EXPERIMENT FEST

HANDBOOK
BIOLOGY

THE UNIVERSITY OF
NEWCASTLE
AUSTRALIA

newcastle.edu.au/experimentfest

INTRODUCTION

Experiment Fest is an experiment program designed to provide enriching educational experiences for senior high school students who are studying Physics, Chemistry, Biology, Earth and Environmental Science, and Food Science.

Experiment Fest is supported by the University of Newcastle's College of Engineering, Science and Environment, and takes place at both the Callaghan and Ourimbah (Central Coast) campuses of the University of Newcastle.

All experiments are complemented by notes, follow-up discussions and questions to enhance your learning experience.

CONTENTS

WELCOME

Welcome to the College of Engineering, Science and Environment at the University of Newcastle.

Experiment Fest is a wonderful chance to give you practical experience which complements your classroom learning while giving you a first-hand look at University life and facilities. Science is an exciting field of study, allowing you to move with the times and contribute actively and responsibly to society. There are many education opportunities in science after high school. Here in the College we provide study and research programs in fast-moving modern fields that make our world work.

The College staff and students who will be taking you through the experiments today are involved in contemporary science research. Please ask questions and utilise your time with them.

Take this day to enjoy being out of the classroom, exploring science with fellow students and participating in valuable experiments and discussions which will help you in your HSC and beyond.

I wish you well in your studies. I hope you apply yourselves to the learning process with enthusiasm and you enjoy your time at the University. We hope to see you studying with us in the future!

Best wishes,

Professor Craig Simmons

Pro Vice-Chancellor
College of Engineering, Science and Environment
The University of Newcastle

STUDYING BIOLOGY

WHY STUDY BIOLOGY?

Biotechnology and Biological Sciences investigate and utilise the science of life. Biologists study the world of living organisms, ranging from microbes to human beings. Biotechnologists utilise their biology and chemistry based knowledge to use living cells to generate novel products for use in human health, environmental care and agriculture. The discipline of Biological Sciences provides you with a great deal of hands-on learning and exposure to engaging research, moving you beyond just textbook and lecture based learning, and into the laboratory to experience the latest molecular biology techniques. Course work provides you with the fundamental framework of knowledge, while the more research intensive Biotechnology Program also provides you with practical hands-on experience.

OPPORTUNITIES FOR FURTHER STUDIES

The Bachelor of Biotechnology and Bachelor of Science degree programs (Biology major) at the University of Newcastle provide a foundation of knowledge, skills and attributes that allows graduates to be employable not just in today's rapidly changing job market, but into the future, in a way that you will be actively and responsibly contributing to society. Within the Biotechnology and Bachelor of Science Biology major programs, you have the opportunity to sample and/or specialise in any one of the following fields:

- Animal Biology
- Animal Research Science
- Biochemistry
- Biophysics (double major)
- Biostatistics (double major)
- Cell and Molecular Biology
- Environmental Biology
- Genetics
- Molecular Microbiology
- Park and Wildlife Biology
- Patent Law
- Plant Biology and Ecology
- Reproductive Biology and Medicine

RESEARCH IN BIOLOGY

We conduct research on a wide range of contemporary biological problems. Staff are grouped into five major research themes, however, extensive collaborative links exist across these five themes. Our major research includes:

- Drug Design and Discovery
- Environmental and Conservation Biology
- Microbiology
- Plant Biology
- Reproductive Biology

Biology is a strength of the University of Newcastle, recently receiving the highest possible ranking for biological research in the Excellence in Research for Australia by the Australian Research Council. This recognition is testament to the world-class researchers who contribute teaching into both the Bachelor of Science (Biology) and Biotechnology Programs. Biology also continues to maintain strong research partnerships with industry, offering student exposure to these industry partners during the undergraduate programs.

CAREERS IN BIOLOGY

The College of Engineering, Science and Environment cares about our students and are interested in giving as much direction as possible to those making career choices and beyond. The possible career paths listed below include a range of opportunities for graduates at degree, honours, and post graduate study levels.

- Animal Biologist / Research Scientist
- Biochemist
- Biologist / Marine Biologist
- Biotechnologist
- Botanist / Plant Scientist
- Bushland Assessment Officer
- Clinical Research Coordinator
- Conservationist / Ecologist
- Environmental / Ecological Biologist
- Forensic Biologist
- Geneticist
- Laboratory / Research Assistant
- Medical Scientist
- Microbiologist
- Molecular Biologist
- National Parks Ranger / Field Officer
- Pharmaceutical Sales
- Physiologist
- Reproductive Medicine / IVF Chemist
- Research Scientist
- Science Information / Education Officer
- Science / Biology Teacher

For more information on the College of Engineering, Science and Environment, check out our website:

newcastle.edu.au/college/engineering-science-environment

For more information on our degrees visit:

newcastle.edu.au/study

FOR MORE INFORMATION VISIT NEWCASTLE.EDU.AU

EXTRACT FROM HSC SYLLABUS

Module 1: Cells as the Basis of Life

Students examine the structure and function of organisms at both the cellular and tissue levels and investigate the biochemical processes of photosynthesis and respiration.

