Laboratory Project in Biochemistry

The aim of this project is to provide an introduction to the design, plan, implementation and presentation of a laboratory investigation. You will be given background information on the project. You must then take responsibility for the designing and planning of experiments, preparation of reagents, budgeting, collection and analysis of experimental data. You will need to assess the reliability of your data and, if necessary, repeat measurements to ensure reliability. There will be two postgraduate demonstrators, assigned to guide you in experimentation process and discuss methodology, costs etc. Remember that some of the project choices you make are within the field of expertise of these demonstrators. You will also need to liase with the Teaching Lab technician, to order reagents etc.
For the laboratory project, you will be required to work as a member of a team of 2-3 individuals. Each team will be responsible for overall planning of the project, experimental design, time management and budgeting (£150 / pair). You will need to check with the demonstrator assigned or the Teaching Lab Technician or myself before you place any orders). A lab book will be provided for you to keep a record of your day-to-day activities, which will be inspected during the project as part of the assessment process. At the end of the investigation, you must submit an ***individual*** project report. This report should contain all the results obtained by the team but the write-up should be an independent piece of work. Guidelines for the report are provided in this handout.

Project Assessment

50%	Final report
5%	Design of experiments
5%	Critical appraisal skills
5%	Data analysis and interpretation
25%	Ability / performance in lab • 10% Effort • 5% good lab practice • 5% lab records • 5% team work
5%	Development (further) problem solving skills
5%	Originality / flair for experimentation or initiative shown

A good project report requires skill in the handling and presentation of your own results (involving data analysis, the use of tables, figures and plates, their description and interpretation). It requires skill in the use of literature in the field of study and ability to discuss the results in this context. A good project investigation depends on you developing and displaying a variety of skills. Various things will be looked for, such as your technical and experimental capability; your ability to design and carry out experiments; your ability to develop a productive work pattern; your contribution to the development of the investigation; your ability to innovate; team work, independence etc.

Timetable

The laboratory work for this project should ***ideally*** be carried out in weeks 2-8 of the semester, leaving weeks 9-12 for the write-up. The teaching lab is available on *Mondays* and *Fridays*; it might be possible to work for limited periods at other times by *prior arrangement*. Reports must be submitted to the Department office by **Tuesday 8th May 2007**. Retrospective applications for extensions cannot be accepted except under extenuating circumstances.

Laboratory Safety

1. You must not work in the lab without supervision. An academic supervisor (myself), the postgraduate demonstrator or a technician must be present while you are in the lab. If you are unsure about the safety of any procedure or you need assistance in the use of equipment, ask for advice before you begin.

2. All microbiological work must be carried out under conditions of Good Microbiological Practice. You must use appropriate aseptic techniques when handling viable organisms, all spillages must be cleared up using appropriate decontaminants, all unwanted cultures must be autoclaved before disposal.

3. Risk assessments have been carried out for the standard procedures described. Use appropriate precautions (safety glasses, gloves etc) when handling strong acids/ alkalis/ reagents (including acrylamide), mutagens and carcinogens.

4. If you decide to use any additional procedures not described below, you must assess any potential risks involved and special precautions that might be required. Discuss the procedure with a supervisor or demonstrator before beginning any experimental work

Project 1: Extremophile research

Searching for photolyase activity in halophiles

Ultraviolet light is absorbed by nucleic acids producing two major photoproducts, cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts. Although a variety of complex DNA repair mechanisms (e.g. nucleotide excision repair) allow organisms to survive uv radiation, photoreactivation is the simplest and perhaps most important strategy used by some of the oldest species like archaea. It is catalyzed by a single protein called photolyase, which uses the energy in a photon of light to chemically break apart a pyrimidine dimer in DNA. Photoreactivation probably represents the earliest type of UV repair system because many species, from bacteria through marsupials, show the presence of photolyases. Humans and other placental mammals do not seem to have a photoreactivation process, but the photolyase gene is highly conserved and may have evolved to play a role in the excision repair process.

