vectors.