Equipment Name: Protein Expression Factory	**Category:** **B. Particle Labelling**
	Institute: University College Dublin
	Location: Conway Institute
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Short technology description/Overview:

The ExpressionFactory allows large scale, automated production of any one or many selected proteins (from > 10,000) to high purity.

With a unique combination of biology, software and instrumentation, the system fully automates and integrates the processes involved. Vector construction, protein expression and purification are performed within a single platform.

Expression of a particular protein can be difficult to achieve. The processes involved are complex, and specific proteins may require individual strategies for optimal expression. The unique features of the expressionfactory overcome these difficulties and limitations, and improve protein expression.

The expression factory adopts a matrix approach to protein expression and enables the user to investigate different combinations of vector construction, hosts used to express the protein, growth conditions and purification strategies, in a single run. The data generated is organised and controlled by the information management system (IMSTM) underpinning the expressionfactory, which removes the sequence, sample and protein-tracking problems usually associated with highly parallel expression.

The multi-vector strategy enabled by the expressionfactory allows the user to define the expression method which best facilitates production and purification of protein fit for purpose. In addition, the system provides the facility to engineer proteins to express domains of the molecule when the full length protein is insoluble or will not express. These features, in combination with the platform's advanced automation and software facilities, result in the systematic and parallel production of many hundreds of proteins in minimal time.

Within the bionano /nanosafety field, potential applications of the expressionfactory include:
- easily produce larger quantities of desired peptide and proteins efficiently and economically for an extended range of nanoparticle-protein fibrillation studies;
- develop panels of point-mutation proteins to determine exact binding sites with nanoparticles / receptors
- develop panels of point-mutation proteins to determine key sites for nanoparticle-induced protein fibrillation.

Main Features (Equipment Capabilities):

The expressionfactory platform combines all the hardware elements required for protein expression. The features include advanced liquid handling systems and thermocyclers for cloning and gene amplification, an incubator for cell growth, and a centrifuge and proprietary filtration system for protein recovery, solubilisation, and purification. Integrated robotics transfer samples between components, seamlessly linking each process together and removing the need for user supervision or intervention. The system can be accessed remotely, to result in the production of the desired protein in the desired form.

- PCR (including primer design)
- Gateway BP and LR reactions and additional cloning techniques
- Growth of E.coli cells
- Plasmid preparation (minipreps)
- Restriction digestion (single and double enzyme digestion)
- SAP and CIP treatment
- Klenow treatment
- Ligation
- Transformation of plasmids into competent E.coli cells
- Cell growth monitoring for E.coli by OD (600nm) and protein expression by fluorescence (GFP)
- Expression culture induction
- Cell lysis with detergent
- Separation of soluble and insoluble E.coli cell lysis fractions by centrifugation
- Sample preparation for SDS PAGE analysis
- Solubilisation of insoluble inclusion bodies
- Purification of proteins from fractions under native or denaturing conditions
- On-column refolding and purification of denatured proteins on a nickel affinity matrix
- Preparation of transposed baculovirus DNA for use in insect expression cultures (BAC to BAC system)
- Native protein purification on pre-made or commercial resins.
- Insoluble proteins may be solubilised and purified using automated affinity chromatography.

Instrumentation:

- CRS robotic arm
- Sias Xantas liquid handling system comprising of 8 channel pipettes
- Automated plate handling
- Four plate positon thermocyclers
- Cytomat for cell growth and monitoring
- Sias two plate centrifuge system
- Filtration station
- 'Smart Trough' system for purification and recover

Typical Samples & Images:

Any further Information:

This facility can also be accessed without physically coming to UCD, due to the robotic nature of the work. Should a User wish to request proteins be expressed as a service, that would also be considered.