Boost Biology With Bats!

Explore Key Concepts in Genetics, Evolution and Ecology using Bats

Transition Year Syllabus

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Contents

Overview

Genetics, biochemistry and molecular biology are exciting and progressive areas of science, underpinning biology. Groundbreaking advancements have been made in recent years, the human genome project being one, DNA sequencing has unlocked a new vault in biology. This booklet is designed to bridge the gap between the biology curriculum and what is currently going on in molecular and evolutionary biology, within Professor Emma Teelings' Bat Lab in UCD.

This material is an outcome of Science for Schools programme, during which teachers, Olivia Derwin and Clare Lamont joined UCD's Laboratory of Molecular Evolution and Mammalian Phylogenetics aka Batlab. Olivia and Clare received diverse training in cutting-edge research techniques, got familiar with laboratory skills, learnt how to understand and analyse genetic data and participated in field trips. During the Science for Schools programme, the teachers used bat research as a unique learning tool to explore key biological concepts in Comparative Genomics, Evolution, Ecology and Ecosystems and Speciation.

This booklet is aimed at mixed ability Transition Year biology students and assumes only Junior Certificate science prior knowledge. It should give students an insight into why we study molecular biology and how this science is used on a daily basis in biological research. This booklet is designed to act as a project workbook which students can complete throughout the year and present as part of their T.Y. portfolio of work. It contains sufficient theory upon which end of term examinations can be based and is designed so that students can use it for independent learning. There are a number of class and individual projects in the booklet with links to useful websites and videos.

Science for Schools web site: http://www.ucd.ie/scienceforschools/

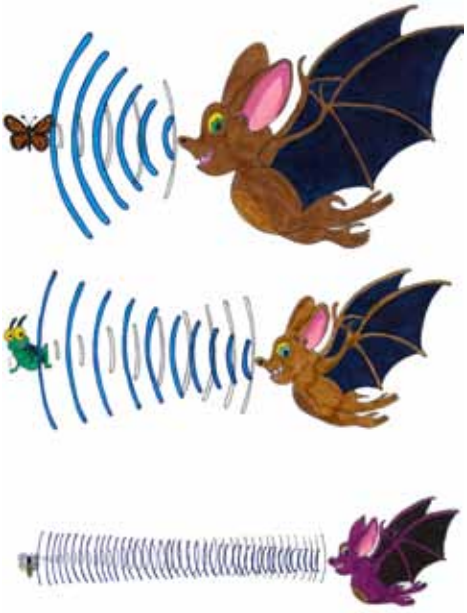
Why Study Bats?

What do you think of when you hear the word bat?

Often synonymous with horror films, Dracula, Halloween, unwelcome visitors in the attic, getting caught in your hair, being creepily nocturnal and fear in general, these fascinating creatures are often just misunderstood. Bats as we know them have been on Earth for a very long time (about 64million years). Bats are mammals. They have fur, usually give birth to one pup a year and suckle their young.

They have several remarkable attributes.

- Bats have a unique ability to use sound to perceive their environment. This is called echolocation. They emit sound waves from their larynx which reflect off objects in their environment. The bats then hear these echoes and they turn these echoes into an acoustic image. This enables them to orientate and find food in complete darkness.
- One of the most unique things that bats do as a mammal is that they fly. In fact, while other mammals can glide, bats are the only mammal capable of true and sustained flight



Echolocation

So why are bats so important to our world?

Economical and Ecological importance of bats.

These mammals are pest controllers and pollinators. Bats are essential for our ecosystems to function correctly. Amazingly, each tiny bat found in Ireland is capable of devouring thousands of insects on a nightly basis. If there were no bats, these insect populations would significantly expand due to the lack of a predator. This in turn would have an impact on agriculture as the crop growers would have to spend a large amount of money on insecticides.

Throughout the world, many plants rely solely on bats for pollination. Bats pollinate the agave plant, the juices of which are distilled to make Tequila and some species of the cacao tree, the seeds of which are used to make chocolate! Bats are also involved in seed dispersal. Therefore, if we remove these mammals from our ecosystems, they simply will not work as they do now.

We can study bats to help us learn about our own health.

Scientists believe that by studying the unique sensory abilities of bats they will gain insight into human diseases of the senses such as blindness and deafness. Throughout this module, you

will be learning about DNA which codes for proteins which are essential for you to function. You will learn about how scientists can now unravel DNA and examine its genetic code. Every animal's DNA is a little bit different and scientists want to figure out if these differences make animals, especially including ourselves, more susceptible to diseases.

For example, scientists can examine the region of the genome that is important for good vision. If they look at that region in a group of mammals that have very good vision and compare it to the same region in mammals that don't see so well, such as certain bats, they may spot a difference and this difference (mutation/variation) could be what is causing the disease.

In the UCD Bat Lab, scientists are researching blindness and deafness. There are many underlying genetic causes for these disorders. Scientists have been looking at the unique sensory specialists, the bats, and have analysed the genes that enable bats and other mammals to see and hear. In bats and other mammals that do not see that well, the scientists have searched for the genetic defects that may break these genes and could lead to blindness. They can then use these data to predict which sites are most likely to cause disease in humans. Therefore, bats are important for our health as they enable us to better understand how our genome functions.

Does Bat DNA contain the secret to everlasting youth?

As people age, their bodies and health deteriorate. Ageing is inevitable and a huge amount of money is spent by people trying to avoid the ageing process. Studies have reported that an increase in oxidative stress levels due to high metabolic rates have a huge impact on the ageing process. Typically in mammals, there is a relationship between body size, metabolic rate and how long you can live for. Small mammals tend to have a shorter life span predicted by their small size and their often fast metabolic rates. Bats are small mammals that fly and this flight uses up a lot of energy resulting in high metabolic rates. For most mammals, the combination of high metabolic rates, increased oxidative stress and small body size would predict a short lifespan but amazingly, the Myotis brantii can live for up to 42 years. In fact, bats can live up to 9 times longer than expected despite having a really high metabolic rate. There are 19 mammal species that live longer than man, given their body size, and 18 of those are bats. Therefore, they must have something within their DNA that enables them to deal with metabolic stresses of ageing, particularly of flight. They expend 3 times more energy than mammals of the same size but don't seem to suffer the same consequences or the effects.

In the UCD Bat Lab, scientists are combining state-of-the-art bat field technology (going out and catching the long-lived bats) with the most up to date modern molecular technology to

better understand how they seem to defy the inevitable process of ageing and achieve extraordinary longevity. If this secret is understood, it could have huge implications for gene therapy in humans.

Ageing is a big problem for humanity. If we find out what these amazing little bats are doing, through gene therapy, we may be enabled to do the same thing. This means that we could potentially halt aging or maybe even reverse it.

So now, after reading this, you decide.

Bats are flying demons? OR Bats are superheroes?

For more information check out Prof. Emma Teeling's TED Talk – The Secret of the Bat genome.
http://www.ted.com/talks/emma_teeling_the_secret_of_the_bat_genome

Section 1. Genetics

1.1 Cells-the building blocks of all living organisms.

Introduction to cells.

If you studied Junior Cert science you will know that anything which is considered living (an organism) is made up of units called cells. Some organisms are made up of many different types of cells (humans, bats etc) and some organisms are made of only one cell-an Amoeba is an example of a unicellular organism.

Eukaryotic Cell

You will have examined using the light microscopes in your lab the differences between plant cells and animal cells, you have even prepared, stained and examined onion cells on slides and you may even have prepared and examined slides with your own cheek cells. Cells are more complex and contain many parts each with a role vital to the cells' survival.

An Advanced look at cells.

There are two types of cells-**Eukaryotic** and **Prokaryotic**.

Eukaryotic cells include most cells that you would think of, so all plant cells and animal cells are eukaryotic. They are eukaryotic because they have **Do** have a Membrane bound nucleus and they **Do** have membrane bound organelles. So if you were to look inside eukaryotic cells with a very powerful microscope (**example: an electron microscope**) you would see lots of bundle like structures called organelles and these are all surrounded by their own little membranes-**They are Membrane Bound.**

Take a tour inside a cell with medical animator David Bollinsky.
TED Talk-"Visualizing the wonder of the cell"
http://www.ted.com/talks/david_bolinsky_animates_a_cell

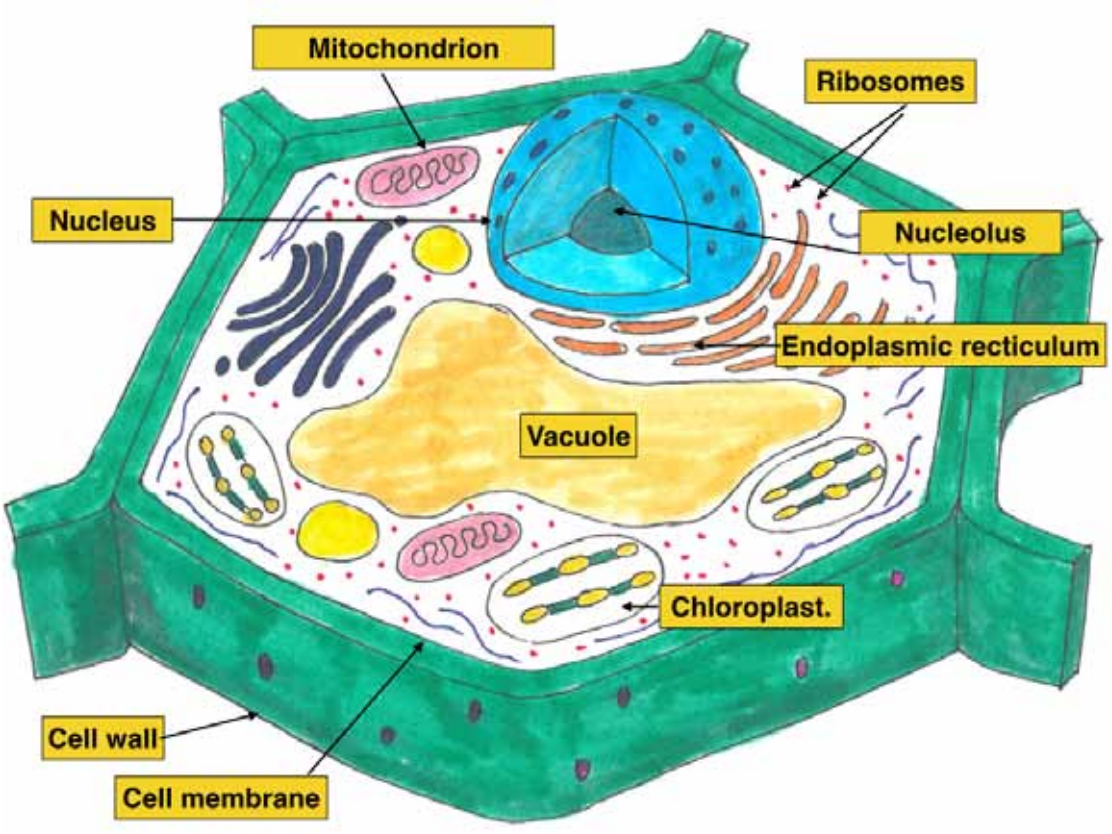
Movie clip demonstrating how an electron microscope works.
http://www.youtube.com/watch?v=GY9lfO-tVfE

Eukaryotic Cells-Organelles to note.