Module 5: Heredity

Students investigate reproduction and inheritance patterns in plants and animals. They investigate the role of DNA in polypeptide synthesis and the uses of technologies in the study of inheritance patterns.

Module 6: Genetic Change

Students investigate genetic change, including mutations, environmental pressure and uses of biotechnology. They investigate how the processes of inheritance and evolution are applied.

Module 8: Non-infectious Diseases and Disorders

Students investigate non-infectious diseases and disorders including their causes and effects on human health. They explore technologies and their uses in treating disease and disorders as well as the epidemiology of non-infectious disease in populations.

ASSISTED REPRODUCTION TECHNOLOGIES

Sexual reproduction is the engine behind genetic diversity and therefore the adaptability of eukaryotic organisms including animals. The sperm and eggs, referred to as 'gametes' or 'germ' cells, are very different from normal somatic (body) cells.

This primarily because they carry half the genetic material (haploid) of somatic cells (diploid) in unique combinations, which recombine during the process of fertilisation, supporting a new 'haploid' embryo. There are several biological processes that each gamete must successfully complete before they can participate in fertilisation.

These processes are designed to support the germ cells finding each other and selecting the best quality gametes for fertilisation.

We will look at key chemical factors that are required for sea snail sperm to activate, so they can initiate fertilisation with the egg. (Modules 1, 5, 6).

Experiment 1: In this experiment, you will become a reproductive or 'IVF' scientist. You will use isolated sperm and eggs from the common sea snail and understand how current IVF (in vitro fertilisation) techniques are used to produce fertilised eggs. Importantly, these techniques are always being improved, based on the cellular biology knowledge of gametes (sperm and eggs) we are continually uncovering as researchers.

