TECHNIQUES AND STRATEGIES IN MOLECULAR MEDICINE

Date: 1,2 December 2010
Venue: UCD Conway Institute Lecture Theatre

Wednesday, 1 December (09:30–16:20)	
Session Chair: Dr Shane Duggan, TCD	
0930	**RNA detection and quantitation** Dr Shane Duggan, TCD
1010	**Differential gene expression: overview of relevant methods** Dr Karen Power, UCD
1050	Coffee/Tea
1120	**Polymorphism association with disease** Prof. Denis Shields, UCD
1200	**Model organisms** Dr Alison Reynolds, UCD
1240	Lunch break
Session Chair: Dr Ross McManus, TCD	
1320	**DNA cloning strategies** Dr Ross McManus, TCD
1400	**Imaging using Fluorescent/Confocal Microscopy** Dr Gavin McManus, TCD
1440	Coffee/Tea
1500	**RNA interference** Dr Stephen Maher, TCD
1540	**Systems Biology Approaches to Understanding Mammalian Signal Transduction** Dr Marc Birtwistle, UCD

Thursday, 2 December (09:30–16:50)	
Session Chair: Prof. Niamh Moran, RCSI	
0930	**Determining protein:protein interactions in biology** Prof Niamh Moran, RCSI
1010	**Protein expression and purification** Dr Henry Windle, TCD
1050	Coffee/Tea
1120	**Mass Spectrometry** Dr Giuliano Elia, UCD
1150	**Expression proteomics: biomedical applications** Prof. Steve Pennington, UCD
1230	Lunch break
Session Chair: Dr Anthony Davies, TCD	
1310	**Immunodetection methods on cell and tissue extracts** Dr Leonie Young, RCSI
1350	**Flow cytometry, cell sorting and flow imagine** Dr Alfonso Blanco, UCD
1430	**Stem Cells - Biology and Applications** Dr Linda Howard, NUI Galway
1510	Coffee/Tea
1530	**High Content Screening and Analysis Platform Technologies** Dr Anthony Davies, TCD
1610	**Transgenics and knockouts** Dr Tom Moore, UCC

Session 1

RNA Detection and quantitation
Dr Shane Duggan (Institute of Molecular Medicine, TCD)

The protein components of the cell are derived by numerous processes indirectly interpreted from a genetic element known as the "gene" which is coded in the cellular DNA. This element is interpreted by the cell in a process called "transcription" where the genetic code for a particular gene is converted into a molecular code known as messenger RNA (mRNA). This mRNA molecule can now be utilised in the creation of a new protein via the translation process. In this lecture the nature and analysis of Ribonucleic acid (RNA) in biological systems will be explored. The understanding of this has allowed the laboratory scientist to interrogate and explore gene expression as it may relate to diseases or cell signalling. Extraction and quantitation of good quality RNA will be discussed as they are the first step in any investigation of gene expression. Standard techniques in common use such as Northern blotting and cycle limited RT-PCR shall also be described as well as more modern techniques such as real time RT-PCR analysis. This lecture will allow the interpretation of published literature utilising these techniques and introduce the steps involved in performing RNA related techniques in your laboratory.

Differential gene expression: overview of relevant methods
Dr Karen Power (UCD Conway Institute)

This lecture will summarise the main approaches used to determine alterations in gene expression at the RNA level. Emphasis will be placed in this context on global approaches that attempt to map differences in the transcriptome, i.e. entire complement of transcripts in a cell. Methodologies that will be described include the more recent and popular approaches of DNA microarrays and high-throughput sequencing.

Review articles:
The use of profiling gene expression patterns;
Van't Veer, L.J. and Bernards, R. (2008)
Enabling personalized cancer medicine through analysis of gene-expression patterns
Nature, 453, 564-570

Challenges face in profiling tumour transcriptomes;
Coe, B.P., Chari, R., Lockwood, W.W. and Lam, W.L. (2008)
Evolving strategies for global gene expression analysis of cancer.
Journal of Cellular Physiology,

Experimental Design, Data preprocessing, Differential Expression Analysis, etc;
Grant, G.R., Manduchi, E. and Stoeckert, C.J. (2007)
Analysis and Management of Microarray Gene Expression Data
Current Protocols in Molecular Biology

Review of microarrays;
Schulze, A. and Downward, J. (2001).
Navigating gene expression using microarrays – a technology review.
Nature Cell Biology, 3, E190-E195.