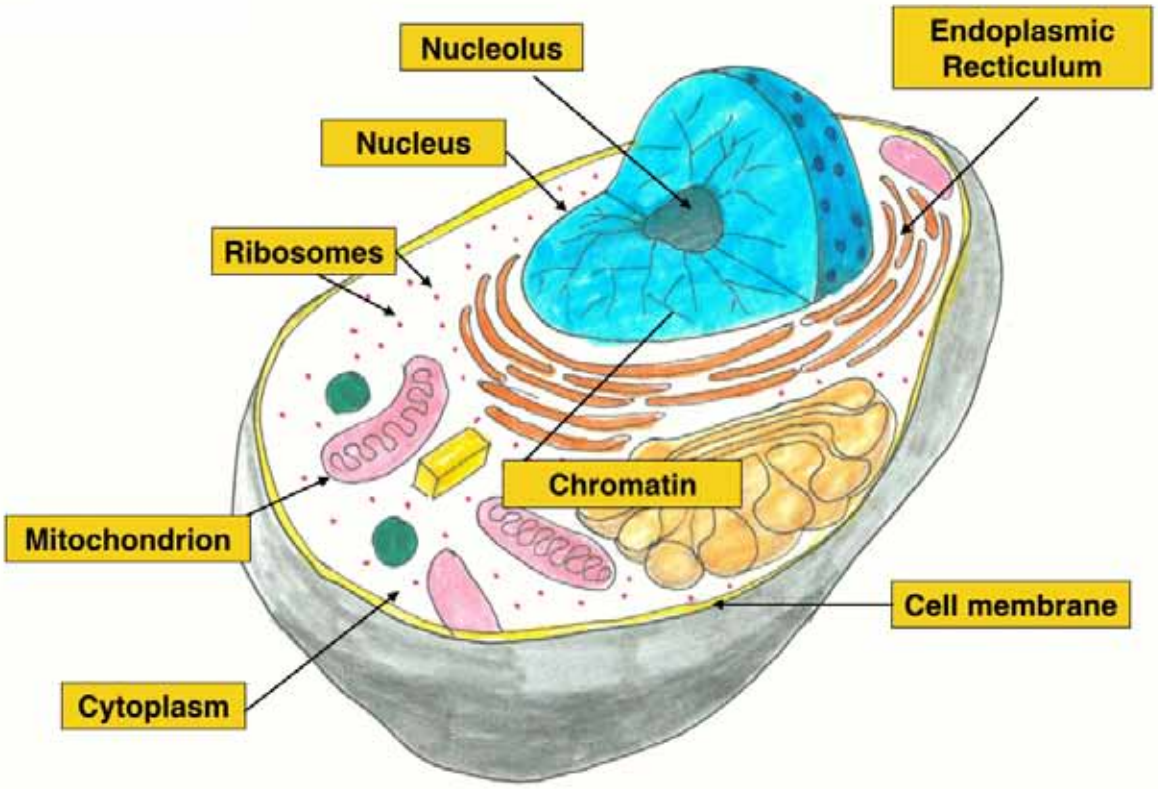
- **The nucleus**- the control centre of the cell. It contains most of the genetic material of the cell i.e. its DNA.
- Nucleolus-found in the nucleus it is responsible for making making ribosomes.
- **The ribosomes**- small structures found in the cytoplasm which carry out protein synthesis (making/ assembling proteins)
- **Endoplasmic reticulum**-has many functions (it plays a role in protein folding).
- **Mitochondrion**-cellular respiration, also contains DNA which originated from the maternal line (mothers' side, as most of the male mitochondria in mammals is found in the tails of the sperm which never make it into the egg and if they do are usually destroyed in the fertilisation process).
- Only plant cells and algal cells have **chloroplasts**-where photosynthesis occurs and chloroplasts also contain some DNA.

Note: Plant cells have a cell wall which gives strength and support BUT animal cells do not and you also remember that plant cells have one large vacuole and animal cells can have many smaller vacuoles.

Plant Cell

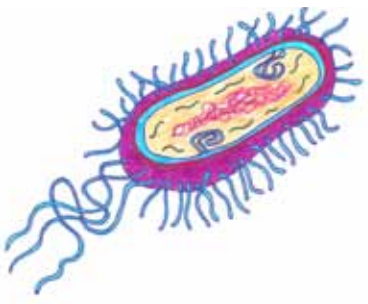


Animal Cell



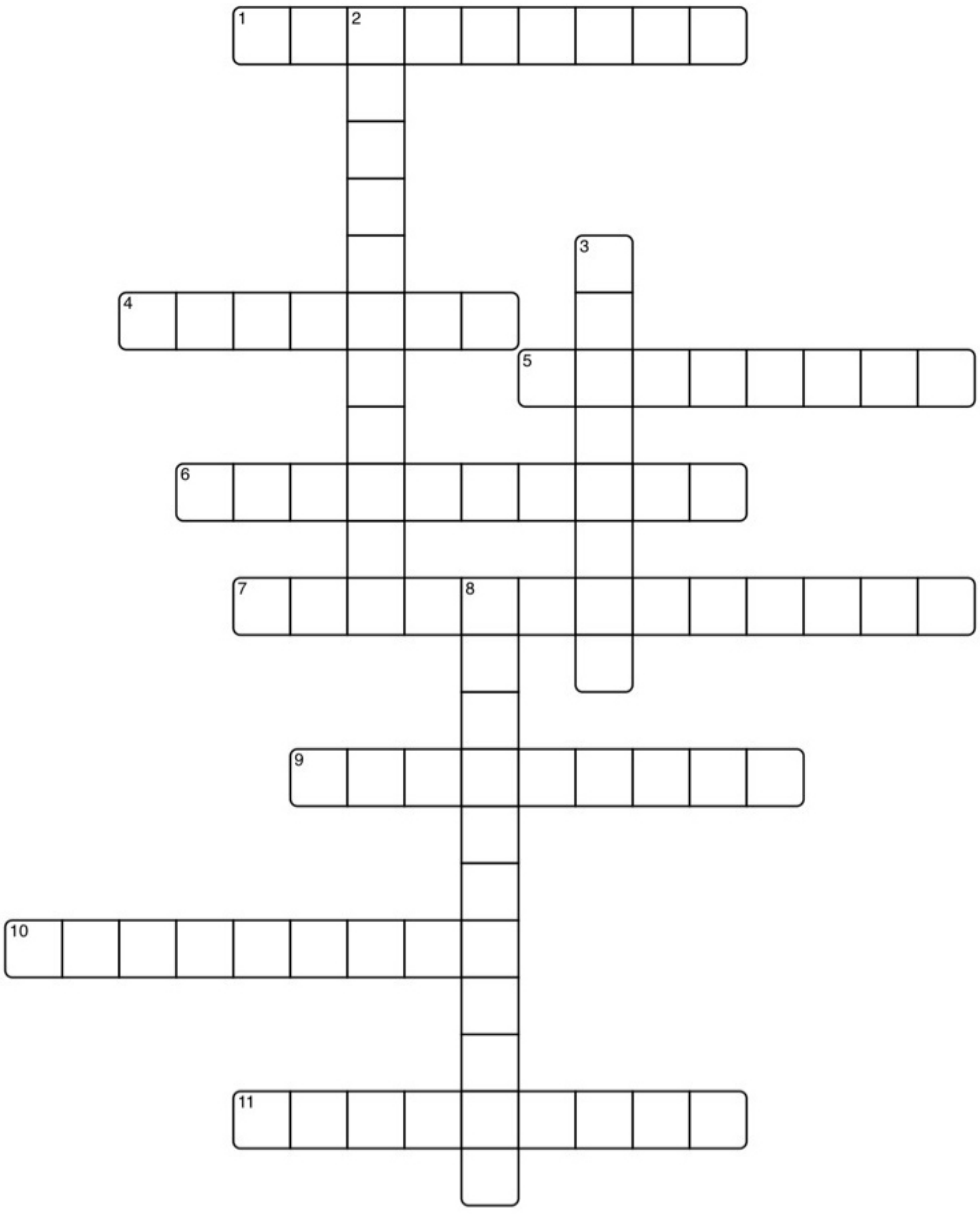
Scientists can look at the DNA in the mitochondria and nucleus across many animals including bats using DNA sequencing and make comparisons. (see section 3.1)

Prokaryotic Cells-Do not have a membrane bound nucleus and they do not have membrane bound organelles. The best example of a prokaryotic organism is a bacterium, for example *E coli* , Streptococcus.



Why all this cell science? Although the research in Bat Lab at UCD involves catching and watching animals in the wild, to truly answer the scientific questions you need to look inside the cell. This enables the scientist to understand the DNA code that makes the organisms do what they do! Therefore we have to understand how cells work. If you don't know your cell structure you won't understand the processes that occur in them. It's a wise investment to know your cells!

Test Your Knowledge



Across Hints

1. Found at the centre of the nucleus
4. The cell wall does this for the plant cell
5. Keeps the contents of the cell inside.
6. Has a true nucleus
7. Place where respiration occurs
9. Liquid found in cells
10. Organelles involved in protein synthesis
11. Example of a prokaryotic cell

Down Hints

2. Organelle in plant cells which has DNA
3. Type of microscope
8. Made up of DNA and protein

How did scientists learn about the organelles and their functions?

Cell Fractionation.

Scientists use a process called cell fractionation to separate the contents of the cells.

The cells are first broken up by blending, this gives you a mixture called the homogenate.

The homogenate is placed into small tubes and spun at extremely high speeds in the centrifuge.

Centrifuge

A centrifuge is one of a research scientists' main tools. Spinning the tubes at a slight angle at great speed creates a force. This force causes the components of the cells to settle in layers.

First spin you see tiny solid pellets at the bottom of the tube-these are the nuclei of cells, there is a liquid suspension on top of these pellets which contains other less dense organelles.

To isolate the other organelles you very carefully remove the liquid and add it to clean tubes. Centrifuge at a higher speed for a few minutes and the result is again tiny solid pellets and a liquid. This time however the pellets are rich in mitochondria.

Loading samples in the centrifuge

The Iiquid suspension is removed and transferred to clean tubes and centrifuged at an even higher speed.

The result is tiny pellets at the bottom and liquid suspension on top. The pellets in this fraction contain fragments of the cell membrane.

The liquid suspension is removed and centrifuged at an even higher speed than previous and the resulting pellets are rich in ribosomes. You now have the organelles which were once inside cells. Scientists can now use specific cell organelles for molecular research.

Centrifuge

The genetic research in the UCD Bat lab often involves analysing DNA (found in the nucleus and in the mitochondria). The DNA is extracted from bat blood samples or skin membrane samples (clipped from the wing). The DNA is extracted and purified(cleaned). The centrifuge is used to isolate the chemicals used in the purification process into a liquid layer and to gain a DNA pellet at the bottom of the tube. The liquid is easily removed leaving a pure DNA pellet.

There are many pieces of equipment which the scientists in the Bat Lab use: the centrifuge (used in extracting DNA) and the micropipette (used for measuring very small quantities) are essential tools.

Tiny volumes

Each one measures a different range of volumes

8 µl

The micropipette is used in every process in the genetic lab. Volumes used in Bat Lab are extremely small. The only way to work with these small volumes is by using the micropipette.
The standard unit of measurement is the µl (micro-litre).

1000µl = 1 ml.

1 µl = 0.001cm3

Extremely tiny quantities, sometimes even smaller than a drop.

1.2 Structure of DNA.

At the end of this section, you should be able to:

- Describe the structure of DNA
- List the four different bases found in DNA
- Explain what is meant by complementary base pairing

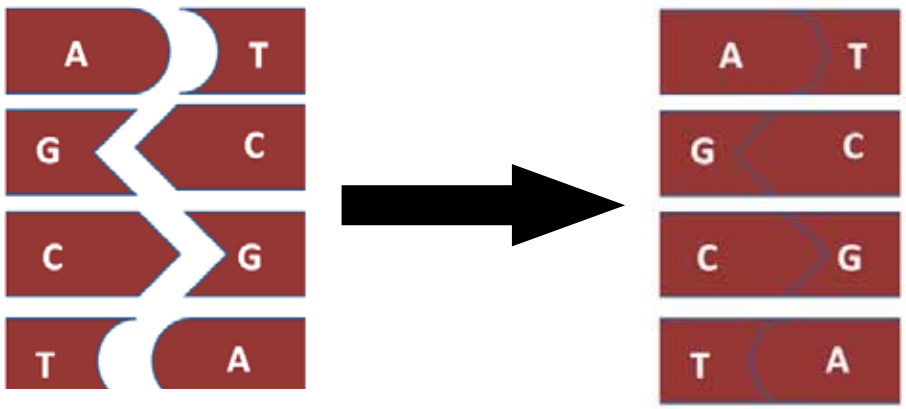
For your Junior Cert, you would have learned that chromosomes contain genetic material that is passed from parent to child. You also learned that chromosomes are found in the nucleus of a cell and are made up of DNA and protein. Now, we are going to study these concepts in a little more detail.

