

Pre-masters Research Skills Course Timetable (Draft)

This timetable is a draft designed to provide you with an overview of the activities you will undertake. There may be slight variations on the final timetable.

Week 1

Molecular cloning and protein biochemistry

Students will be presented with a sample of GFP cDNA which contains a genetic mutation. Over the next five practical sessions they will amplify the cDNA using PCR, perform a ligation reaction and transform the cDNA into E.coli cells to generate cloned recombinant DNA. Recombinant and non-recombinant vectors will be distinguished using 'blue-white' screening. The recombinant vectors will be purified using an alkaline lysis procedure based on original concepts first developed in 1979 by Birnboim and Doly. The recombinant DNA will be analysed using restriction digest patterns to determine if the insert has been cloned in an orientation suitable for protein expression. The insert will also be sequenced to identify the mutations present at the nucleotide level. The cloned DNA will be expressed and purified using nickel affinity chromatography followed by SDS-PAGE analysis to estimate the molecular weight of the purified protein using molecular weight standards. Mass-spectrometry analysis will be performed to detect amino acid changes in the protein sequence followed by further bioinformatics analysis of the purified protein.

Staff: Chris Thompson (Day 1), Shazia Chaudhry and Dave Thornton

M	Intro to the lab Orientation Lab safety, basic pipetting, weighing, making solutions Prepare protein standard curve to check can do accurately	Lab skills Lab calculations Data Analysis Skills Excel spreadsheet and statistical analysis
T	PCR & Molecular Cloning Introductory Lecture: Molecular Cloning Part I (this will include concepts of how PCR works, how to design primers and steps required in order to perform cloning – will also include an overview of the next 5 days and the scenario). • PCR - To design and use the PCR	Techniques <ul style="list-style-type: none">• Molecular cloning skills• PCR• Agarose gel electrophoresis• Ligation• Transformation• Sterile Technique Lab skills <ul style="list-style-type: none">• Making solutions

	<p>reaction to amplify specific alleles in cDNA</p> <ul style="list-style-type: none"> • Primer Design – design two 21bp primers adding restriction enzymes sites • Agarose Gel Electrophoresis – analyse PCR results • Ligation • Transformation/Plate Cells (Grow o/n) - To distinguish between recombinant and non-recombinant vectors using 'blue-white' screening 	<ul style="list-style-type: none"> • Modify or design related experiments • Lab basics (calculations, pipetting, using a balance, etc) <p>Experimental design</p> <ul style="list-style-type: none"> • To develop experimental design skills by making experimental choices • Understand importance of experimental controls and how to interpret data by comparison with controls
W	<p>Clone Analysis Introductory Lecture: Molecular Cloning Part II (this will include the theory behind the mini-prep, analysing restriction digest patterns and how to determine if insert is in correct orientation and how sequencing works)</p> <ul style="list-style-type: none"> • Pick colonies early in day 8.00 - 8.30 am - Use sterile techniques to culture <i>E. coli</i> cells • Mini-prep DNA • Restriction Digests • Agarose Gel Electrophoresis - To demonstrate the success of the ligation of a DNA insert into a plasmid • Analyse results - To determine the orientation of the DNA insert relative to the plasmid backbone • Setup Sequencing (ON) - To determine the underlying genetic mutation 	<p>Techniques</p> <ul style="list-style-type: none"> • Mini-preps • Restriction Digests • Agarose gel electrophoresis • Sequencing <p>Lab skills</p> <ul style="list-style-type: none"> • Making solutions • Modify or design related experiments • Lab basics (calculations, pipetting, using a balance, etc) <p>Experimental design</p> <ul style="list-style-type: none"> • To develop experimental design skills by making experimental choices • Understand importance of experimental controls and how to interpret data by comparison with controls
T	<p>Protein Analysis</p> <p>Introductory lecture: 'How to purify a protein'</p> <ul style="list-style-type: none"> • Purify poly His-tagged protein 	<p>Techniques</p> <ul style="list-style-type: none"> • Protein purification by Ni²⁺ affinity chromatography • SDS-PAGE • Western blotting • Proteinase digestion