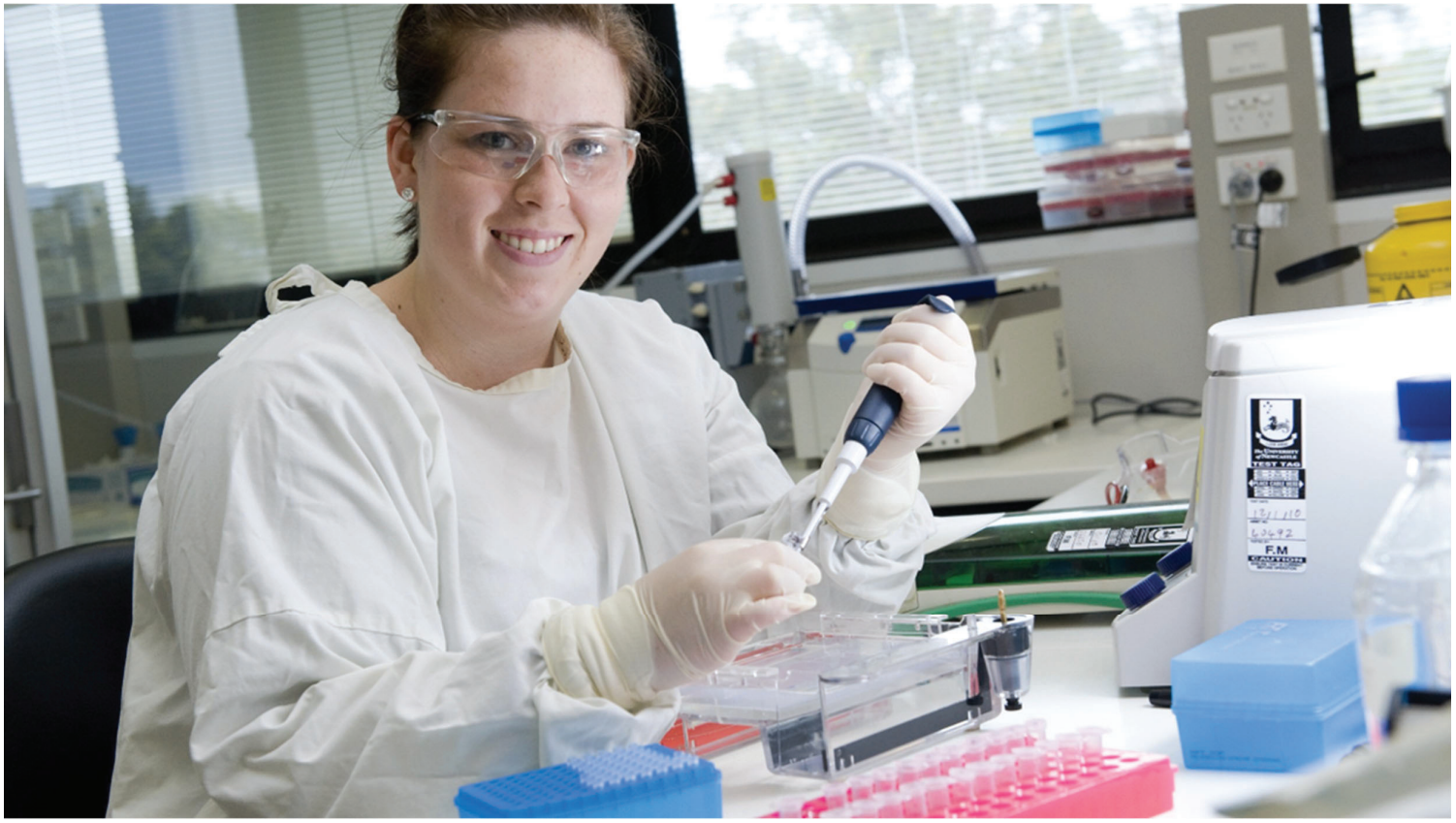
DNA AND THE GENE

DNA is the repository for data storage in biology. The code imbedded within its structure via the sequence of four different nucleotides, encodes the information that comprises a gene.

Generally, a gene encodes information for the production of a protein. As we now appreciate, proteins are the primary biomolecule responsible for carrying out cell function, including catalysing reactions.

The manipulation and characterisation of DNA in lab organisms including bacteria and cell lines, is a key aspect of modern biology and medical research. This can be used to manipulate cell function and to understand patterns of inheritance or to characterise the types of species present in a location (HSC Biology Syllabus Modules 5 and 6).

Experiment 2: In this experiment you will isolate DNA from plant cells using household reagents, which are remarkably good substitutes for many standard lab reagents typically used for DNA extraction across many cell types or tissues. You will understand which reagents fulfil which purpose for this isolation. You will then manipulate DNA using molecular techniques, visualise the DNA using gel electrophoresis, and understand how this methodology is used to help us understand and engineer the genome of a range of organisms to provide solutions for human health and for the betterment of the environment.



EXPERIMENT 1: ASSISTED REPRODUCTIVE TECHNOLOGIES

INTRODUCTION

Assisted reproductive technologies are now a critical industry in modern society. IVF is the common term used to describe all assisted reproduction technologies (ART) but IVF actually stands for a common technique called '*in vitro* fertilisation'.

These technologies are critical for the birth of many types of animals, and of course for humans. ART (or IVF) has provided the opportunity for 100's of thousands of people around the globe to have their own children, however, much of the biology that underpins the seemingly growing incidence of infertility, in our modern population, and why significant proportions of people repeatedly fail to achieve pregnancies and live births via ART, is still poorly understood.

With about 1.5 children out of every classroom (of 30) now conceived via ART, the goals toward improving our understanding and the efficiency of the technology is clear but the emerging possibility of health risks in the children born from ART, amplify the urgency of research in the area, including research on model systems.

THE EXPERIMENT

PURPOSE

In this experiment you will analyse gametes from sea snails, assess sperm quality and factors that support fertilisation. For the ART of any animal species, whether it be implemented to improve the efficiency and genetics of livestock animals or species for conservation efforts, or even to assist people in starting and/or growing a family, the conditions in which gametes (sperm and eggs) are cultured (maintained or activated), as well as the factors available for these cells in the laboratory setting, are critical. Additionally, observations of cell shape (morphology), is a key tool employed by embryologists to deliver the best possible reproductive outcome.

BACKGROUND

Reproductive Medicine and research in this field is greatly supported by the use of model systems. Not only used to perform new experiments and gain new insights, but model systems have also provided the platform of knowledge we now build upon.

Much of the biology that describes the union of the male and female gametes (spermatozoa and oocytes, respectively) has been established in sea animals such as the anemone.

The zebra sea snail (Austrocochlea obtusa) is one of most common molluscs in Australia and is abundantly found on rocky shores of Western Australia, Queensland, and New South Wales. The animal is herbivorous and feeds mainly on seaweed and often on a vegetable scum that coats platform rocks. The species is dioecious, the male having testis containing sperm, while the female has a compartment where eggs are stored.

Besides the processes we are investigating in this laboratory, other aspects of reproductive biology are still being researched and uncovered.

EQUIPMENT AND MATERIALS USED

Each bench will have:

- Culture media and reagents
    - Natural seawater
    - Artificial (Na+ and Ca2+-free) seawater
    - Ingredients: Glycerol – 68.3 ml (1 ml = 1.26 g); MgCl2.6H2O – 11.20 g; KCl – 0.77 g; KHCO3 – 0.21 g; made up to 1 L with water.
- Sea snail gamete preparations in 1.5 ml microfuge tubes
- Microscope
- Disposable Petri dishes
- **Non-disposable** glass well slides and coverslips
- Cell staining reagents and disposable microscope slides

METHODOLOGY

PROCEDURE A: SPERM MATURATION FACTORS

1. Set up your Microscope(s) before you prepare your gamete cultures. Please get your demonstrator to help with this or consult the instruction sheets near each scope.