While *Escherichia coli* is a model organism for investigation in the domain bacteria, archaeal DNA repair mechanisms still remain to be characterised and their relationship to bacteria or eukarya are still largely unknown. Halophilic archaea that thrive in hypersaline habitats, in particular, are subjected to intense solar radiation in their natural environment, making them good models for investigating DNA repair. The archaeon *Haloferax volcanii* is a halophile that thrives in high salt environments, requiring salinities up to ten times those of seawater. The *Halobacterium* NRC-1 genome contains two photolyase homologues, *phr1* and *phr2*, both members of the Class I family of photolyases

Work done do far:

Previous studies in the lab have identified novel *phr1* and *phr2* photolyase-like homologues for the first time in *H. volcanii,* but their structure/function is yet to be determined. It is thought that they could encode CPD photolyases, (6-4) photolyases, or one of the closely related cryptochromes.

NOTE: **This project has a few subprojects that can be carried out by a pair of students in any one of the following areas**

1) Clone and express the full length *phr1* or *phr 2* from *H.volcanii* in a suitable vector system (Charlotte Bennnett report 2005).
2) Structural modelling of phr1 and phr2 proteins (this is for anyone interested in structural bioinformatics and they MUST have done the ***Proteins and Immunochemistry*** option)
3) Development of *in vitro* photoreactivation assays in *E.coli* that can be applied to halophiles.
4) Expression and purification of recombinant photolyases from an *E.coli* construct supplied by Aziz Sancar group

References:

1) DNA repair & mutagenesis *by EC Friedberg* et al (1995) pgs 91-169; 367-399
2) Menck CFM Shining a light on photolyases NAT GENET 32 (3): 338-339 NOV 2002 (http://wos7.mimas.ac.uk/isicgi/CIW.cgi?PigeIoJYy@AAAAFfJas_C75A62C7_PigeIoJYy@AAAAFfJas-0&Func=Abstract&doc=1/9)
3) Park, H.W et al. "Crystal Structure of DNA Photolyase from E coli". Science, **268**, 1866-1872 (1995)
4) http://www.microbiol.unimelb.edu.au/staff/mds/HaloHandbook/index.html **(methodology on growth conditions of *Haloferax volcanii*)**

PROJECT 2 Neuroscience: Genetic and behavioural studies in *C.elegans*

As you may remember from the BB20028 practical, the model organism *C. elegans* is very useful for studying the molecular and genetic basis of behaviour, such as movement, mechanosensation and chemosensation. We have mutants in genes encoding various receptor subunits and genes that remain uncharacterised in both genetic and behavioural characteristics.

Project 2A: Role of *hif-1* and *adar* genes in mediating response to hypoxic stress

Hypoxia is an important physiological state that has serious implications in various conditions such as stroke, resistance to tumour radio and chemotherapy as well as a risk factor in the onset of dementias such as Alzheimer's or Parkinson's disease. The key transcription factor involved in the regulation of hypoxic pathways is HIF-1 (hypoxia inducible factor), a heterodimer made of a αregulated by cellular oxygen) and β (constitutively expressed) subunits. A *D.melanogaster* screen identified another adenosine deaminase RNA editing enzyme (*adar-1/2*) as being sensitive to hypoxia. This enzyme is known to edit the mRNA of glutamate-gated chloride channels (GluCl), which are inhibitory ionotropic receptors found in invertebrates. Mutations in these channels result in resistance to ivermection, an anthelmintic drug. Mutations in genes encoding this channel (*avr-14* and *avr-15*) are known to confer resistance to ivermectin. Currently however, neither homologue of the *C.elegans* ADAR genes (adar-1/2) have been shown to be responsible for GluCl channel editing in *C.elegans*. Therefore, ivermectin resistance in *adr-1/2* or *hif-1* mutants may indicate a role of these enzymes in GluCl channel editing and may also indicate a common pathway in hypoxia recovery. Recent preliminary data suggests that *hif-1* mutants are hypersensitive to ivermectin whereas *adar* mutants are more resistant (*C.elegans* projects by R Pratt, A Hartt, S Harker and J Grey 2005). The aim of this project is therefore to expand this observation further and devise novel assays of examining this effect. At least 2 pairs of students could be working on this project.