	<ul style="list-style-type: none"> • To analyse the column fractions under UV light to detect which fractions contain the GFP-tagged fusion protein • Analysis of purification by SDS-PAGE and Western blotting - To estimate the molecular weight of the fusion protein by comparing its electrophoretic mobility with that of several proteins of known molecular weights • Digest protein for mass spectrometry 	
F	<p>Protein Analysis</p> <ul style="list-style-type: none"> • Analysis of purification by SDS-PAGE and Western blotting <p>Introductory lecture: 'Protein identification by mass spectrometry'</p> <ul style="list-style-type: none"> • Perform MALDI-TOF and tandem mass spectrometry analysis of protein digest 	<p>Techniques</p> <ul style="list-style-type: none"> • Western blotting (continued) • Reverse phase chromatography (Zip tips) for the purification of tryptic peptides • Preparation of purified peptides (and standard peptide mixture) for MALDI-TOF and tandem mass spectrometry <p>Assessment</p> <p>Determine molecular weight of the His-tagged protein from the SDS-PAGE. Prepare a slide to present the data on Monday. How does this compare with the value estimated from the insert size from the cloning data?</p>

Week 2

M	<p>MONDAY OF WEEK 2</p> <p>Informatic analysis of protein, DNA and RNA data</p> <p>Introductory lecture: 'Analysis of mass spectrometry data'</p> <ul style="list-style-type: none">• Analyse mass spectrometry results• Using mass spectrometry data to determine protein sequence <p>Bioinformatic analysis of DNA and RNA</p> <ul style="list-style-type: none">• Analyse Sequencing results (from day 3)	<p>Data Analysis Skills (Protein)</p> <ul style="list-style-type: none">• Protein identification from mass spectrometry data <p>Techniques (Protein)</p> <ul style="list-style-type: none">• Bioinformatic analysis of data<ul style="list-style-type: none">• Introduction to BLAST searching• Introduction to MASCOT• Introduction to SCAFFOLD• <i>De novo</i> protein sequencing <p>Assessment (Protein)</p> <ul style="list-style-type: none">• Determine the sequence of peptides from model tandem mass spectro-metry data and then perform a BLAST search to identify the protein. <p>Data Analysis Skills (DNA & RNA)</p> <ul style="list-style-type: none">• Detection of mutation using DNA sequence <p>Techniques (DNA & RNA)</p> <ul style="list-style-type: none">• Comparing wild-type to normal sequence by hand and also using CLUSTALW etc alignment software to corroborate findings <p>Assessment (DNA & RNA)</p> <ul style="list-style-type: none">• Determine the mutated sequence and what type of mutation is present highlighting exactly how the wild-type sequence has changed
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Week 2 continued

Cell biology: Protein localisation using immunofluorescence and green fluorescent protein chimeras

Students are provided with cDNA constructs encoding a variety of proteins that have been tagged with green fluorescent protein, and will express these by transient transfection of tissue culture cells. The aim is to **determine the localisation of the chimeric molecule** with reference to antibodies to a range of subcellular organelles and cytoskeletal structures.

Lisa Swanton

T	<p>Cell biology revealed by immunofluorescence</p> <p>10:00 – 11:00 am Talk: overview of the practical and introduction to immunofluorescence microscopy.</p> <p>11:00 – Practical: fix cells & perform immunofluorescence labelling of untransfected cells (emphasis on controls). Split cells onto glass coverslips ready for tomorrow.</p> <p>Data Analysis: prepare electronic 'atlas' consisting of fluorescence light microscopy images of different organelles/structures, obtained from papers in Journal of Cell Biology.</p>	<p>Techniques use of antibodies in immunofluorescence microscopy</p> <p>Lab skills</p> <ul style="list-style-type: none">• Making solutions• Lab basics (calculations, pipetting, using a balance, etc)• Mammalian cell culture <p>Experimental design</p> <ul style="list-style-type: none">• To develop experimental design skills by making experimental choices• Understand importance of experimental controls <p>Data Analysis Skills Use of the literature Figure preparation</p>
W	<p>Transfection with GFP labelled chimera</p> <p>10:00 – 10:30 Talk: introduction to transfection</p> <p>10:30 – 12:00 Practical: transfection of cells with chimera.</p> <p>1:00 – 1:30 Talk: demonstration of image acquisition using fluorescence microscope.</p> <p>1:30 – 3:30 Practical: fluorescence microscopy of labelled cells (from yesterday), and cells expressing chimera. Take image of cells</p>	<p>Techniques transfection, immunofluorescence microscopy</p> <p>Experimental design</p> <ul style="list-style-type: none">• To develop experimental design skills by making experimental choices• Understand importance of experimental controls and how to interpret data by comparison with controls <p>Data Analysis Skills how to interpret data by comparison with controls</p>