2. For the spermatozoa culture, clearly label 2 Petri dishes, one with natural seawater (e.g. 'NW') and the other with artificial seawater (e.g. 'AW'). Add ~1 ml of each respective media to the dishes.

3. For oocyte culture, prepare two additional Petri dishes as in Step 2 (above); add 1 ml of each respective media to the dishes and label accordingly

4. You will be provided with 4 x 1.5 ml microtubes each containing either sperm or eggs in either natural or artificial sea water.

5. As soon as you can, gently invert the tubes containing spermatozoa (to disperse cells evenly) then add spermatozoa (100 ul) into each of your culture dish preparations and record the time (as t=0)

6. Examine the sperm under a low magnification first (10 X objective), then increasing to 20 X or 40 to get a closer look. At regular 5-minute intervals for about 20minutes (time permitting), score the percentage of sperm that are motile in the respective natural and Na-free artificial seawater.

7. At 20 minutes (or your last time point) determine how many cells are still alive in each medium. (see staining protocol below)

   Try and count a minimum of 50 cells for each culture.

TIME (MINUTES)	NATURAL SEAWATER	ARTIFICIAL FREE SEAWATER
	% motile	**% motile**
0		
5		
10		
15		
20		
	% motile	**% motile**
20		

SPERM VIABILITY

1. Aliquot 5 uL of the sperm sample to a normal microscope slide and apply coverslip. Let the sample settle for 3 mins

2. Prepare an eosin exclusion assay for sea snail sperm by adding 5 uL of eosin to your sperm preparation(s) directly on the slide

3. Count how many sperm are stained red with eosin (dead cells) out of 50-100 cells. Report the percentage live cells for your sample in the table above.

CONCLUSIONS

What can you interpret about the importance of factors such as Na^+ and Ca^{2+} for sea snail sperm function?

PROCEDURE B: SPERM AND EGG JELLY INTERACTION

Observation of sperm penetrating the egg jelly in both natural and artificial sea water culture media

1. Pick up about 50 eggs in natural sea water using a pipettor (~50 uL of settled eggs) and transfer them onto a well slide.
   Note: one well should be for natural the other for artificial seawater. Add 1 drop (~10-20 ul) of sperm in natural sea water (from tubes obtained in Procedure A) onto the well containing eggs and cover with coverslip. Place the well slide onto microscope immediately and examine the cells under natural sea water under the low power (10 X) objective, then you may increase the magnification carefully.

2. Refer to the information sheet provided on fertilisation stages. Examine the eggs at regular 1 minute intervals for about 10 –15 minutes, and record when the following events occur and how many sperm are involved in for each culture medium:

   - sperm attach to outer boundary of the egg jelly
   - sperm penetrate the egg jelly
   - sperm penetrate the fertilisation envelope
   - sperm penetrate or attach to the egg plasma membrane

   You may take photos through the eyepiece using your phone. Please observe strict instruction from the demonstrator before proceeding.

3. Repeat steps (1) and (2) above using sperm and eggs stored in the artificial (Na- & Ca-free) seawater, in the adjacent well (or sperate well slide) and enter your data in the table below.

	TIME (MINUTES) / NUMBER OF SPERM	TIME (MINUTES) / NUMBER OF SPERM
	NATURAL SEAWATER	**ARTIFICIAL FREE SEAWATER**
SPERM ATTACH	_____ / _____	_____ / _____
PENETRATE JELLY	_____ / _____	_____ / _____
FERT ENVELOPE	_____ / _____	_____ / _____
PLASMA MEMBRANE	_____ / _____	_____ / _____

CONCLUSIONS

How might the proper support of sperm maturation *in vitro* (in culture) translate to fertilisation success?

Cells that do not progress through the maturation processes and therefore are unable to participate in fertilisation and pass on their paternal genome to the next generation. Can you speculate on why some sperm may be unable to complete maturation and what this may tell us about the underling quality of the cell?

EXPERIMENT 1: ISOLATION OF DNA FROM STRAWBERRIES AND THE MANIPULATION AND VISUALISATION OF DNA FROM 3 DIFFERENT ORGANISMS

INTRODUCTION

RESTRICTION DIGESTION

As part of their natural defence against invasive agents, bacteria produce restriction endonucleases. These enzymes cleave DNA in a sequence specific manner. That is, they recognise a specific sequence of nucleotides. A large number of these enzymes have been isolated from various bacteria. The enzyme nomenclature is based on the bacteria, the strain and in the order the enzyme was isolated (in Roman numerals). Thus, the enzyme isolated from Escherichia coli, strain R, 1st enzyme isolated is given the name: EcoRI. Recombinant technology (protein production in a laboratory) has allowed for the production of large amounts of these enzymes. They are now routine tools for DNA manipulation. We see the result of one of these enzymes in this laboratory.