1) Nature (1998) 394: pp485-490 (Role of HIF-1 in apoptosis etc)
2) PNAS (2000) 97: 2674-2679 (genetics of ivermectin resistance)
3) Nature Reviews Genetics (2003) 4: 181-194 (ADAR and hypoxia)
4) ANYAS (2003) 995: 191-199 (hypoxia signalling in C.elegans)

Project 2B: Glutamate receptors and the control of *C. elegans* locomotion

The nematode also has a plethora of glutamate receptors, both excitatory (AMPA and NMDA receptors) and inhibitory (glutamate-gated chloride channels). We have mutations in several genes encoding these receptors, some of which have opposite effects on behaviour. The question arises, what happens if you make double mutants? Is one phenotype dominant over the other? Are the worms still viable? Then project will involve simple behavioural assays and some genetics. Since the phenotype of many of the mutants is not obvious, the results of genetic crosses may need to be analysed via genomic PCR, so some molecular biology will also be involved.

Project 3: Neuroscience – does tyrosine hydroxylase act as an oxygen sensor

The pathological hallmark of Parkinson's disease is the loss of mainly dopaminergic neurons in the substantia nigra pars compacta, resulting in severe depletion of dopamine in the striatum. The first rate-limiting enzyme involved in dopamine synthesis is a highly specific $_L$-tyrosine hydroxylase (TH), which converts $_L$-tyrosine to $_L$-DOPA (l-dihydroxyphenylalanine), which is then converted to dopamine by a more general decarboxylase. Crucially however, the key rate-limiting enzyme, TH, depends on molecular oxygen for catalysis, in the presence of tetrahydrobiopterin. Oxygen-sensitive cells, such as the carotid body, are also known to up-regulate TH levels under hypoxic conditions. However, the fundamental question of how neurons sense reduced oxygen levels outside the cell is still unknown. The pivotal pathway shown to mediate hypoxia sensing intracellularly, involves a key transcription factor called hypoxia-inducible factor1 (HIF-1), which functions as a master switch that allows neurons to respond to falling levels of oxygen. HIF1 is a heterodimeric protein consisting of 2 subunits, HIF1α and HIF1β of which the alpha subunit acts as a key regulator in the cellular adaptation to hypoxia. Degradation of HIF-1α is triggered by the post-translational hydroxylation of conserved amino acid residues within key polypeptide domains. Studies so far have suggested that prolyl and asparaginyl hydroxylases (enzymes that hydroxylate conserved proline and asparagine residues respectively on HIF1α) modify the oxygen-dependent degradation domain (ODD) and second carboxyl-terminal transactivation domain (C-TAD) of the HIF-1αsubunit respectively. Hypoxia, however, blocks the activity of both these enzymes, thereby allowing HIF-1α stabilisation and expression of target genes. Both enzymes use oxygen as a key substrate for catalysis. Critically, since oxygen is the key rate-limiting step for enzyme activity, the suggestion is that these enzymes act as oxygen sensors that provide a direct link between extracellular oxygen concentration and intracellular hypoxia-responsive signalling pathways.

Tyrosine hydroxylase as an oxygen sensor: Tyrosine hydroxylase (TH) is one enzyme whose role as an oxygen sensor has been largely ignored so far. This is especially relevant in Parkinson's disease considering the selective degeneration of TH-containing dopaminergic terminals in hypoxia-related PD. There is strong evidence in favour of TH enzyme may act as an oxygen sensor (outlined below)

1) *Specificity in localisation*: TH is specifically localised in dopaminergic terminals in the nigro-striatal pathway, which selectively degenerate in PD.
2) *High specificity for oxygen*: Tyrosine hydroxylase is the first rate-limiting enzyme for the synthesis of dopamine, crucially dependent on molecular oxygen for catalysis.
3) TH is a HIF1 target gene, with several hypoxia response elements (HREs) in its promoter sequence, thereby suggesting that TH would be responsive to hypoxia.
4) *Highly conserved tyrosine*: The amino acid residue tyrosine 798 (Y798) is highly conserved across all HIF□ species and could potentially be the critical residue that mediates silencing of the HIF1□ subunit

The objective of this project is to initially show the differential expression of TH under hypoxic conditions in either an oxygen sensitive cell line or embryonic neuronal cultures using RT-PCR. If this is successful, siRNA techniques could be developed to show the functional relevance of this enzyme as an oxygen sensor.