	<p>expressing chimera, and controls if sufficient time. <i>NB: timing here depends on ratio of students to microscopes. If there is plenty of time, and students enjoy the microscopy, could also look at other group's slides.</i></p> <p>Experimental design: develop hypothesis as to localisation of chimera with reference to electronic atlas. Choose primary antibodies to test localisation hypothesis.</p>	<p>How to develop a hypothesis</p>
T	<p>Immunofluorescence labelling 10:00 – 11:00 Group discussion: students outline their hypothesis (with reference to electronic atlas) and experiment to test this.</p> <p>11:00 – Practical: fix transfected cells & perform immunofluorescence labelling using antibodies to subcellular markers.</p>	<p>Techniques immunofluorescence labelling</p> <p>Lab skills</p> <ul style="list-style-type: none"> • Making solutions • Modify or design related experiments • Lab basics (calculations, pipetting, using a balance, etc) <p>Experimental design</p> <ul style="list-style-type: none"> • To develop experimental design skills by making experimental choices • Design experiments to test a hypothesis.
F	<p>Fluorescence microscopy</p> <p>10:00-3:00 Practical: fluorescence microscopy of transfected, antibody-labelled cells.</p> <p>3:00 – 5:00 Group discussion: comparison of data - does it support hypothesis or does hypothesis need revision? Why additional experiments to support hypothesis, and types of approaches that could be taken.</p> <p>Homework: revise hypothesis as appropriate, design experiment to provide additional evidence to support localisation hypothesis.</p>	<p>Techniques Fluorescence microscopy</p> <p>Data Analysis Skills</p> <ul style="list-style-type: none"> • How to interpret data in relation to a hypothesis.

Week 3

From genotype to phenotype: Using mutants to understand the regulation of gene expression during growth and development

Students will be given gene sequences and instructed how to use blast searches to determine the organism they came from and to formulate hypotheses about their potential function. To test their ideas they will be introduced to the importance of understanding when and where these genes are expressed and the power of reverse genetics to test their function. Model systems will be *Neurospora* and *Drosophila*

Sue Crosthwaite and Hilary Ashe

M	BANK HOLIDAY	BANK HOLIDAY
T	<p>9am -11am Database searches (ncbi, <i>Neurospora</i> genome sequence) to identify a gene (<i>heat shock factor-2</i>) and obtain information on its possible function</p> <p>11-11.30 Introductory Lecture “Development in <i>Neurospora</i>- a model eukaryote”</p> <p>11.30am-12 noon Observe wildtype and <i>hsf2</i> mutant under the light microscope.</p> <p>1pm-5pm Harvest tissue and compare the expression of specific genes (<i>hsf2</i>, <i>vegetative and asexual development-2</i>, <i>actin</i>) over a developmental timecourse in wildtype and <i>hsf2</i> strains.</p> <p>Extract RNA</p> <p>Assay expression of genes with roles in development by semi-quantitative RTPCR.</p> <p>Homework: bioinformatics analysis of the sequence of a <i>Drosophila</i> developmental gene (encoding the Bicoid transcription factor)</p>	<p>Techniques Blast and domain searches RNA extraction Semi-quantitative RTPCR</p> <p>Lab skills Lab basics (calculations, pipetting, etc)</p> <p>Experimental design Using bioinformatics to inform experimental design. Understand importance of experimental controls</p>
W	<p>9am-12.30 Analyse and compare expression profile of genes in the WT and mutant</p>	<p>Techniques Agarose gel electrophoresis In situ hybridisation</p>