The structure of DNA is often likened to that of a ladder. It consists of 2 outer strands which are the backbone of the ladder and bases that link together to make up the steps of the ladder.

There are 4 different bases in DNA: adenine (A), thymine (T), cytosine (C) and guanine (G).

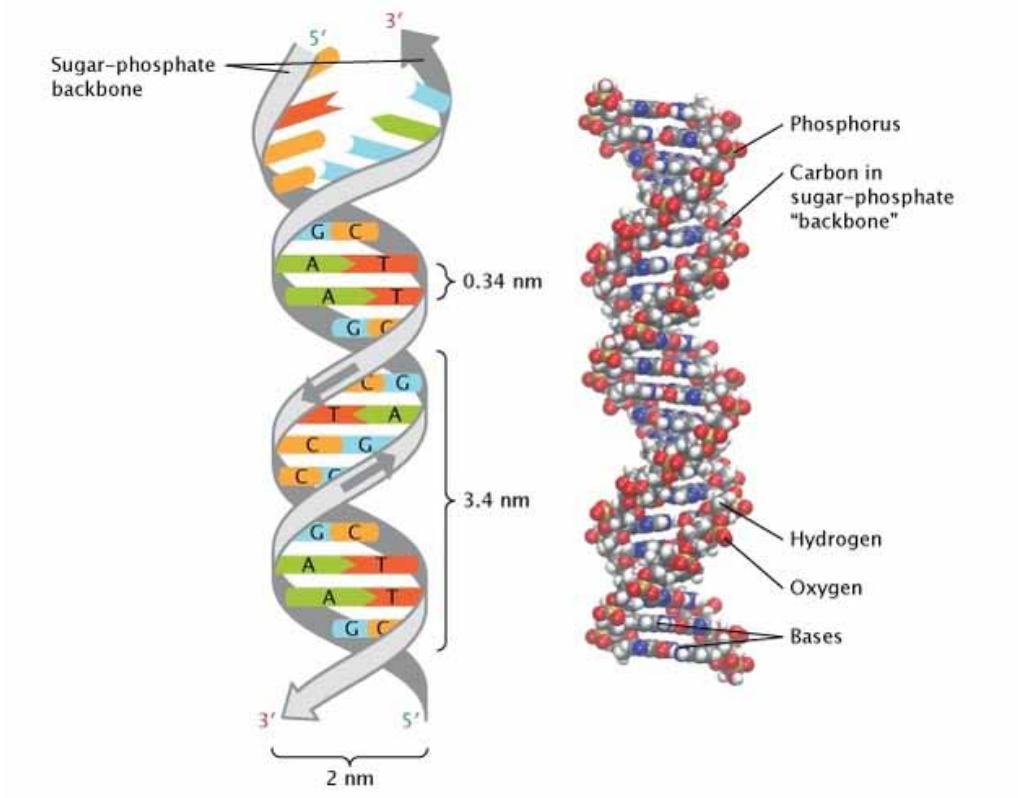
A always pairs with T
C always pairs with G

These are called complementary base pairs

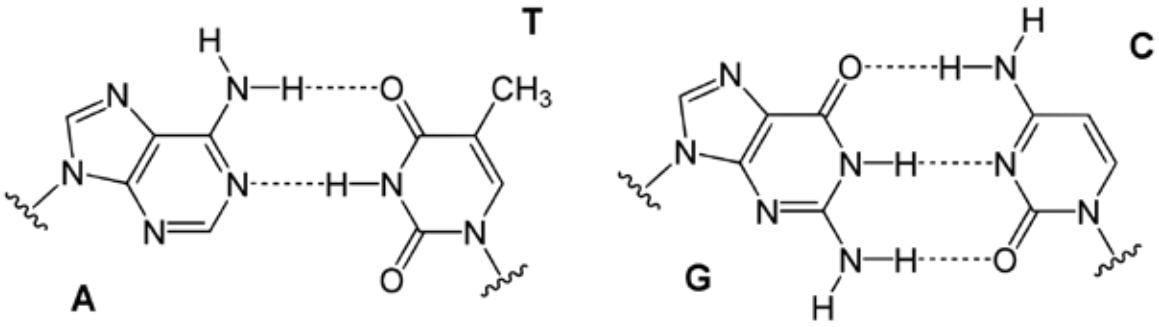


In this way, opposite strands of DNA are said to be complementary.

The DNA ladder is also known as a double helix as it is twisted into a helical shape.
In a little more detail, the 2 outer strands are made up of a phosphate and a 5-carbon sugar. One base then bonds to the sugar. This trio of phosphate, sugar and base is known as a nucleotide.



The double-helical structure of DNA. © 2013 Nature Education



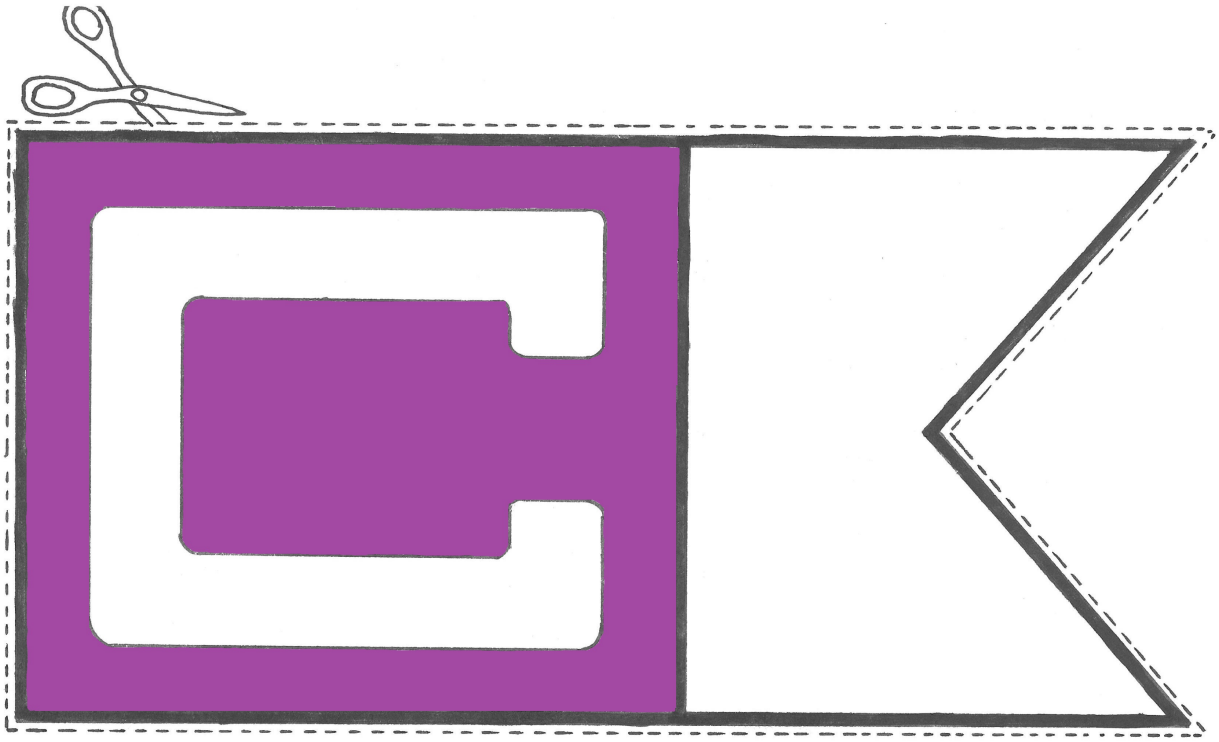
H bonds by Yikrazuul. Public domain via Wikimedia Commons

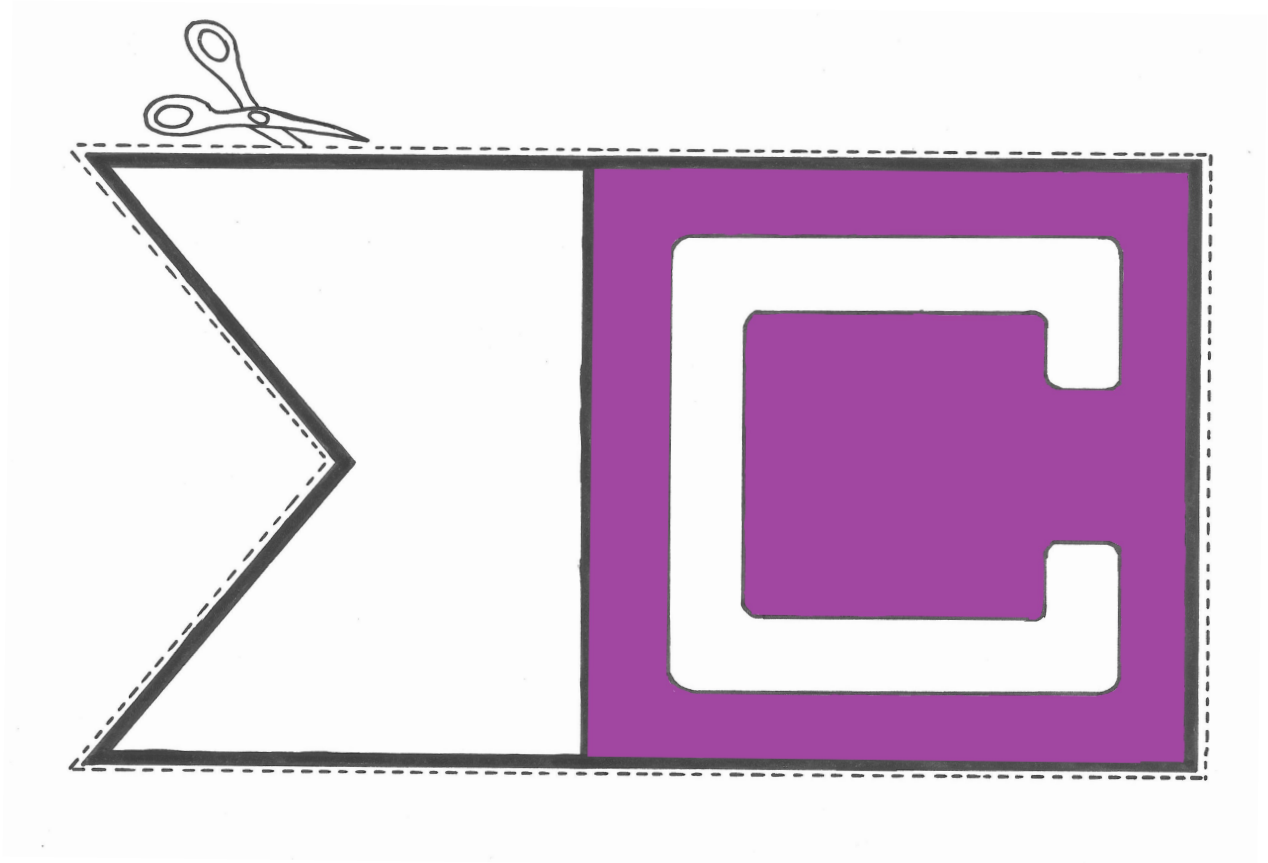
The bond that is formed between complementary base pairs is known as hydrogen bonding. There are two hydrogen bonds between A and T. There are 3 hydrogen bonds between C and G. While individual hydrogen bonds are quite weak, the vast amount of hydrogen bonding that occurs in DNA makes it a stable molecule.