References:

1) Semenza GL. Targeting HIF-1 for cancer therapy. ***Nat Rev Cancer*** (2003 Oct) 3(10):721-32.
2) Semenza GL. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. ***Cell.*** (2001 Oct) 5; 107(1):1-3.

PROJECT 4: Does hypoxia induce expression of proapoptotic BNIP3 in neurons?

The Nip3/BNIP3 protein is a pro-apoptotic member of the Bcl-2 family induced during hypoxia via the hypoxia-inducible factor (HIF) 1. BNIP3 has been linked to both apoptotic and necrotic cell death involving mitochondrial permeability transition. Apoptosis can be induced in response to hypoxia. The severity of hypoxia determines whether cells become apoptotic or adapt to hypoxia and survive. Neurons however, are especially sensitive to any fluctuations in oxygen level and show cell death via apoptosis. Constitutively expressed BNIP3 may participate in apoptotic and necrotic processes after brain ischemia. This project involves identifying a novel role for BNIP in hypoxia-induced apoptosis in neuronal cultures. Initial characterisation will be done in neuronal cell culture models and then repeated in rat primary cortical cultures, if possible. The techniques involved will be RNA preps, RT-PCR, Western Blots and function survival assays in vitro.

References:

Sharp FR, Bernaudin M. HIF1 and oxygen sensing in the brain NATURE REVIEWS NEUROSCIENCE 5 (6): 437-448 JUN 2004

Bruik R. K. Expression of the gene encoding the proapoptotic BNIP3 protein is induced by hypoxia. Proc. Natl. Acad. Sci. USA, *97:* 9082-9087, 2000.

PROJECT 5: Tail regeneration in Xenopus laevis: Gene cloning and expression analyses

Xenopus laevis embryos have been the favoured model for investigation into early embryogenesis for over 100 years. This is mainly due to their large size and ease of micro-dissection. Many of the fundamental principles of vertebrate development arose from experiments on these embryos. Moreover, Xenopus tadpole tail is an excellent model to study regeneration. Recent results show there is no de-differentiation or transdifferentiation during tail regeneration, suggesting that cellular processes in tadpole regeneration is very similar to tissue repair in mammals (*Gargioli 2004,* and *Chen, 2006)*.

The aim of this project is to clone potentially important genes (*Xsna* and *N-cam*) from Xenopus embryos. After we get full-length sequence of these genes, we make antisense RNA probe to detect their expression pattern both in normal embryos and regenerating tails by in situ hybridization (ISH). Whole-mount in situ hybridization is used to detect the spatial and temporal activation of specific gene in embryos or tissues. The principle ISH is the specific annealing of a labelled nucleic acid probe to complementary sequences in fixed tissue, followed by visualization of the location of the probe. Compared to immunohistochemistry, it is straightforward to derive the specific probes of ISH from known gene sequence. Thus ISH has become an invaluable tool for analyzing experimentally manipulated embryos or tissues.

General reference for the project:

1) Early development of Xenopus laevis: A Laboratory Manual by *HL. Sive* et al (2000) pgs 249-274, COLD SPRING HARBOR LABORATORY PRESS
2) Molecular Cloning: A Laboratory Manual by *J Sambrook* et. al (2001), COLD SPRING HARBOR LABORATORY PRESS
3) *Gargioli C and Slack JM.* Cell lineage tracing during Xenopus tail regeneration. *Development.* 2004, 131(11):2669-79.
4) *Chen Y, Lin G, Slack JM* Control of muscle regeneration in the Xenopus tadpole tail by Pax7. *Development*. 2006 Jun; 133(12):2303-13.

PROJECT A: *Xsna (Xenopus snail)*

The neural crest (NC) yields pluripotent cells endowed with migratory properties. During embryonic development, they give rise to neurons, glia, melanocytes and endocrine cells, and to diverse 'mesenchymal' derivatives. The earliest sign of the prospective neural crest of *Xenopus* is the expression of the ectodermal component of *Xsna* (the Xenopus homologue of snail) in a low arc on the dorsal aspect of stage 11 embryos. Professor Jonathan Slack's group is very interested in spinal chord regeneration of *Xenopus* tail. Whether and how neural cells participate in regeneration of spinal chord remains unclear. *Xsna* can be used as a marker for neural crest cell to trace the role of neural cells during regeneration.