GEL ELECTROPHORESIS

DNA fragments can be separated on the basis of size using gel electrophoresis. The gel can be made of a polymer of acrylamide or agarose (an extract from seaweed). The gel is made with wells that allow the addition of the DNA. An electrical current is then applied to the gel and the DNA migrates according to size (see below).

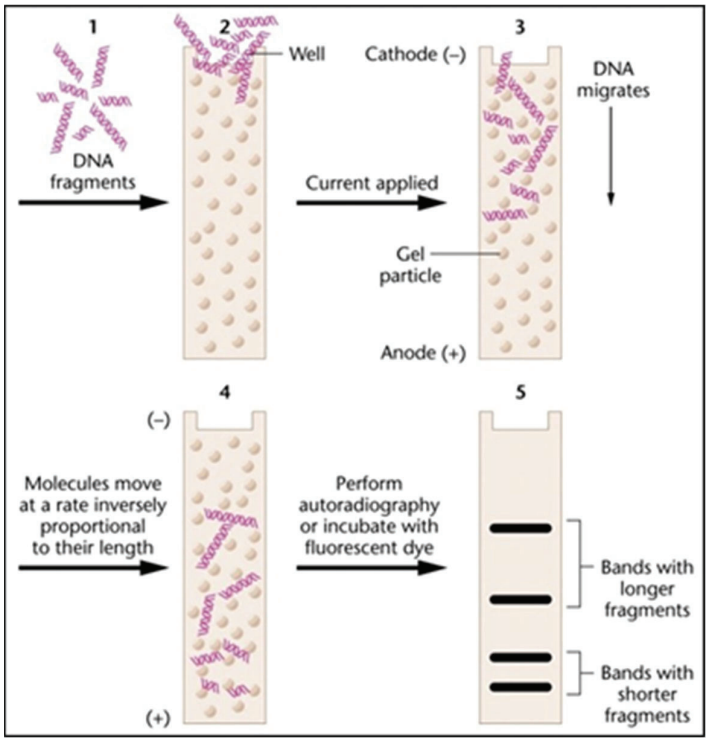


Figure 2.1: Fragments (1) of DNA are loaded into a well (2). A current is applied (3).

The phosphate backbone of DNA gives the molecule a (-) charge. Thus, DNA migrates towards the anode. However, the gel particles impair migration.

The smaller the DNA molecule the quicker it moves around the gel particles and makes its way towards the anode (4). DNA fragments within the gel can then be visualised by the binding of a fluorescent dye (5). Klug & Cummings

DNA ISOLATION

General DNA isolation procedures can be divided into three stages;

1. Disruption of the cell membrane and release of the DNA into a medium in which it is soluble and protected from degradation

2. The dissociation of the protein-DNA complexes

3. The separation of the DNA from other soluble cellular components

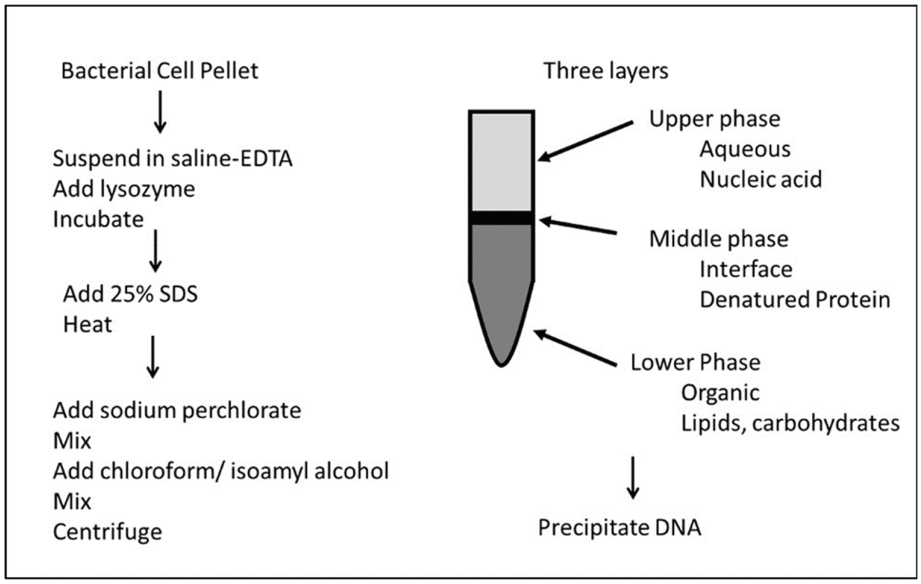


Figure 2.2: A general DNA isolation protocol for isolating DNA from bacteria

THE EXPERIMENT

PURPOSE AND BACKGROUND

In this experiment you will isolate DNA from plant cells (strawberries) using household reagents, which will replace some of the lab reagents identified above in Fig 2.2. You will understand which reagents fulfil which purpose for this isolation. Variations of these isolation techniques are used routinely in many laboratories to isolate DNA from their target cell type or tissue.