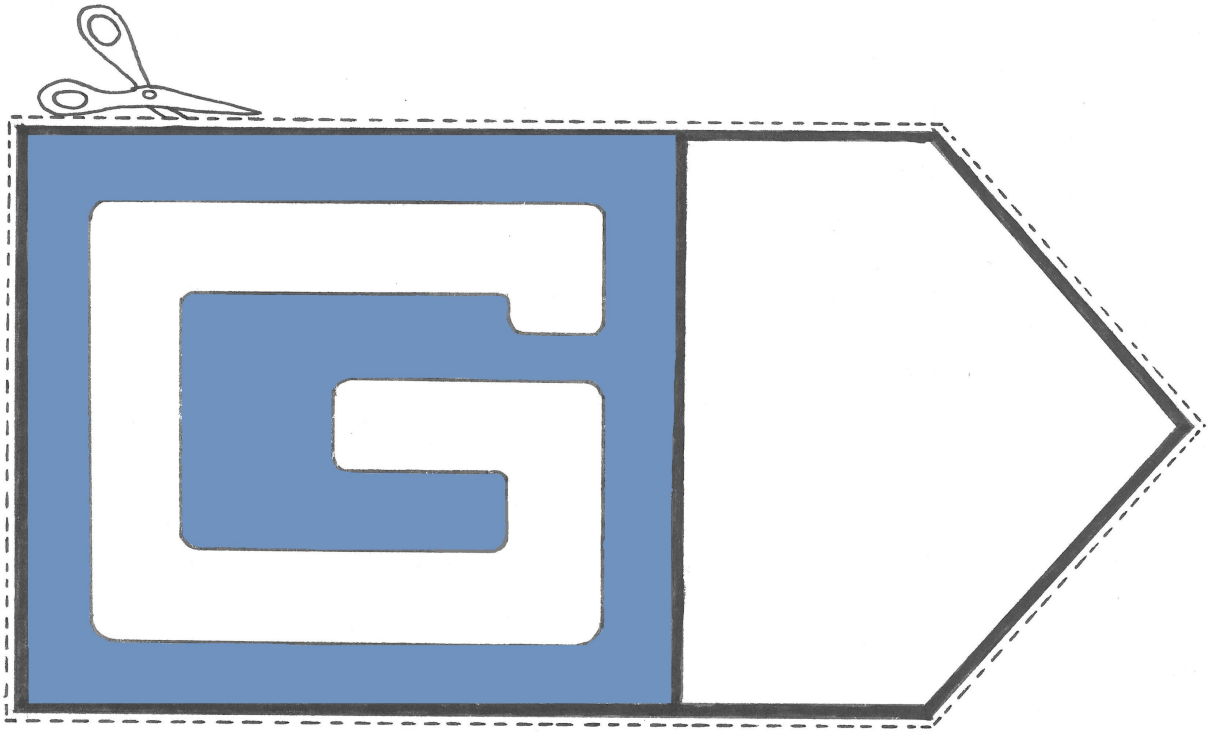
STUDENT TASK

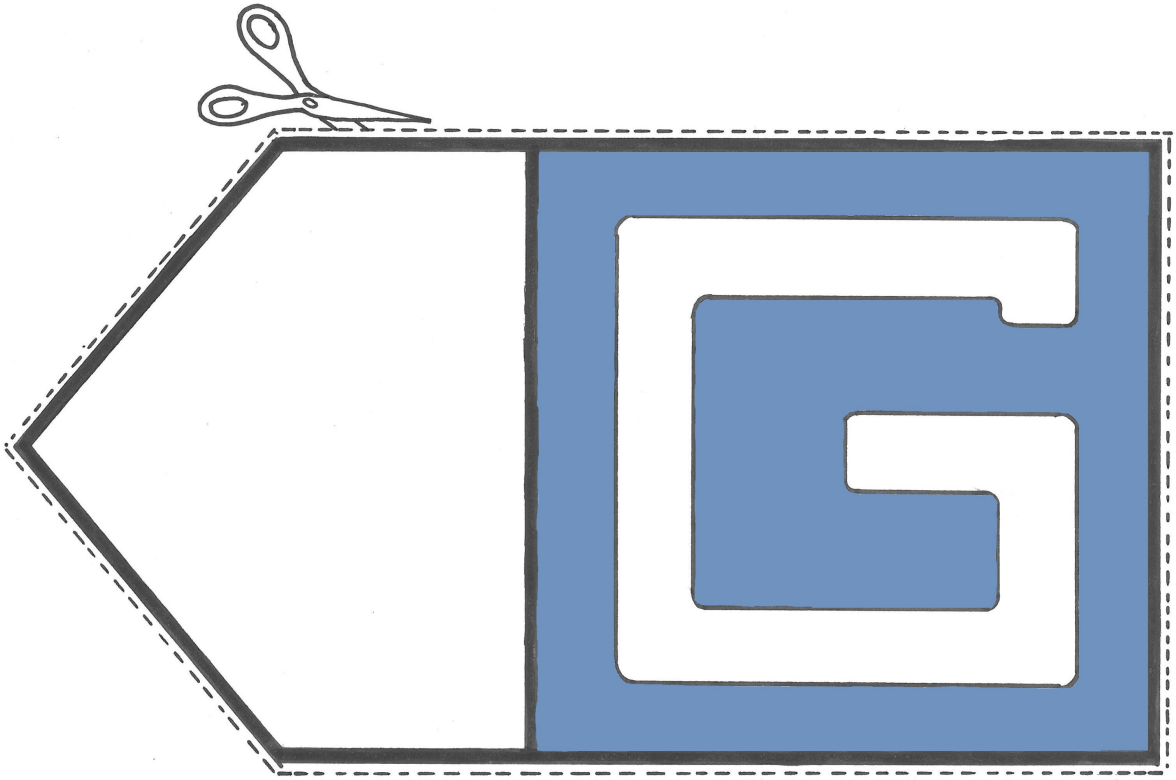
Your challenge is to build a DNA model from everyday materials. There are plenty of websites that are full of great suggestions about materials and designs that you can use. You can also use our guide for making a DNA model provided on the following pages. Your models can then be displayed in your lab. Good Luck!