1) Expression of Xenopus snail in mesoderm and prospective neural fold ectoderm. LJ Essex, *et al. Dev Dyn.* 1993, 98(2):108-22.
2) Neural crest cell plasticity and its limits. NM LeDouarin, *et al Development,* 2004 131(19):4637-50.

PROJECT B: NCAM

The Neural-cell adhesion molecule (NCAM) is a cell-surface glycoprotein. It could mediate some intercellular adhesive interactions in the nervous system. According to immunohistochemistry work, the anti-NCAM were found to specifically recognise the satellite cells on mouse muscle tissue. Muscle satellite cells are shown to be responsible for muscle regeneration. Upon injury, muscle satellite cells are activated and withdraw from quiescent state to proliferate, differentiate and fuse to form new myofibers.

Professor Jonathan Slack's group is also interested to study molecular and cellular events in muscle satellite cells during regeneration. NCAM could be a cell specific marker of satellite cells to study their roles in muscle regeneration.

1) Regenerating and denervated human muscle fibres and satellite cells express neural cell adhesion molecule recognized by monoclonal antibodies to natural killer cells. *Illa I et al. Ann Neurol.* 1992, 31(1):46-52.
2) Neural cell adhesion molecule in normal, denervated, and myopathic human muscle. *Cashman NR et al. Ann Neurol.* 1987; 21(5):481-9.

PROJECT 6: The Grb10 Mysteries

Grb10 is a cytoplasmic adaptor protein which mediates growth factor signalling. Grb10 plays a fundamental role in regulating growth, development and metabolism, and mis-regulation of the *Grb10* gene has been implicated in some cancers. *Grb10* is also interesting because it is an imprinted gene. In most tissues of the mouse embryo, expression of *Grb10* is predominantly from the maternally-derived allele, but in the central nervous system, expression is from the paternally-derived allele. The reasons for this unique expression pattern, which has not been observed for any other gene, are not clear but are likely to be highly significant in mouse development.

Project 6a: *Grb10* and the Mystery of the Short Transcript

Key techniques:

- RNA isolation
- Probe design, amplification and cloning
- Northern blotting

We have generated a transgenic mouse line, termed 'Smudger', in which full-length transcripts of *Grb10* have been ablated. Heterozygous Smudger mice in which the deletion allele has been inherited from the mother are a third larger than their wild type siblings at birth, illustrating the huge influence *Grb10* has on growth and development. However, Northern blotting has revealed a mysterious short transcript of the gene which has not been ablated in Smudger.

To characterise this short transcript, we generated a second transgenic line termed 'Jumble' which aimed to ablate all the *Grb10* transcripts. Comparison of Jumble and Smudger reveal some significant differences in growth, development and even in behaviour, but we have not yet unequivocally shown that the short transcript has been successfully ablated in Jumble.

Project 1 addresses this issue. Students will design, clone and purify probes, and use them in Northern blotting to confirm that the short transcript is not expressed in Jumble mice. Once this has been shown, the project can be extended to examine the expression of other genes known or suspected to interact with *Grb10*, using the same technique. These experiments are fundamental to our studies if we are to attribute the phenotype of Jumble mice to the absence of the short transcript. Help solve the mystery of the *Grb10* short transcript!

Project 6b: *Grb10* and the Puzzle of the Lean Mice

Key techniques:

- RNA isolation
- Probe design, amplification and cloning
- Northern blotting
- Histology

This project is closely linked to Project 1. Previous experiments in our lab have shown that both *Grb10* knock-out lines, Smudger and Jumble, have less adipose tissue than wild type mice. These lean mice present us with an interesting hypothesis: *Grb10* might be partly responsible for the regulation of fat metabolism and storage.
We want to investigate this intriguing possibility by asking if the leanness is due to a lack of adipocyte differentiation. This project will involve the design, amplification and cloning of probes for fat markers, which will subsequently be used in Northern blots. If time/materials permit, we hope to perform histological examination of some tissues for fat content.

Project 6c: *Grb10* and the Enigma of CNS Expression

Key technique:

- Cloning (restriction digests, ligations, transformations, DNA purification etc.)

As described in the General Background, the expression pattern of *Grb10* in the mouse embryo (paternal expression in the CNS, mainly maternal elsewhere) has not been observed in any other gene to-date. One key question we want to address is how this complex system of expression is regulated.