A series of DNA samples are also provided for comparison. These were isolated by second 3rd year UON students, from bacteria (plasmid DNA) and genomic (E.coli) DNA as well as phage DNA, then digested (chopped) with the restriction nuclease, HindIII.

You will load these samples into an agarose gel (Procedure A below) which will allow you to visualise the different size DNA fragments contained in each sample (Procedure C). The different size fragments will tell us about the presence/absence or the frequency of 'restriction' sites. Remember, this site is the specific DNA recognition sequence of HindIII, on each type of DNA samples.

If we know the position of the restriction sequences in the organism, you could use this simple experiment to identify which type of organisms may be present in a sample (eg, one obtained from soil). The digestion patterns can therefore be useful for many applications including DNA fingerprinting or the characterisation of 'eDNA' (DNA obtained from the environment).

This kind of information can tell us if organisms are related or which type of organisms are present in a sample area (water, soil etc).

EQUIPMENT

Each bench will have:

• Household solutions for making DNA isolation from strawberries (DNA extraction buff er 20 mLs)

- liquid dish washing detergent or shampoo
- water
- salt
- ethanol kept on ice

• Materials for one DNA extraction

- Zip lock plastic bag
- 1 strawberry (fresh or defrosted)
- Gauze, cut into squares
- Funnel
- Plastic transfer pipettes
- Clear test tube, plastic/glass
- Long wooden skewer

• DNA samples in sterile tubes stored on ice for gel electrophoresis

• Gel loading buffer

• Gel electrophoresis and visualisation equipment (shared with class)

METHODOLOGY

The DNA samples you will analyse via gel electrophoresis, have already been isolated for you and digested using the restriction nuclease as set out in table 2.1 below. The 'plasmid' and 'E.coli' DNA samples represents two discreet types of DNA. The plasmid is a small DNA polymer, while E.coli chromosomal DNA being much larger (tubes 2 and 3 respectively). The phage DNA is from a simple bacteria-specific virus (tube 1).

Table 2.1: How your DNA samples were digested

TUBE NO.	1	2	3
Species origin of the DNA	**Phage DNA**	**Plasmid DNA**	**E.coli DNA**
DNA amount µL	**5**	**5**	**5**
d H2O µL	**11**	**11**	**11**
Buffer *µL	**2**	**2**	**2**
Amount HindIII µL	**2**	**2**	**2**

* Specific HindIII buffer supplied by the manufacturer

PROCEDURE A: LOAD DNA SAMPLES INTO AGAROSE GEL

ELECTROPHORESIS:

1. Recover your tubes 1-3
2. Heat the tubes at 65 ^{o}C for 10 minutes on the heating block or designated water bath (this ensures separation of the cut fragments).
3. Add 5µl of loading buffer to each tube.
4. Mix gently by moving the liquid slowly up and down in the pipette tip 2 or 3 times.
5. Slowly load 20µl of the DNA/loading buff er mixture onto the agarose gel in the designated well.

   Note: you will need the help from a demonstrator to perform this step.
6. Write down which gel you load your samples on and which lane each tube number is loaded.

Once all samples are loaded, the demonstrator will start the gel by applying a DC current in the electrophoresis apparatus. While this gel is running (~45 minutes) please continue with Procedure B, below)

PROCEDURE B: ISOLATION OF DNA FROM STRAWBERRIES

DNA EXTRACTION COORDINATOR:

Makes 20mL (enough for 2 extractions):

- 2 mL liquid dish washing detergent or shampoo
- 0.30 g salt
- 18 mL water

The active ingredient in shampoo or detergent is a molecule called Sodium dodecyl sulfate (SDS) or close analogues of this. Purified SDS is also primarily used in research labs when isolating DNA.

DNA ISOLATION FROM STRAWBERRY:

1. Wash the strawberry, remove the sepals (the green leaves) and put it into a zip lock plastic bag. Add 10mL of the DNA extraction buffer and seal the bag tightly, making sure any air bubbles are pushed out and crush the strawberries with your fingers on the bench surface for 1 minute.
   Note: Do not crush the strawberries too much as this will cause the DNA to shear/degrade

What is happening in step 1?