	<p>Neurospora strains</p> <p>Begin preparation of RTPCR results in figure format</p> <p>(Afternoon) Introductory lecture 'Drosophila development'</p> <p>Start in situ hybridization of Drosophila embryos</p> <ul style="list-style-type: none"> • Prepare mixed stage embryos for hybridization with RNA probes • Hybridise sense and antisense RNA probes overnight 	<p>Lab Skills Lab basics (calculations, pipetting, etc) Understand importance of experimental controls</p> <p>Data Analysis Skills NIH image for quantification of PCR products Statistical analysis of class results</p>
T	<p>In situ hybridization of Drosophila embryos</p> <ul style="list-style-type: none"> • Wash off probe and incubate with an AP-conjugated antibody which recognizes the probe • Remove the antibody, wash and detect specifically bound antibody using reagents which generate a coloured product <p>Analysis of the phenotype of mutant Drosophila embryos by cuticle analysis: prepare cuticles of wildtype and bicoid mutant embryos, photograph</p> <p>Homework: predict expression patterns of Bcd target genes based on the Bcd binding consensus and enhancer sequences</p>	<p>Techniques Drosophila in situ hybridisation Cuticle preparation of Drosophila embryos</p> <p>Lab Skills Lab basics (calculations, pipetting, etc) Understand importance of experimental controls</p> <p>Experimental Design •To develop experimental design skills by making experimental choices •Understand importance of experimental controls and how to interpret data by comparison with controls</p>
F	<p>9am-12.30 Collection and analysis of in situ results Preparation of figure Integration of data to make a hypothesis about the developmental role of Bicoid</p> <p>1pm-5pm Presentation of figures and conclusions from RTPCR and in situ experiments</p>	<p>Lab Skills Acquiring embryo images</p> <p>Data Analysis Skills Figure preparation</p> <p>Assessment Feedback on analysis and presentation of gene expression results.</p>

Week 4

Evolutionary biology and microbial genetics

Students will be tasked with trying to determine what might limit evolutionary success (e.g costs/pleiotropy). Two examples they will be given are antibiotic resistance (in *E.coli*) and social cheating (in *Dictyostelium*). In both cases it is intuitive to imagine that both antibiotic resistance and cheating are advantageous. The students will study this by measuring fitness (counting success) between genetically distinct strains under different competitive situations or environments. They will measure success after competition by genotyping the strains given gene sequences. Different techniques for genotyping will be used (e.g. visual markers, reporter genes, PCR, etc). Because the measures are quantitative, students will be introduced to more quantitative skills (e.g. serial dilutions, statistical analyses)

Chris Thompson and Danny Rozen

M	<p>Lecture: Fitness and pleiotropy in evolution</p> <p>Antibiotic Resistance in E.coli: determine antibiotic sensitivity of different <i>E.coli</i> strains by serial dilution of antibiotic</p> <p>E coli growth competition: Set up competitions between different <i>E.coli</i> strains +/- antibiotic in liquid culture</p> <p>Lecture: Reporter genes</p> <p>Competition in Dictyostelium: Set up clonal and chimeric development between different marked <i>Dictyostelium</i> strains</p>	<p>Techniques</p> <p>microbiological sterile technique</p> <p>Lab skills</p> <ol style="list-style-type: none">1.Cell dilutions and volumes3.Use of controls in experiments4.Serial dilutions <p>Experimental design</p> <ul style="list-style-type: none">•To develop experimental design skills by making experimental choices•Understand importance of experimental controls and how to interpret data by comparison with controls
T	<p>Determine resistance of bacteria to antibiotic (MIC)</p> <p>Fix and stain <i>Dictyostelium</i> slugs and fruiting bodies</p> <p>Plate out <i>E.coli</i> to determine numbers of different <i>E.coli</i> strains after growth</p>	<p>Techniques</p> <p>microbiological sterile technique Whole mount lacZ embryo staining</p> <p>Lab skills</p> <ol style="list-style-type: none">1.Cell dilutions and volumes2.Use of controls in experiments3.Serial dilutions <p>Experimental design</p>

		<ul style="list-style-type: none"> •To develop experimental design skills by making experimental choices •Understand importance of experimental controls and how to interpret data by comparison with controls
W	<p>Lecture: qPCR</p> <p>Set up qPCR to genotype abundance of Dictyostelium</p> <p>Microscopy: take pictures of Dictyostelium slugs and fruiting bodies</p> <p>Quantify numbers of different E.coli strains</p>	<p>Techniques</p> <p>qPCR</p> <p>Data Analysis Skills</p> <p>Use of the literature</p> <p>Figure preparation</p>
T	<p>Workshop: How to develop your own research proposal</p> <p>Give students different research questions that they are to go away and develop a research proposal</p> <p>Time to work on research proposal</p>	
F	<p>Presentation day!</p>	