We aim to generate a mutant mouse line in which a regulatory region of the *Grb10* gene is disrupted. Our hypothesis is that this regulatory region is responsible for expression from the paternally-derived allele in the CNS. If this is true, disruption of this region should generate a phenotype which might also help us address the significance of paternal expression in development.

Project 3 involves a key contribution to this work, helping to build the targeting construct which contains the modified regulatory region. A range of cloning techniques will be employed to 'stick' components of the construct together, providing a grounding in fundamental molecular biology techniques.

The expression pattern of *Grb10* is interesting both intellectually and in the context of mammalian development and disease. The implications of fully understanding this expression might well help to shape future directions in imprinting research.

Starter ref for Project 6:

Charalambous, M. *et al* (2003). Disruption of the imprinted *Grb10* gene leads to disproportionate overgrowth by an *Igf2*-independent mechanism. Proc. Nat. Acad. Sci.100 (14):8292-8297.

Guide to Laboratory Project Write-up

The project report

Reports should follow the standard format of a final year project, comprising of the following

One single copy should be submitted. The first page must be a title page, clearly outlining the full title of the project, unit code, name of investigator, year, date, degree and name of unit convenor.

The report should be typed (single sided, double-spaced) on A4 paper, pages numbered at the top. All pages, including graphs, diagrams, tables must carry a clear margin of not less than ½" (1cm) on all sides, preferably 1 ½" (3cm) LH margin. Failure to observe this may result in material being cut out during page trimming. The exact **title** of the report must be submitted to me 5 weeks before the end of semester 2, so that covers can be typed. Students must ensure that this title and that on the title page agree and that the supervisor has approved this. Please indicate clearly which course you are doing at the time of registering your title.

Where possible, reports should be concise. **Projects should not exceed 8,000 words (c.32 pages) of text**. Although this does not include diagrams, tables, references and appendices, these should be kept within reasonable bounds. Ingenuity in data presentation is rewarded. An over-long project report may be viewed as evidence of inability to cope with the challenge of presentation.

The report should be written concisely, avoiding undue verbosity, especially in the 'Introduction'. Keep sentences short and avoid pompous phrases such as "at this moment in time". Avoid all jargon and do not try to make the text more interesting by using familiar language. If you can précis a paragraph then do so. You should put each section to one side and reread it a couple of weeks later to judge its clarity objectively.

The project usually contains the following sections:

- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- References
- Acknowledgements
- Appendices

- **Abstract**

The length is usually no longer than 300 words and summaries the theme of the problem and the results. Do not use abbreviations (other than for units) unless you first make clear what it means. Do not include references. Write the abstract **last** when you know what you have described in the report.

- **Introduction**

This section should consist of two main parts, the review of literature and the aims & objectives.

Review of Literature

Aim for about 10% of the report. It should appraise the work relevant to the subject of the project. It should serve as a preamble to the problems being investigated. Avoid too much detail of material that will be included in the discussion section. The main directions of your experimental work should dictate the amount of detail on each aspect. It is the background to this experimental work that must be reviewed in depth here. See later section on references for the preferred method of quoting references.

Aims & objectives
This should describe in 200-400 words the theme of the project at the start of the work and your main objectives. This should clearly demonstrate the link from the previous work into what you are reporting on. It is difficult to write but it should be good because it is often the first part of the report to be read and, therefore, gives the first impression. The introduction can be divided into sections dealing with particular aspects if this is advantageous but try and maintain continuity.

- **Materials and Methods**

This section should describe clearly and concisely, but above all clearly, the main materials and methods used in the research so that someone else could repeat your experiments. You should include details of all bacterial strains used in your work and information on suppliers & reagents. There should be a brief description and a reference to any standard methods, with complete details of any modifications you have introduced or new methods that you have developed.
You **should not** give experimental results in this section unless they explain or illustrate special points, provide data for calibration curves or serve similar purposes. Quotation of references should be kept to a minimum. Include a description of the statistical methods employed with relevant references. Do not make this a story of what you did each week.
This is the section in which a good deal of sub-division and writing as notes are acceptable provided that both are not over-done. Take care to state exactly the reagents used, their concentration and, where relevant, how they are used e.g. $MgSO_4.7H_2O$ and not $MgSO_4$ when the hydrated salt is used. Although you must include all necessary detail, avoid irrelevancies.