Crushing the strawberries breaks open many of the strawberry cells, releasing the nuclei where the DNA is stored.
The soap in the detergent or shampoo in the extraction buffer breaks down the phospholipid membranes of the cells, breaks open the nuclear membrane and releases the DNA into solution.
The salt makes the DNA molecules stick together and separate from the proteins that are also released from the cells.

2. Place the funnel lined with gauze into the test tube.

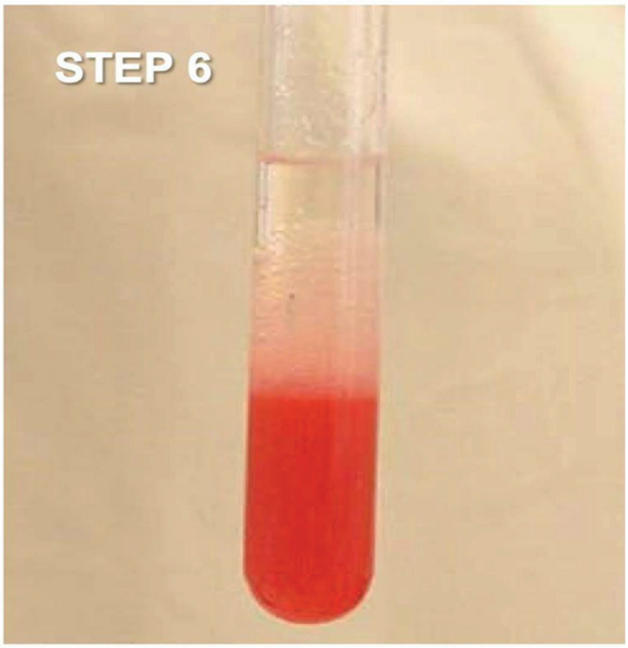
3. Pour the strawberry DNA extraction buffer mixture into the gauze and filter the mixture into the tube through the gauze.

4. Keep the liquid filtered into the tube in the tube and discard the gauze and the strawberry pulp.

What is happening in step 3?

The gauze retains strawberry cell debris. The DNA is dissolved in the extraction buffer, which passes through the gauze into the test tube.

5. Layer an equal volume of ice-cold ethanol on top of the strawberry solution in the test tube using the plastic transfer pipette provided.



6. Observe what happens at the interface of the alcohol and strawberry solution when you twirl a long wooden skewer through the interface. Keep the tube still at eye level and do not shake it.

What is happening in step 6?

The DNA partitions (or collects) between the layers of strawberry extract and ethanol.
DNA is quite insoluble in alcohol, so it precipitates. These long thread-like strands of DNA collect here and will stick to a wooden skewer.

7. You can collect the DNA strands onto a wooden skewer. The whitish, gooey, stringy stuff is DNA containing strawberry genes! This contains the code for all of the proteins required by the strawberry throughout its life.
   ** Procedure B is adapted from document supplied by Carolyn Jones*

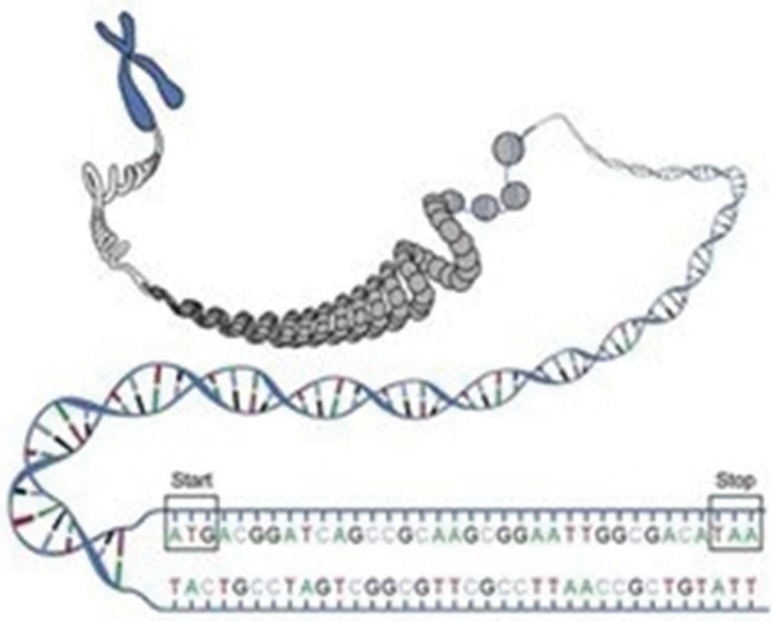


Figure 2.3: the lower section shows a gene encoded in a DNA strand, with the start and stop regions indicated. Genes provide all the function in biology including the instructions for building proteins. (http://commons.wikimedia.org/wiki/Image:DNA_ORF.gif)