Review on RNA-sequencing;
Wang, Z., Gerstein, M. and Snyder, M. (2009)
RNA-Seq: a revolutionary tool for transcriptomics
Nature Reviews Genetics, 10, 57-63

Polymorphism association with disease
Prof Denis Shields (UCD Conway Institute of Biomolecular & Biomedical Research)

Different strategies are required to identify rare and common genetic variants underlying both rare and common diseases. For common genetic variants, there is now a very rich dataset of identified common single nucleotide polymorphisms (SNPs). These can be investigated in disease groups (compared to controls) in candidate genes, or by whole genome association analysis, using chips with one million different SNPs on them. Analysis of these variants requires careful attention to the patterns of association of SNPs that are chromosomally adjacent (in linkage disequilibrium, meaning that the variant at one site is associated with a particular variant at another site, arising from the population history of the origin of the mutation, its spread through the population, and the gradual breaking down of the association of adjacent variants by recombination events). Linkage analysis (tracking in families the disease co-inheritance with widely spaced gene markers) is the traditional approach of choice for rare mutations that have strong phenotypic effects. High throughput sequencing of candidate regions (and in future whole genomes) are accelerating the rate of data accumulation.

Model organisms

Dr Alison Reynolds (UCD School of Biomedical and Biomolecular Sciences, UCD Conway Institute)

The aim of this lecture is give an overview of the advantages and disadvantages of the various model organisms which are routinely used in biomedical research. We will discuss the use of vertebrate and mammalian models and their application to the study of development and disease research. We will cover the generation of transgenic models of disease using both targeted methods of genetic manipulation and random screens. These methods have greatly improved our understanding of gene function. The various models used in vision research will be used as case studies.

Session 2

DNA cloning strategies

Dr Ross McManus (Institute of Molecular Medicine, TCD)

Even in the post genome era, DNA cloning is essential to the manipulation and stable propagation of genetic material. This talk will cover the basic aspects of DNA cloning, ranging from the anatomy of cloning vectors to the choice of vectors based on the cloning strategy employed. The strategy employed will depend on the overall objectives of the project and the nature of the starting information or material available. Thus different choices and approaches would be employed for a sequencing project compared with a genome mapping project or production of RNA or protein. I will discuss a number of basic and specialised cloning strategies to illustrate some of the options and possibilities available.

Imaging using Fluorescent/Confocal Microscopy

Dr Gavin McManus (TCD)

Fluorescence microscopy is an important and fundamental tool for biomedical research. Optical microscopy is almost non-invasive and allows highly spatially resolved images of organisms, cells, macromolecular complexes and biomolecules to be obtained. Generally speaking, the architecture of the observed structures is not significantly modified and the environmental conditions can be kept very close to physiological reality. The development of fluorescence microscopy was revolutionised with the invention of Laser Scanning Confocal Microscopy (LSCM). With its unique three-dimensional representation and analysis capabilities, this technology gives us a more real view of the world.

Suggested reading material:

Microscopy Primer Website http://micro.magnet.fsu.edu/primer/index.html (very good site with tutorials)
Handbook of Biological Confocal Microscopy, edited by James B. Pawley
Protein Localisation by Fluorescence Microscopy, edited by V.J. Allan, Practical Approach Series

RNA interference

Dr Stephen Maher (Institute of Molecular Medicine, TCD)

RNA interference (RNAi) is an evolutionally highly conserved process of post-transcriptional gene silencing, whereby double stranded RNA (dsRNA), when introduced into a cell, causes sequence-specific inhibition of mRNA sequences. The RNAi machinery, once it encounters a dsRNA molecule, cleaves it, separates the two strands, and then proceeds to target RNA molecules that are complementary to one of those segments. Target RNA molecules can be regulated by two mechanisms, degradation, or translational repression. RNAi has recently been applied as an experimental technique to "knockdown" gene expression in model organisms, allowing for experimental loss-of-function analysis in determining the cellular functions of specific genes. Therefore, dsRNA that trigger the RNAi mechanism have significant potential in disease therapeutics. This presentation will outline and discuss techniques and strategies underpinning the use of RNAi in molecular medicine.

Systems Biology Approaches to Understanding Mammalian Signal Transduction

Dr Marc Birtwistle (UCD Conway Institute)

Deregulation of mammalian signal transduction underlies a host of diseases such as cancer and type II diabetes. Although much work has gone into characterizing the pieces that make up such deregulated signaling networks, relatively little effort has gone into synthesizing the resultant data into a coherent and predictive view of how the signaling system functions as a whole to dictate cellular behavior. In this talk, I will outline how systems biology approaches can and are helping us to perform this synthesis task.