- **Results**

This section should describe what you did and should be arranged in a **logical** order that need not be chronological. The design of this section provides the backbone of the report. The section is usually divided into sub-sections of related experiments. Avoid excessive division.
An experiment is usually best described as follows
1. Give a short statement of objectives
2. Describe briefly how you did the experiment
3. Show the results in writing, or usually in Tables or Figures (do not repeat data in both tables and figures)
4. Abstract and summarise the significant results from (3).
5. End, if appropriate, with a short statement of conclusions and follow with a lead sentence or paragraph to the next experiment. ***Do not give Discussion here i.e. interpretation or speculation.***

Tables and Figures
must be self-contained and understandable on their own, i.e. ***there must be a figure legend***.
Equally it should be possible to know the main findings from the text.
Should be carefully compiled and designed to leave no room for misinterpretation and to lighten the reader's task. The simpler they are the better. If possible, arrange the Table so that important results occur close together and well separated from less important results.
Title should be concise but give the key facts about its contents.
Headings of columns should be accurate, ***including units of measurement*** where appropriate, and, if necessary, explained by footnotes.
Numerical data must be supplemented by statistics unless the latter are omitted for good reasons that should then be given in the text.
Figures should be used only when they have advantages over Tables
Figures should be self-explanatory, both axes and contents should be labelled clearly and accurately.
For graphs, use sufficiently large symbols and connecting lines that will not confuse the reader.
Always refer to Figures, Graphs or tables in the text.
It is legitimate to give brief numerical results in the text if they do not justify a table or figure.

Photos of gels should have all lanes labelled and a relevant explanation in the legend. Molecular weights should be given in numbers down the side of the gel (not just a lane labelled MWT!). Bands discussed in the text should be indicated with an arrow or other appropriate sign.

- **Discussion**

For many people this is the most difficult part of the report to write. It is usually difficult because it requires analysis, not a statement, of the results. There should be an assessment of the significance of your work, especially in relation to that of others, a restatement of the problem as a consequence of your findings and a forecast of how the research might be developed. Avoid a discussion that is too long and little more than a shortened version of parts of the Experimental section. Aim for no more than 10% of the report and preferably less. Discussions can be divided to correspond with major divisions of the Experimental section but do so sparingly because the main function of the discussion is to present a coherent and critical view of the particular problem that has been studied. Make sure you state what the results do not mean as well as what they do.

- **References**

Any one of the following 2 methods is acceptable and should be quoted in the text
1) The Harvard system, as detailed in handouts from the library.
2) References may be numbered in ascending order in the text and numerically listed in References.

- **Acknowledgements**

You should acknowledge the help given by people who have provided you with materials for your work, who have helped you with ideas or time.

- **Appendices**

This can contain recipes for different solutions that were used in the research, detailed descriptions of techniques that were described briefly in the Materials and Methods section. This section may also contain raw data, which appears in the processed form in the report. These are useful repositories and can keep the project shorter.

University Policy on Plagiarism

Please see the University's web site for its policy on plagiarism. The following is intended only as a helpful summary.

1. Coursework, dissertations and essays submitted for assessment must be the student's own work, unless in the case of group projects a joint effort is expected and is indicated as such.

2. Unacknowledged direct copying from the work of another person, or the close paraphrasing of somebody else's work, is called plagiarism and is a serious offence, equated with cheating in examinations. This applies to copying both from other students' work and from published sources such as books, reports or journal articles. Plagiarised material may originate from any source. It is as serious to use material from the World Wide Web or from a computer based encyclopaedia or literature archive as it is to use material from a printed source if it is not properly acknowledged.

3. Use of quotations or data from the work of others is entirely acceptable, and is often very valuable provided that the source of the quotation or data is given. Failure to provide a source or put quotation marks around material that is taken from elsewhere gives the appearance that the comments are ostensibly one's own. When quoting word-for-word from the work of another person quotation marks or indenting (setting the quotation in from the margin) must be used and the source of the quoted material must be acknowledged.