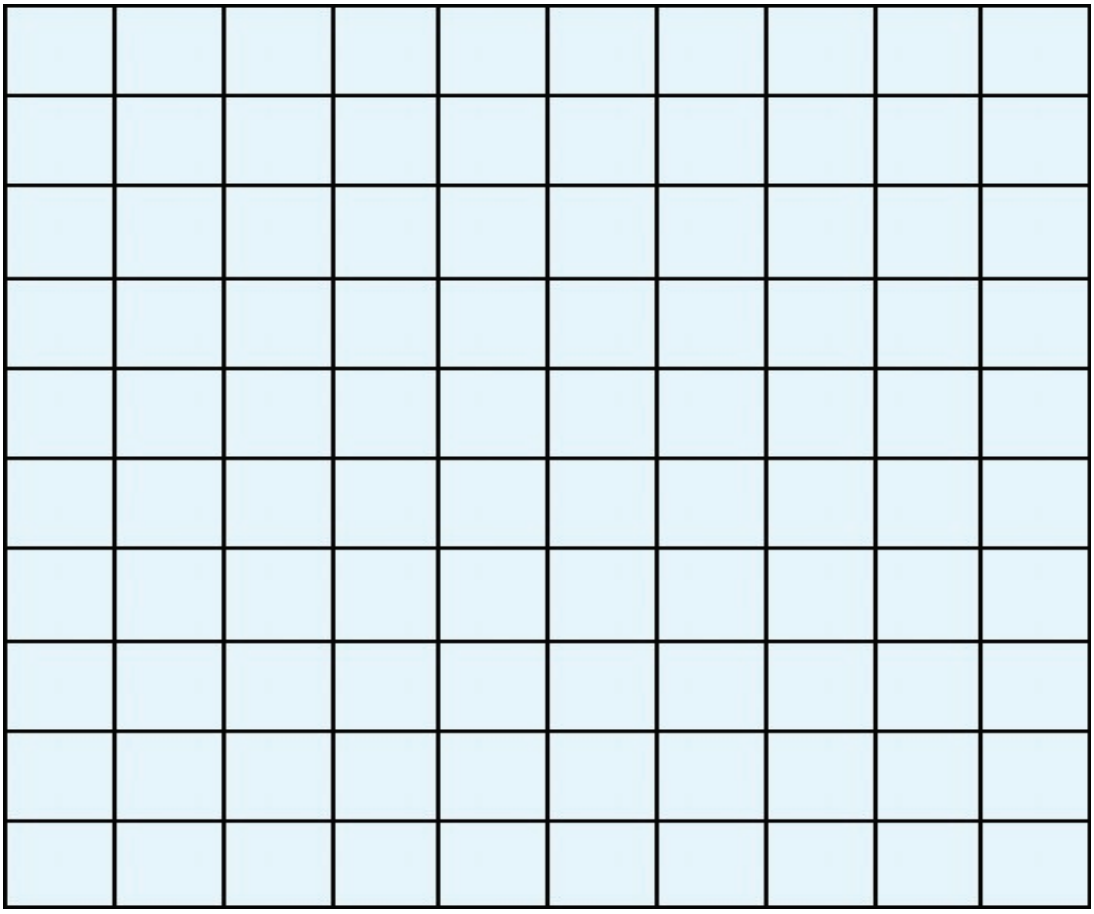
PROCEDURE C: VISUALISE DNA VIA GEL ELECTROPHORESIS

While preparing your strawberry DNA, the DNA prepared in Procedure A has been travelling through the agarose gel, via electrophoresis. The DNA will migrate according to size. The larger the segment of DNA, the less distance it will travel due hindrance moving through the agarose matrix in the gel. Therefore, smaller DNA fragments will move further. In this way, DNA can be conveniently separated according to size.

The gel also contains a DNA specific dye which will stick to any DNA fragments in the gel. The DNA can be visualised using fluorescence photography. Your gel image will be provided for you. Please sketch your data and answer the questions below.

RESULTS
GRAPH YOUR RESULTS

Make a quick sketch of your gel electrophoresis banding or pattern



QUESTIONS

1. Why does the DNA travel toward the positive electrode
   (HINT: DNA is an acid.)

2. Examine the gel photo of the digested genomes. What differences do you see?

3. How many bands can you resolve for the different samples?

4. How many restriction sites can you predict based on Q3 above?

DISCUSSION AND CONCLUSIONS

1. Given the different gel electrophoresis patterns, what can you say about the number (or presence or absence) of HindIII cleavage sites on the 3 different types of DNA provided?

2. Which organism(s) contain the most HindIII restriction sites?

3. Is plasmid DNA more similar to phage or genomic DNA?

Acknowledgement

The University of Newcastle extends sincere gratitude to Orica for financially supporting Experiment Fest through Orica's Community Investment Program. Orica's investment in the future of our region's students, continues to promote the value of education and lifelong learning, fostering a brighter future for all.

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THE UNIVERSITY OF NEWCASTLE AUSTRALIA