Recommended reading:

Kholodenko, BN, Hancock, JF, Kolch, W. Signalling ballet in space and time. Nat Rev Mol Cell Biol. 11(6): 414-26, 2010.
Alon U. Network Motifs: theory and experimental approaches. Nat Rev Genet. 8(6): 450-61, 2007.
Janes KA, Yaffe, MB. Data-driven modelling of signal-transduction networks. Nat Rev Mol Cell Biol. 7(11): 820-8, 2006.

Session 3

Determining protein:protein interactions in biology

Prof Niamh Moran (RCSI Research Institute)

Whereas the Human genome Project has successfully identified all human genes and their corresponding proteins, the next challenge in biology is to understand how these proteins interact with each other in a dynamic cell system. My lab is interested in the molecular mechanisms of integrin activation in the human platelet in order to better understand thrombotic disease. Using the platelet as a model system therefore, we explored various mechanisms of elucidating relevant protein interactions with integrin cytoplasmic tails. Current conventional methods are discussed in this presentation including co-immunoprecipitation, FRET, yeast-two hybrid system, TAP-Tags and protein chip technology. In addition, the emerging protein interaction websites provide a useful tool in this process. Verification of newly identified interactions is the next bottle-neck in the process and needs to be tailor made for each specific protein. This involves a combination of literature-searching, bio-informatics and basic cell-biology techniques. The conclusion of this presentation will highlight the importance of choosing an appropriate system for exploring protein:protein interactions; identifying relevant association and eliminating non-specific interactions, and finally emphasising the importance of relevant verification in this process. We have termed this process Protein ENONOMICS from the Greek term for interaction and claim that it is the next emerging 'omic' after proteomics!

Protein expression and purification

Dr Henry Windle (Institute of Molecular Medicine, TCD)

This lecture will cover the basics of protein expression and purification. Emphasis will be placed on alternative strategies and issues that should be considered prior to selection of specific expression systems and purification strategies. As protein purification methodologies are generally well described and accessible, only a brief overview of these will be given but with emphasis on common problems that can arise, particularly for those about to attempt purification for the first time. The following books from The Practical Approach series by IRL Press are an invaluable aid with detailed and reliable protocols: Protein Purification Applications; Protein Purification Methods (2001, Editor Simon Roe).
The always excellent Methods in Enzymology has recently published a comprehensive overview of protein expression and purification (Methods in Enzymology 463, 1-851 (2009): RR. Burgess and M P. Deutscher, Eds).

Mass Spectrometry

Dr Giuliano Elia (UCD Conway Institute of Biomolecular & Biomedical Research)

Mass spectrometry is a powerful technique for the precise and accurate determination of the molecular mass of a vast range of chemical compounds. The introduction, about two decades ago, of soft ionization techniques like MALDI (Matrix-Assisted Laser Desorption-Ionization) and ESI (ElectroSpray Ionization) allowed the widespread application of mass spectrometry to biological macromolecules, including proteins and peptides. Mass spectrometry is truly at the origin of the enormous success of proteomic science.
This lecture will present basic concepts in mass spectrometry, introduce the most common types of mass spectrometers used in biological research and discuss some simple mass spectrometry-based proteomic approaches.

Expression proteomics: biomedical applications

Prof. Stephen Pennington (UCD Conway Institute of Biomolecular & Biomedical Research)

Following the sequencing of the genomes of a large number of organisms including the landmark publications of the human genome it has become increasingly apparent that the study of their encoded proteins - on a genome-wide scale - is required. This is the field of proteomics. When, in 1994, Marc Wilkins coined the term 'proteome', defined as the protein complement of a genome, the methods for investigating protein expression on such a scale were in their infancy and today (a decade and a half later) they are still in rapid evolution.
The technique of two-dimensional gel electrophoresis (2-DE) remains a core for many applied proteomic projects due to its ability to separate simultaneously thousands of proteins and to indicate post-translational modifications that result in alterations in protein pI and/or Mr. Moreover, recent developments for 2-DE including the use of fluorescent dyes that facilitate the multiplex analysis of samples make it possible to achieve greater proteomic coverage combined with more accurate differential expression analysis.
Liquid chromatography (LC) based methods have also emerged as powerful approaches for protein expression analysis. Multi-dimensional LC approaches potentially have the advantage of higher coverage and sensitivity but are only recently being applied to the routine separation and quantification of very complex mixtures of proteins. In this presentation several biomedical applications will be described to show the utility of gel and LC based proteomics approaches including the use of isotope coded affinity tags. Together, these should illustrate the diversity of approaches available for the measurement of protein expression. The very recent development of a mass spectrometry based method to potentially measure all human proteins will be introduced.