DNA-TEACHING MODEL TEMPLATE



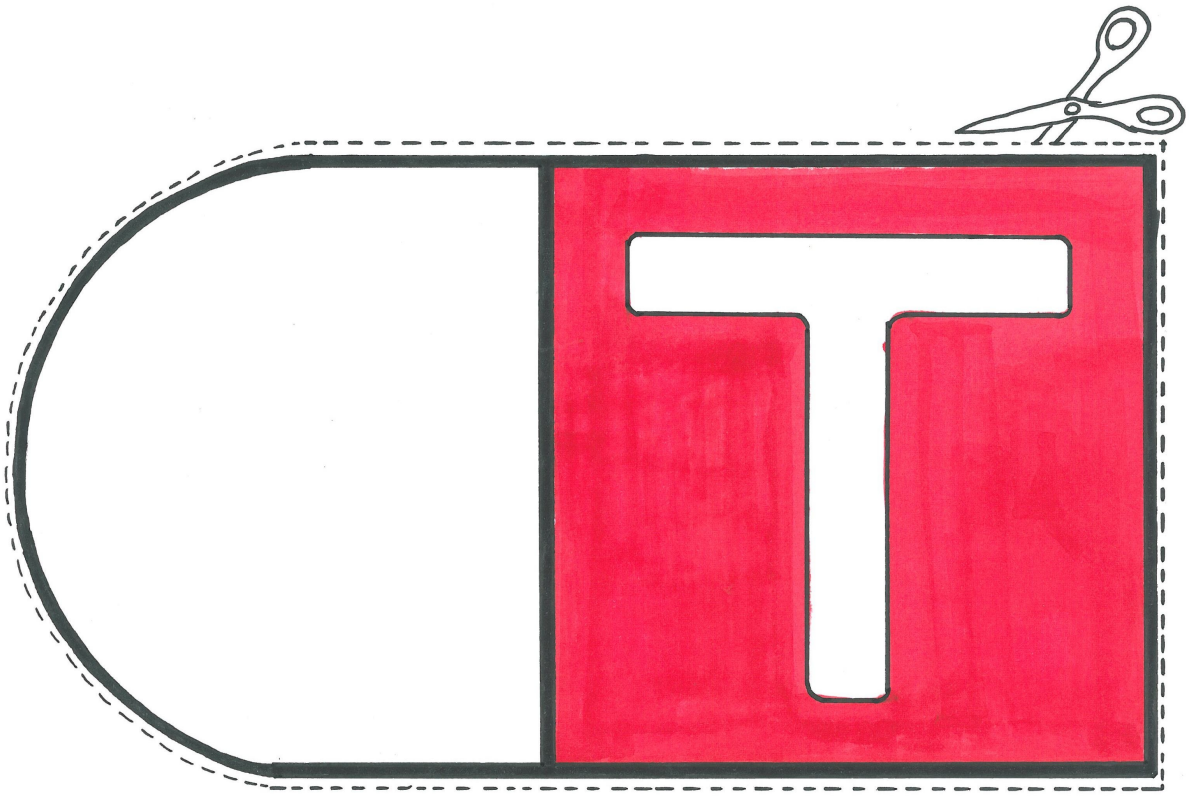







A


A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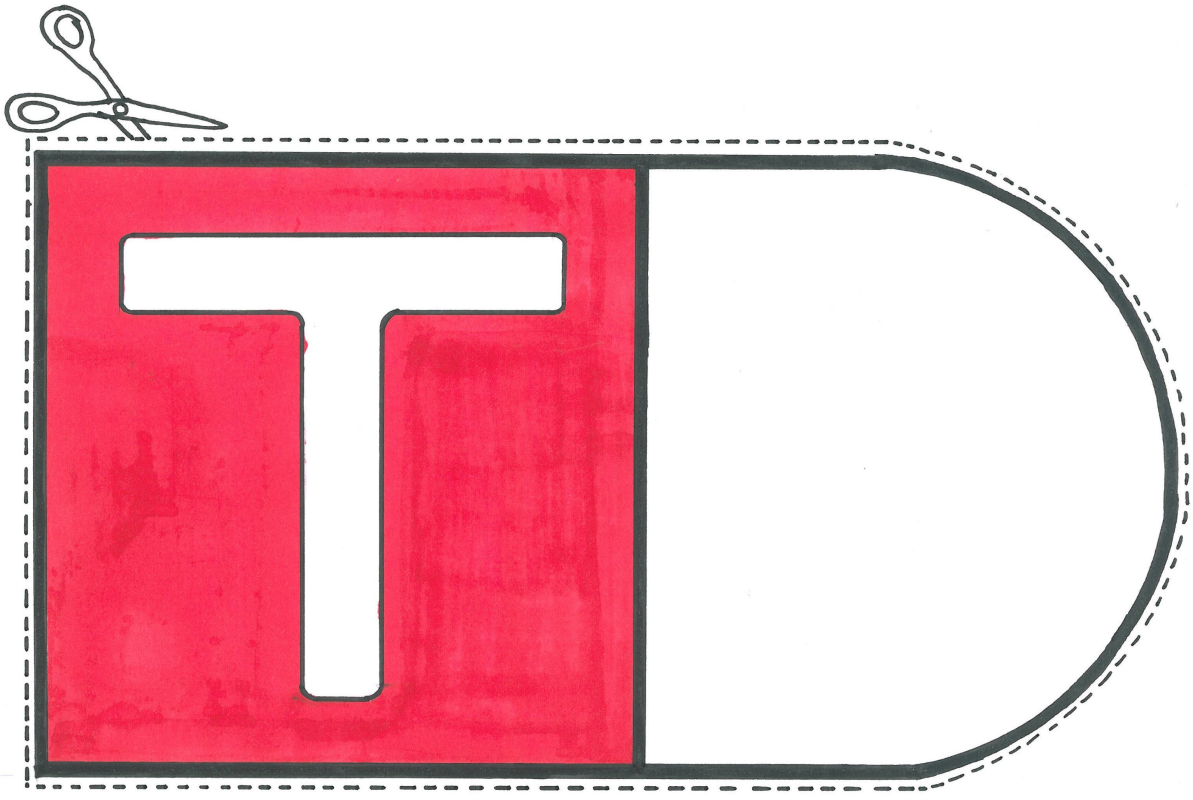
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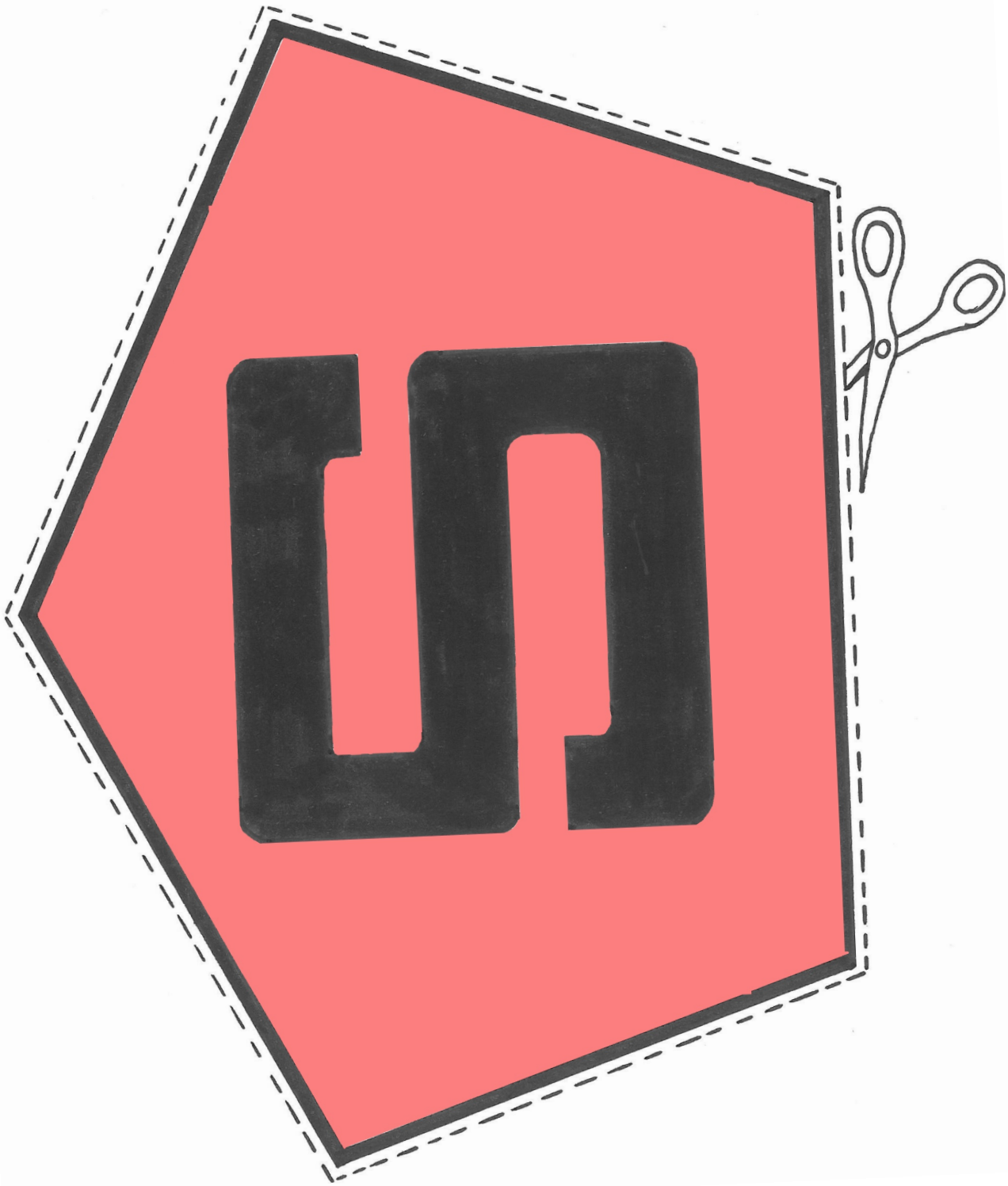
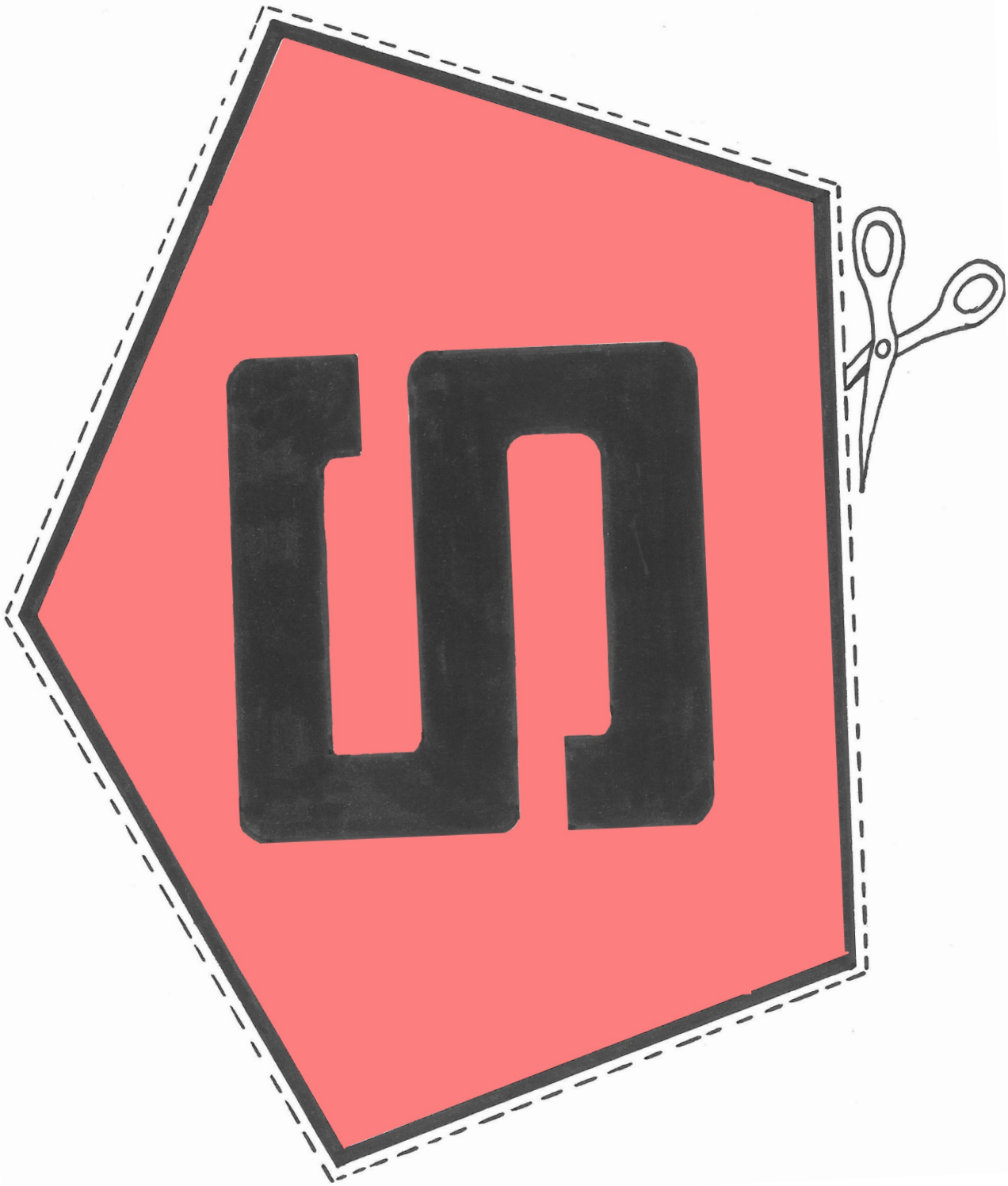
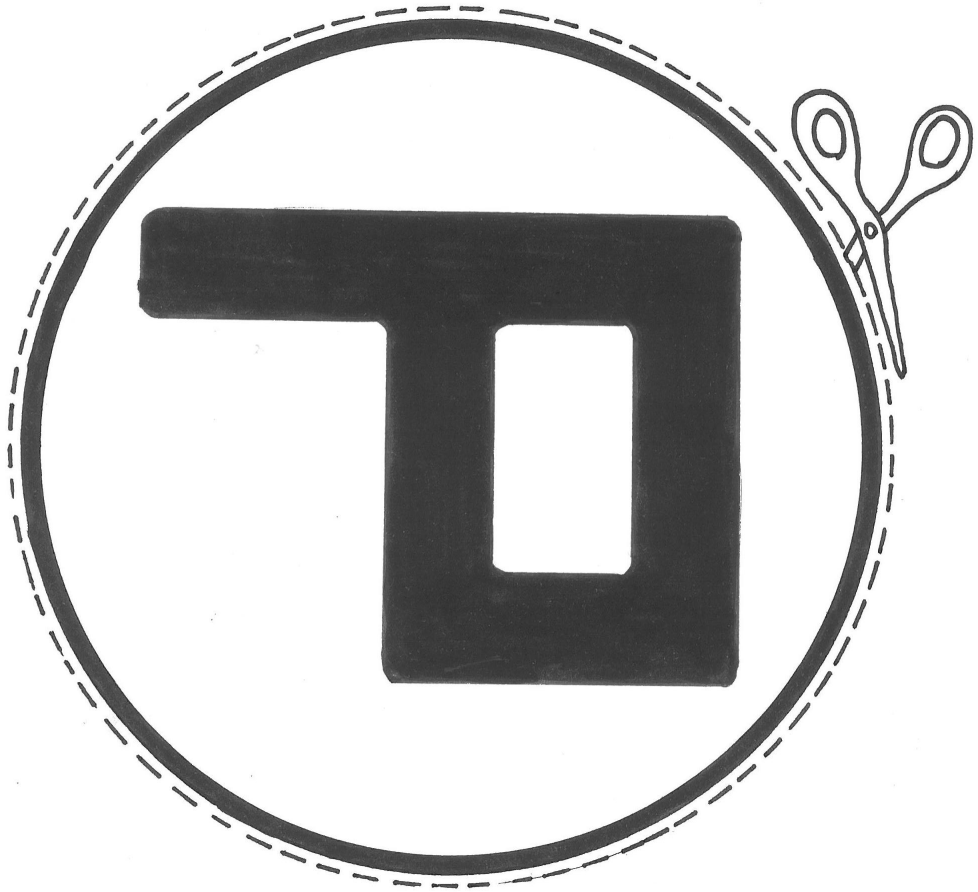
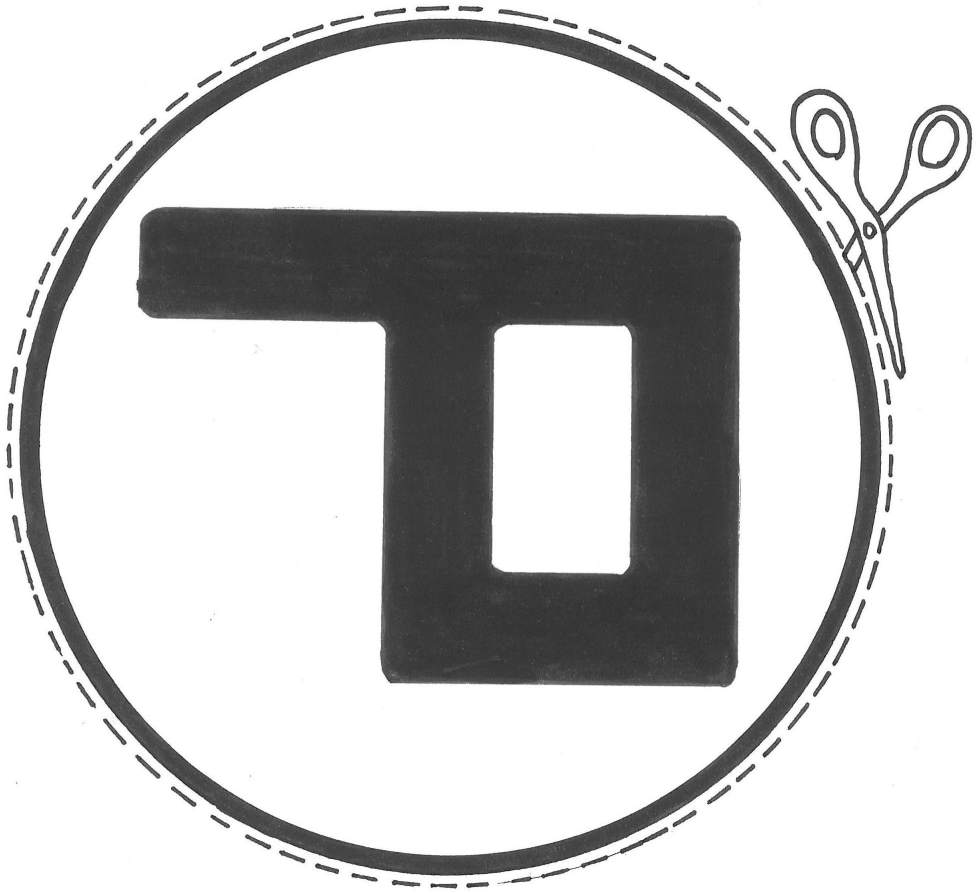