4. Paraphrasing, when the original statement is still identifiable and has no acknowledgement, is plagiarism. Taking a piece of text, from whatever source, and substituting words or phrases with other words or phrases is plagiarism. Any paraphrase of another person's work must have an acknowledgement to the source. It is not acceptable to put together unacknowledged passages from the same or from different sources linking these together with a few words or sentences of your own and changing a few words from the original text: This is regarded as over-dependence on other sources, which is a form of plagiarism.

5. Direct quotations from an earlier piece of the student's own work, if unattributed, suggests that the work is original, when in fact it is not. The direct copying of one's own writings qualifies as plagiarism if the fact that the work has been or is to be presented elsewhere is not acknowledged.

6. Sources of quotations used should be listed in full in a bibliography at the end of the piece of work and in a style required by the student's discipline area.

7. Plagiarism is a serious offence and will always result in imposition of a penalty. In deciding upon the penalty, the University will take into account factors such as the year of study, the extent and proportion of the work that has been plagiarised and the apparent intent of the student. The Student Discipline Committee can impose a range of penalties for plagiarism.

Laboratory code for project students (by Felicity Veazey)

I hope you enjoy your time while working on your project in the teaching laboratories. As we will not be able to supervise you at all times, even if you needed it at this stage in your career, here are a few guidelines so that we can all work in the same labs without any conflict.

Firstly please keep an eye on the teaching timetable. There will be times when the lab is full in which case for your comfort and ours please stay away. Usually we should be able to accommodate you in one lab or the other but please check before moving in. If you are working while another class is taking place please respect this and keep quiet and out of their way. Remember that these other students will be learning from your example so for this reason and for your own safety please remember to obey the lab safety rules.

When you start your project you will be given a safety sheet which you will be expected to read and must sign, so agreeing to abide by the safety rules of the lab. This is for all our protection. Please hand these sheets in to the laboratory staff before you start work. You have a duty of care to the prep. room, decontamination staff and the porters so please obey the disposal rules. No glass or sharps in the autoclave bags, hypodermic needles and razor blades must be placed directly into sharps bins. Nothing carrying bacterial contamination must be put down on the bench. Please make sure that used pipettor tips are discarded immediately into autoclave bags and not placed on the bench. We will provide you with autoclave bags and labelled containers for washing up and decontamination so please dispose of used plastics and glassware appropriately.

If you borrow equipment, which you are usually welcome to do, please return it in good condition or let us know if any repair is needed. You may have to book certain items of equipment if they are very popular or if they are needed for class. Please be prepared to return anything if asked to do so, we will try to give you adequate warning. There are incubators and water baths in the labs but please check before changing the temperatures and please remove anything you have put into them when it is finished with. If you are using one over a long period please put a note on it in case we think you have forgotten about it. You may also use the fridges and freezers in the labs but again please label everything clearly and throw away left over solutions etc when you have finished with them. Please clear out the fridges and freezers completely at the end of your project.

Please keep your work surfaces clean and put everything into cupboards when you are not working in the labs. I'm afraid there is not enough space for you to have a dedicated piece of bench to yourself. There are lap tops available for use. These may be connected to the internet at some of the points on the back bench. Please be careful and do not take these computers onto benches where lab work is taking place.

If you need something you can't find come to the prep. room and we will find it or order it for you. All the teaching technicians are first aiders so please let us know if you need treatment. The lab is usually open from 8.30 and officially closes at 5.30 however if you need to negotiate for a later finish come and see us, it is usually possible to arrange this. Project students are not admitted to the labs at weekends except by special arrangement and in the company of their supervisor. When you start your project you will be given a costing sheet. Please write down the consumables and chemicals you have used on this as you go along so that we can reclaim the cost.

Thank you and good luck

Felicity Veazey (Chief Technician)

PROJECT CHOICES FORM

NAME:.....

COURSE:.....

PROJECT PARTNER NAME (OPTIONAL):.....

PROJECT CHOICES

1.....

2.....

3.....

I will try and match your project according to your choices. In case there is more than one group interested in the same project, the allocation will be based on a separate discussion / meeting of the groups with the supervisor.

If you need any further clarifications, please get in touch.

Projects 5 and 6 will be especially suitable for students who have not done a placement since they will be supervised in the lab on a regular basis by the postgraduate demonstrators.

Dr. Momna V Hejmadi