Review articles
Cravatt BF, Simon GM, Yates JR 3rd. The biological impact of mass-spectrometry-based proteomics. Nature. 2007 Dec 13;450(7172):991-1000. Review.
PMID: 18075578
Elliott MH, Smith DS, Parker CE, Borchers C. Current trends in quantitative proteomics. J Mass Spectrom. 2009 Dec;44(12):1637-60. Review.
PubMed PMID:19957301.

Session 4

Immunodetection methods on cell and tissue extracts

Dr Leonie Young (RCSI)

The use of antibodies to detect and characterise proteins has been well established. With the development of high through-put techniques such as tissue microarrays (TMA), a real challenge now exists to determine the cellular location, level of expression and the function of these identified proteins. In this lecture, principles fundamental to immunodetection will be outlined. Common pitfalls and measures to avoid these will be discussed. Applications of immunodetection in a modern molecular context will be illustrated, including: western blotting, ELISA, immunohistochemistry/ immunofluorescence, tissue microarrays, co-immunprecipitation, Electromobility shift assays, chromatin immunoprecipitation (ChIP) and antibody arrays.

Flow cytometry, cell sorting and flow imagine

Dr Alfonso Blanco (UCD Conway Institute of Biomolecular & Biomedical Research)

Flow cytometry is a method for qualitative and quantitative analysis of components or structural features of cells, primarily by optical means, but also particles. Although it makes measurements on one cell at a time, it can process thousands of cells per second. Since cell types can be distinguished by quantitating structural and/or physiological features, flow cytometry can be used to count prokaryotic or eukaryotic cells of different types in complex mixtures.
Flow cytometry overview (PDF)

Stem Cells - Biology and Applications

Dr Linda Howard (NUI Galway)

Stem cells are relatively unspecialised cells lacking tissue-specific characteristics. Under appropriate conditions they can generate one or multiple specialised cell types in a process called 'differentiation'. Stem cells show enormous therapeutic potential based on their ability to generate cells for repair or regeneration of damaged tissues and organs. Stem cells can also be used as a source of human cells to study development and to look at human-specific toxicity testing if drugs.
This lecture will describe stem cells sources, characteristics and properties. Finally it will cover ongoing clinical trials designed to evaluate the safety and efficacy of stem cell therapies.
Other sources of reading
Online book on stem cells (free)
http://stemcells.nih.gov/info/scireport/2001report.htm
Online book on regenerative medicine (free)
http://stemcells.nih.gov/info/scireport/2006report.htm

High Content Screening and Analysis Platform technologies

Dr Anthony Davies (Institute of Molecular Medicine, TCD)

High Content Screening and Analysis (HCS(A)) is becoming synonymous with the reporting of biochemical events occurring within individual cells. This new technology now permits visualisation and quantitation of a wide range of target-specific responses on a per cell basis. HCA technologies are now capable of providing a highly detailed picture of a cells physiological state. This technology allows for the first time a quantitative assessment of gross morphology, sub-cellular target localisation, cell viability, apoptosis and cytoskeletal rearrangement of single cells in populations.
Here at IMM Trinity we have already successfully utilised HCA technologies in cell-based cancer, inflammation and infection cardiovascular and immunity studies.
In the Techniques and Strategies in Molecular Medicine course, we will cover the fundamental aspects of HCA technology deployment and utilisation. This will form a solid foundation, on which to build further knowledge and experience in this new and exciting scientific discipline.

Manipulating the rodent genome using transgenic and gene targeting techniques

Dr Tom Moore (Dept. of Biochemistry, University College Cork)

Manipulation of gene expression and function in living rodents is virtually a prerequisite for studying developmental genetics and pathology and the creation of rational models of human genetic diseases. However, the available techniques remain challenging and expensive. I will provide an overview of the underlying principles and practical applications of these techniques, which are usually applied to mice, but with increasing frequency also to rats and, to a lesser extent, primates. Techniques covered in this lecture will include electroporation, oocyte microinjection and lentivirus mediated transgenesis, gene targeting in embryonic stem cells, gene trap libraries, inducible vectors, shRNA, zinc finger nucleases, and an overview of available national and international centres engaged in large-scale production of mutants.