How to make your model.

This is a great class project, students can work in teams to create the nucleotides and ensure complementary base pairing is adhered to.
A great tool for teaching Replication and Protein Synthesis.

Materials:

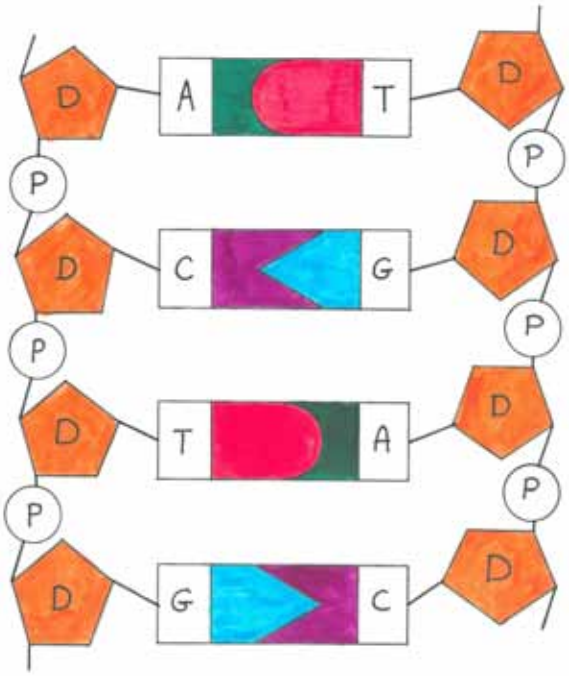
An impressive model which can be suspended from the ceiling should be at least 6 nucleotides long. This template is for the creation of a double-sided model, this has to be considered when calculating the number of templates you need.

- 24 pages of sugar templates
- 12 pages of phosphates
- 12 bases(remember the model is double sided-so write out the genetic code you want and calculate the required pages of bases)
- Laminating sheets (125 micron for a more sturdy model) and laminator.
- Hole punch
- Small paper clips for the Hydrogen Bonds. Larger paper clips can be used to clip the nucleotides together-although string prevents tangles!
- Paper glue

Instructions.

- Print the required pages and cut out each template.
- As you cut out each template glue each pair so that you have doubled sided sugars,phosphates,As,Ts,Gs,Cs (model should read from the back also) **Important: There will be some trimming to size required, G base and C base mostly.**
- When cutting is complete each part should be laminated. (A number of the templates can be placed together in an A 4 laminating pouch to save costs, even better if there is an A3 laminator available).
- Trim each laminated piece to size.
- Before you begin to put the model together it is best to lay it out on the lab bench as per the scheme diagram below) ensuring that the bases are aligned correctly.
- Using a paper punch make holes in the templates as indicated on the scheme below-(3 per sugar, 2 per phosphate and one per base used.
- Assemble the model using linked paper clips for the bonds. In the photograph below we used larger clips to connect the nucleotides but string will work fine.
- Suspend from the ceiling for maximum effect.

Use this picture to help you lay out the model and make the required holes in each template.

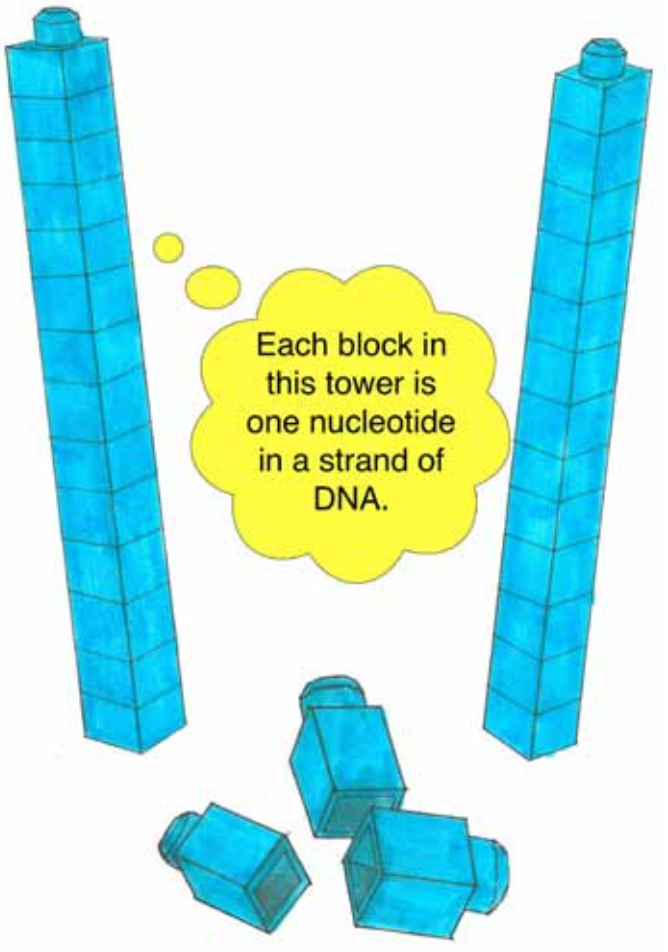


UCD Research students modeling DNA!

Template drawings hand created by R. Dawson & O.Derwin.

1.3 Complementary Base Pairing-Tasks.

- You have learned all about complementary base pairing and its' importance cannot be overstated.
- If you find it difficult to visualise the nucleotides and Hydrogen bonds- Imagine if you were to build the double helix out of building blocks-you would first begin with two towers of blocks stacked one on top of the other. The nucleotides are the blocks and you create two towers. There are Hydrogen bonds holding the two towers together.
- A-Adenine on one tower will always and only bond to T-Thymine on the opposite strand (opposite tower). It will bond using two Hydrogen Bonds.
- G-Guanine on one strand (tower) will always and only bond to C-Cytosine on the opposite strand (opposite tower). It will bond using three hydrogen bonds.
- It is very important to know the number of hydrogen bonds-they never change.
- Scientists in the Bat Lab use this knowledge of complementary base pairing and the hydrogen bonds in picking primers for Polymerase Chain Reaction (PCR). This will be covered a little later in section 2.2.2.



Complementary Base Pairing Task:

There are two tasks involved here the choice is yours-a bracelet or a model for your science lab. A single stranded sequence of DNA is provided, firstly you must write in the complementary bases beneath the provided sequence and you MUST also draw in the relevant lines to show the number of hydrogen bonds.

Gene Sequence-This is an example of a gene sequence similar to those studied by scientists in the bat lab.

ATGTGTGGCTGCATCCCAACACCACACACTCCTAGGCACAAGG

TGGTAGAATGCGGGACGGGGTGGGGTGGGGGTGAGAGTGGG

GAGCTGGCTTAGCTTTTTCCTTAGGGAAAGTCTGAGCTATTT

Instructions

Add in the complementary **DNA strand,** mark in the complementary base and link each base with the correct number of hydrogen bonds.
(Hint: use different coloured pens)

Model.

Materials-What you need.

Polystyrene balls (available in hobby shops and in many sizes-medium sized produces a very striking class display).

Poster paints in 4 colours-allocate a particular colour to each base e.g.

Permanent marker-when painted balls are dry label all the As, Ts, Gs, Cs, front and back. Cocktail sticks or barbecue skewers depending on the size of polystyrene balls. These sticks will be used to join the balls together and to indicate the hydrogen bonds between each strand. Remember 2 sticks to join the A with T and 3 sticks to join the G with C.

Small cocktail sticks with dab of craft glue will secure each ball in a row. Looks great as DNA bunting!

Materials

Bases

Sample Model

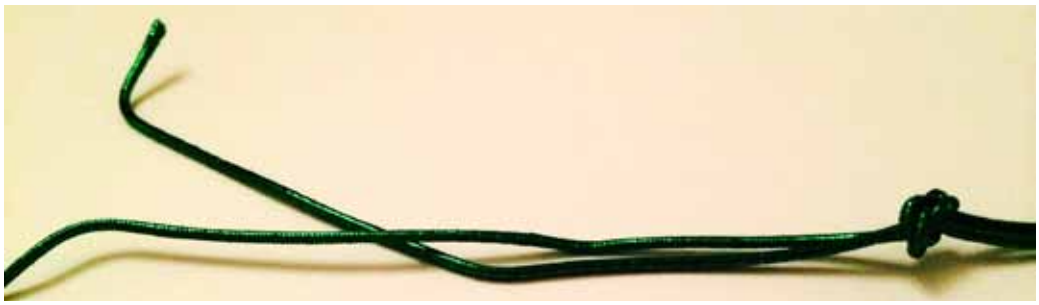
Gene Sequence Bracelet.

Materials
- Coloured beads (4 colours) - available from any art, hobby shop and online.
- Permanent marker-fine tip
- Treading elastic or string, any craft/art/sewing shop or online.

Instructions

- When you have completed the complementary base pairing task above-select a colour that will be A(adenine), T(thymine), C(cytosine), G(guanine)
- Label all the beads with their selected letter, front and back using the marker. (turn beads on side and label front & back)
- Count how many beads you will need (you may need to decide a cut-off point in the sequence if you think it is too long)
- Take two lengths of threading string and tie at one end (leave some length to tie the bracelet one it is completed) thread the first base on one string and it's complementary base on the other string.
- Continue along and secure with knot.
- The bracelet is ready to secure to your wrist, bag or locker keys!
- It's a great show piece for that TY portfolio of work!

Gene structure will be explored in detail in section 1.6 (another cool bracelet idea there!)



Elastic with double knot section of bracelet

Section of bracelet

1.4. Isolating DNA From A Biological Sample.

At the end of this section you should be able to:

- Describe the process of extracting DNA from a tissue sample.
- Explain why particular chemicals are used at certain steps.
- Explain why the incubation times are vitally important.
- Describe what isolated DNA looks like.

For any DNA analysis, you must first have a sample. DNA can be found in body fluids, skin cells, hair follicles etc., basically any sample that contains cells. Once the sample has been collected, it is necessary to isolate the DNA from the rest of the sample. In the BAT LAB, scientists extract DNA from very precious samples of bat blood and wing membranes. Although you don't have samples of bat blood to work with, the principles of DNA extraction are the same.

Extracting DNA from Different Plant Tissues.

What you will need:

- One or more of the following: Onion, Red Onion, Banana, Strawberries, Kiwi Fruit, Peaches. (You could choose one or you could compare several samples)
- Washing-up liquid (make sure it is not concentrated or bacteriocidal).
- Beakers
- Distilled Water
- Balance
- Weigh Boat
- Spatula
- Knife and chopping board
- Water bath (60°C)
- Water bath (ice water)
- Timer
- Blender
- Coffee Filter Paper
- Funnel
- Pasteur Pipettes
- Boiling Tube / Test Tube Rack
- Protease **
- Syringe
- Glass Rod
- Ethanol (that has been kept in the freezer)

** If you don't have protease, you can use contact lens solution. Can you explain why this is the case?

Extracting DNA from Different Plant Tissues (cont...)

Before you start, read through the above list of required materials and equipment. List as many hazards as you can and identify the safety precautions that you can take to minimise the risks involved. Most importantly, wear safety goggles and listen to all the instructions given by your teacher.

Hazard Identified	Safety precautions required to minimise the risk

Procedure:

1. Place 3g of Table Salt and 10cm^3 of washing-up liquid into a beaker and make up to 100 cm^3 with distilled water.
2. Finely chop the onion (or other chosen plant tissue).
3. Add a small amount of the chopped onion to the beaker and stir well.
4. Place the beaker into the 60°C water bath for exactly 15 minutes.
5. Then stand in an ice-water bath for 5 minutes, stirring often.
6. Place the mixture into the blender and blend for a maximum of 3 seconds.
7. Line a funnel with the coffee filter paper and filter the mixture into a clean beaker.
8. Using a Pasteur Pipette, place 10cm^3 of the filtrate into a boiling tube.
9. Add 3 drops of protease to this filtrate and mix gently.
10. Slowly pour 10cm^3 of ethanol down the side of the boiling tube forming a layer on top of the filtrate. (NOTE: The ethanol should be used directly from the freezer). Place the tube in the test tube rack.
11. Gently draw the DNA from the alcohol using a glass rod.

RESULTS AND OBSERVATIONS:

Describe what you can see at the interface of the filtrate and the ethanol.

_____

_____

What does the DNA look like?

_____

_____

Is the any difference in the quantity and appearance of the DNA between the different plant samples?

_____

_____

Was this a fair test? Explain your answer.

_____

_____

If you were to repeat the test, would you do anything differently? Explain your answer.

_____

_____

Think about the structure of a cell, particularly the cell membrane, the nuclear membrane and the fact that the DNA can be found in the nucleus. You are trying to isolate DNA. Each step in the procedure has a specific function to help you achieve this goal. Using the Internet or a Biology textbook, attempt to answer the following questions:

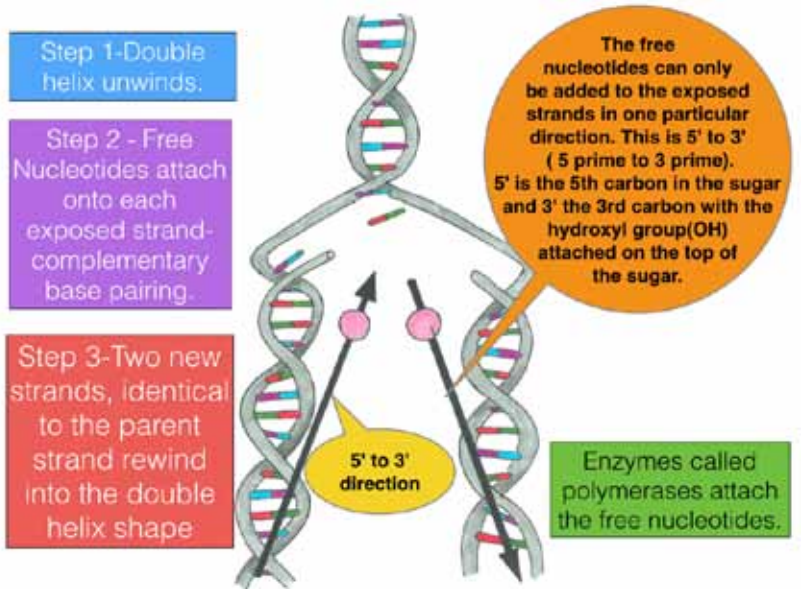
Why do you need to chop the onion?	
Why do you need to use washing-up liquid?	
What is the purpose of using salt?	
Why does the mixture get incubated at 60°C? Why is the incubation for exactly 15 minutes?	
What does blending the mixture do and why can it only be for a maximum of 3 seconds?	
What does the protease do?	
Why is it necessary to filter the mixture?	
What does the ethanol do? Why does it need to be used straight from the freezer?	

1.5 DNA Replication.

Cells are constantly being renewed and created and part of this process involves DNA making exact copies of itself. Some of your cells are at this moment in time making exact copies of their DNA to be passed to the next generation of cells.

Why is replication so important? If the process of DNA replication is flawed this will result in an altered genome, which in turn could result in disease and other negative scenarios. DNA replication can have a number of problems, sometimes too many, too few or incorrect nucleotides are added to the new strands. DNA has a repair mechanism and these mistakes are mostly but not always fixed. When they are not fixed, this results in incorrect nucleotides and if this occurs in a sex cell such as the sperm or egg, the mutation can be introduced to next generations. The importance of DNA replication and repair is essential to life. The introduction of mutations into the next generation of organisms is of particular interest to scientists. It could explain the emergence of new species or traits such as gaining or losing echolocation or alterations in sight. The search for mutations is the basis for molecular evolutionary biology (the study of how evolution occurred by using DNA analysis). It is important to note that some mutations can be harmful whilst others have no effect. Only some of these mutations are important or can change evolution processes.

The Process of DNA Replication - Double helix unwinds at one end, the hydrogen bonds between the base pairs on opposite strands are broken, exposing the strands. Free nucleotides enter and attach to their complementary nucleotide on each exposed strand and enzymes (DNA Ligase) firm up this attachment. The new strands twist into the double helix. Two new strands identical to he original DNA molecule are formed.



DNA Replication

Why not add this to music? This is a novel approach to understanding DNA Replication. Glen Wolkenfeld is a biology teacher in the U.S.-check out his "DNA Replication Rap". YouTube: http://www.youtube.com/watch?v=wdhL-T6tQco

1.6 What Are Genes?

Let's build the answer to that question.

- DNA is made up of molecules called 'Nucleotides'
- Each nucleotide has a sugar (Deoxyribose), a phosphate and one base (A,T,G,C)
- DNA in the nucleus forms long strands called chromosomes.
- Chromosomes are basically 2 very long strands of DNA which at sections have been wrapped around tiny proteins (Histone Proteins). It's all part of natures way of packaging a seriously long molecule into a tiny nucleus, it does have a more complicated role but that is for your future study.
- Unwrap those chromosomes and remove those Histone proteins and you have the double helix.
- Open up the double helix and break these H-bonds that hold the two sides (strands) together and you expose the bases.
- **A gene is a segment of DNA that codes for a product, either a protein or RNA for a certain function**
- In genes you always count the bases (nucleotides) in groups of 3 as the cellular machinery reads in this format. Each group of 3 nucleotides (As,Ts,Gs,Cs) is called a triplet.
- As you run along either strand of the DNA molecule you cross sections of the strand where there are groups of triplets which code-they have the information on how to arrange particular amino acids to produce a particular protein, when 3 bases code for an amino acid they are called Codons. A typical gene has a start codon, lots of codons that are required for the amino acids in the protein and finally a stop codon. (See section 1.8 Protein synthesis)
- You have now built your answer-What is a gene? It is a section of DNA which codes for a protein or RNA. Genes contain the instructions on how to assemble/produce a protein. (Explored in section 1.8)
- **Very important-Not all DNA is made up of genes. There are large sections in our genome that do not contain genes have been known as 'Junk DNA'. These regions have been used in DNA profiling (e.g. identifying a person from their DNA) and now it is thought to have an important role in switching particular genes on and off.**

Genome-this is all the DNA in a cell.

The human genome-this is all the DNA (the genes, the junk DNA) that contains the information needed to build and maintain you. Your genome is approximately 3,200,000,000 base pairs. To print all the As, Ts, Gs, Cs of the human genome it would produce about 5000 paper back books or about 300 boxes of A4 paper (www.yourgenome.org) This includes the DNA in both the nucleus and the mitochondria.

The bat genome-this is all the DNA that contains the information needed to build and maintain a bat. Analysis has shown that the bat genome is smaller than other mammals but contains similar number of genes. The Mitochondrial DNA from the female lineages is of particular importance in evolutionary genetics-tracing back related genetic ancestors, traits, etc.(Reminder: Your mitochondrial DNA is from your mother only, regardless if you are male or female!

Genes and Bat Lab Research.

A new era of science emerged with the sequencing of the human genome. It took many years and great expense to complete but scientists eventually deciphered the order and quantities of each nucleotide which make up the chromosomes in humans. Sequencing the human genome took over 10 years, involved 2000 researchers in 20 labs working in 6 countries. The cost is estimated at $2.7 billion. Today sequencing a genome is much faster and relatively inexpensive, it is claimed that it could be done for $1000 over 2 weeks, this varies greatly but costs have decreased.

Why was this so important?-Sequencing allows scientists to identify the sections of DNA which control disease or traits such as sight and hearing. It gave scientists a tool for comparison, health could be compared with disease. The sequencing of the human genome developed the methods for the sequencing of other mammal genomes-like the bat.

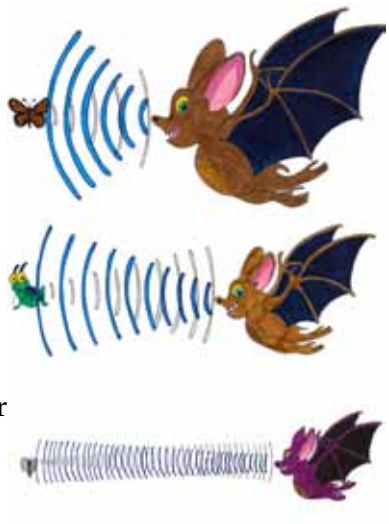
Deciphering an organisms' genome allows genes to be identified and their locations mapped, an amazing tool! Knowing the location is really helpful to research, for example you want to see if the genes for a trait such as colour vision are the same in one type of echolocating and a non echolocating bat, you can isolate that gene in both bats and sequence the gene. Simply, you can take that section of DNA and get the order of the nucleotides and compare the same sections across many animals. This will give you the order of the As,Ts,Gs,Cs and you can work out if the same protein ends up being produced. (This will be further explored in sections 3.1-sequencing)

Genomes that have been sequenced to date are available for free on large databases and you will learn more about these in the Bioinformatics section 3. Genomes also give great insight into speciation and evolution as they hold the secret to understanding the change that occurred in different animals. The genomes of different bat species can be compared to establish just how genetically different/similar they are, the greater their similarity the more closely related they are. It can be used to create phylogenetic trees (gene trees which show evolutionary pathways). Section 3.2-Phylogenetics will develop this further.

This is a great short movie by the History Channel on the Human Genome Project. It will piece it all together. Understanding the human genome will help you understand how other mammals like bats are studied.
http://unlockinglifescode.org/media/videos/544#546

How to Sequence the Human Genome-Ted-Ed
http://youtu.be/MvuYATh7Y74

Genes of interest.



The only way to unravel the mystery of bats is by examination and comparison of similar genes with other bat species. Often scientists are interested in gene changes which result in a different amino acid being inserted into the protein, insertions which change the protein sequence.

An example would include when scientists researching the science of echolocation compare other echolocating mammals such as dolphins with bats. They are looking for similarities at a gene or protein level, is it the same combination of genes in both mammals that results in echolocation?

Scientists have uncovered certain proteins with amino acid changes that are common only to echolocating mammals (bats, dolphins). These genes are expressed in the inner ear and play a role in high frequency echolocation, an example of one such gene is Prestin.

Genes connected to hibernation and ageing are also of interest. It is thought that the "secret to everlasting youth" lies with bats-the gene TERT is of great interest to scientists hoping to gain insight into this genetic secret, could the answers lie within the TERT gene?

Scientists examining why bats live so long are very interested in genes found in the mitochondrial DNA. Do bats have some way of protecting their mitochondria from the stresses of having a high metabolic rate? Scientists are looking to the genes in the mitochondria to answer this question.

Genes located in the liver are currently being studied trying to establish the science behind bat hibernation.

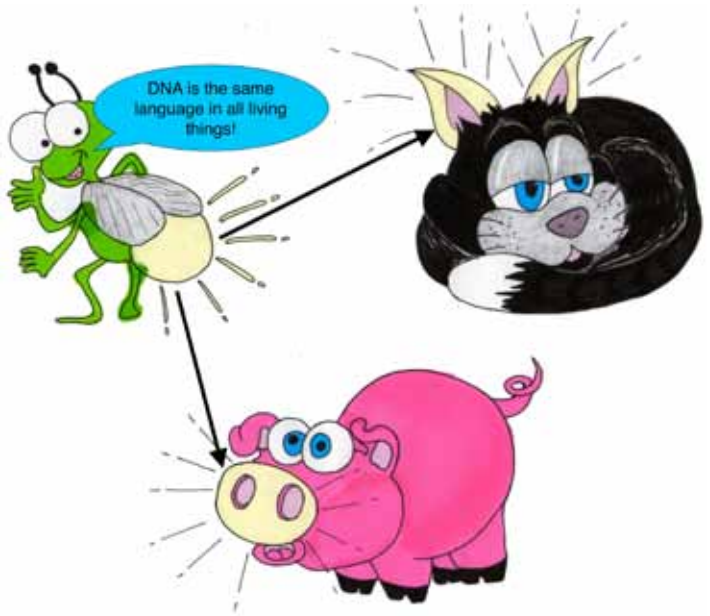
The key to understanding all this genetic research is to remember the genes must be compared with the same gene from other species-so genome comparison is important. Also very important is the protein that is produced from the gene in question. Is the resulting protein the same protein produced even though some of the nucleotide bases are different in one species? If the protein is different-did it result in some new trait? (Colour, sound, responses etc).

When you consider genes always connect it to amino acids and proteins as they are the structures which 'do things' in cells.

Where do genes come from? (TED-Ed) FANTASTIC!
http://youtu.be/z9HIYjRRaDE

1.7 The Genetic Code

- What is the point of genes? The whole point of genes is that they usually act as template for protein production-the end goal is to produce a protein that makes you function correctly!
- You now know that in a gene every 3 bases are called a codon. One codon encodes for a particular amino acid, it says which amino acid is to be added next!
- There are 20 amino acids and they combine in thousands of ways to produce proteins. There are also many different types of protein-the structural protein keratin in hair and nails is very different to metabolic proteins - enzymes, which are also proteins and act as biological catalysts to speed up our metabolic reactions (our chemical reactions). Without enzymes processes such as digestion and respiration could not happen.
- Proteins are made of long chains of amino acids and these chains are then folded into very specific shapes.
- The genetic code is the same language in all living organisms. GCA codes for the amino acid Alanine regardless of species.
- You could take the genes that allow bioluminescence from a firefly and insert it into a cat or pig and they will bioluminesce (glow). It has been tested and there is photographic evidence of cats with glowing ears and pigs with glowing snouts!



- Scientists from Taiwan National University created the world's first fully bioluminescent transgenic pigs in 2005. DNA from bioluminescent jelly fish was added to pig embryos and the resulting piglets born, looked green which turned to a glow when shone with blue light. Amazingly even their internal organs glowed! They were designed to trace human disease by inserting stem cells from the pigs into other organisms, the resulting proteins will show up green, no need for biopsies or other invasive painful procedures.
- There are 3 types of codon that you should be aware of:
  A **start** Codon-it identifies the beginning of the sequence and it is always ATG-which is the amino acid methionine.
  One that **codes** for an amino acid
  A **stop** codon-it marks the end of sequence. It is usually either of these TAA, TGA, TAG
- **Always keep foremost in your mind that it is the production of a protein which is important when considering genes** (Section 1.8-Protein Synthesis will cover this in detail.)

This table shows the which codons code for an amino acid. Notice that there can be quite a few different codons for the same amino acid. (Keep this in mind-it will help you understand variation and evolution)

Codons and Amino Acids

3 Letter Symbol for Amino acid	Single Letter for Amino Acid	Name of Amino Acid	Codons
Ala	A	Alanine	GCA,GCC, GCG, GCT
Asx	N	Asparagine/Aspartic Acid	ACC, AAT, GAC, GAT
Cys	C	Cysteine	TGC, TGT
Asp	D	Aspartic acid	GAC, GAT
Glu	E	Glutamic Acid	GAA, GAG
Phe	F	Phenylalanine	TTC, TTT
Gly	G	Glycine	GGA, GGC, GGG, GGT
His	H	Histidine	CAC, CAT
Ile	I	Isoleucine	ATA, ATC, ATT
Lys	K	Lysine	AAA, AAG
Met	M	Methionine	ATG
Asn	N	Asparagine	AAC, AAT
Pro	P	Proline	CCA, CCC, CCG,CCT
Gln	Q	Glutamine	CAA, CAG
Arg	R	Arginine	AGA, AGG, CGA,CGC, CGG, CGT
Ser	S	Serine	AGC, AGT, TCA,TCC, TCG, TCT
Thr	T	Threonine	ACA, ACC, ACG, ACT
Val	V	Valine	GTA, GTC, GTG, GTT
Trp	W	Tryptophan	TGG
Tyr	Y	Tyrosine	TAC, TAT
Glx	E	Glutamine or Glutamic Acid	CAA, CAG, GAA, GAG
*		Stop codon	TAA, TAG, TGA

Coding Task-This is a gene sequence from the *Myotis brandii* Bat-using the table above-write the appreciated name of each amino acid beneath the codon.

(Hint: use 3 different coloured pens-draw a line under each codon and alternate-first codon underlined beneath, next codon above and change colours-it will be easier to read and you can visualise your emerging code-very nice for your TY portfolio)

ATGGCCCACCGAAGGGGCCCCCAAAGGCTTGCAGGTGGGCAGCTGCAGGCCGGC

TTTGAGGACAGCACCCTTGCGAGCATCTTCACCTACACCAACAGCAACGCCACCAGA

GGCCCCTTTGAAGGCCCCAATTACCACATTGCCCCCAGATGGGTGTACCACCTCACC

AGTGCCTGGATGGTCTTCGTGGTCATTGCGTCTGTCTTCACTAATGGGCTCGTGCTG

GTGGCCACCATGAGGTTCAAGAAGCTGCGCCACCCTCTAAACTGGATCCTGGTGAAC

TTGGCTGTGGCTGACCTGGCAGAGACCCTCATCGCCAGCACCATCAGCGTCGTGAA

CCAGATCTATGGCTACTTTGTGCTGGGCCACCCTCTGTGCGTTGTGGAGGGCTACAC

TGTCTCCCTGTGCGGGATCACGGGGCTCTGGTCCCTGGCCATCATTTCCTGGGAGA

GGTGGCTGGTGGTCTGCAAGCCTTTTGGCAACGTGAGATTTGATGCCAAGCTGGCCA

TCGCAGGCATCACCTTCTCCTGGGTCTGGTCTGCTGTATGGACAGCCCCGC

Coding Task (cont...)

CCATCTTTGGTTGGAGCAGGTACTGGCCCCATGGCCTGAAGACTTCATGCGGCCCA

GACGTGTTCAGCGGTAGCTCGTACCCGGGGGTGCAGTCATACATGATTGTCCTCATG

ACCACGTGCTGCATCATCCCACTCAGCGTCATCGTGCTTTGCTACCTCCAAGTGTGG

CTGGCCATCCGAGCTGTGGCGAAGCAGCAGAAAGAATCCGAGTCCACCCAGAAG

GCAGAGAAGGAGGTGACGCGCATGGTGGTGGTGATGATCCTGGCATACTGCCTC

TGCTGGGGGCCCTACACTTTCTTTGCATGCTTCGCTGCTGCCCACCCTGGCTACGCC

TTCCACCCTCTGGTGGCCGCACTGCCAGCCTACTTTGCCAAAAGTGCCACTATCTAC

AACCCCATTATCTATGTCTTTATGAACCGGCAGTTTCGAAACTGCATCTTGCAGCTTTT

TGGGAAGAGAGTGGATGATAGCTCTGAACTCTCCAGCACCTCCAGAACGGA

Task-Be a code cracker! Using the amino acid table compose a code name using the single letters in the second column. Write your code name using the codons. This creates a line of codons which your partner has to decipher. Make it a challenge by setting a time limit-use your phone as a timer! Remember all letters are not represented there will be gaps-perhaps use a star symbol here. Crack the code or Crack Up! (You could even make a bracelet with a secret name, coded by nucleotides see section 1.3)

4 - Video Clips Which Serve to Educate and Entertain.

Crash Course - DNA

http://www.youtube.com/watch?v=8kK2zwjRV0M

DNA - The Scret Life - PBS Documentary

http://www.youtube.com/watch?v=d7ET4bbkTm0

Living the Wildlife: Bats

This is a great Irish clip (RTE) which shows how extremely cute and very intriguing these little mammals are.

http://www.youtube.com/watch?v=3BtbS9JC8x8

The Animated Genome

This is a great animation which covers everything in this section.

http://unlockinglifescode.org/media/animations/659#660

The Genetic Code and Bats.

The genetic code controls the production of proteins, sometimes one of the DNA bases changes, it can be deleted completely, swapped for another or extra bases can be inserted. You can see how this could (not always) result in a change to the amino acid and this in turn would change the protein.

Scientists in the bat lab can used the genes/genetic code to compare two bats, the bats may look similar when you catch them in a trap but genetic analysis can show that they are in fact very different-they are different species, such as a horse and a camel. It's very important to correctly identify bat species-not doing so would make years of research worthless and we would not be able to estimate how many individuals there are.

The researchers in UCD could continue with comparing the genes of bats to uncover how species of bat echolocate but why another does not. The gene sequences of known different species of bat could be compared to investigate why their echolocation calls are at completely different frequencies and was it always so. How exactly do the gene sequences for echolocation compare to those same gene sequences in other species of bat? Are there more or different As, Ts,Gs, Cs, in the sequence of one of the species vs another? Or are there less, are they present in very different combinations and if so are the amino acids the same or different? Could the findings have implications for humans? Is there any similarity in the human genes which regulate speech and hearing? These are the questions research scientists in the bat lab are asking.

Researchers are also using knowledge of the genetic code to investigate the biochemistry of bats. Bats are very tiny and live to a good age, this is unusual-Why? Flying is a very active process and such extremes of activity usually result in the production of harmful chemicals called free radicals. Free radicals have the potential to change the DNA in an organism-to alter the gene sequence. In most mammals this alteration has a negative effect, it usually results in illness, damage or death. What is it about bats that protects them from these free radicals when due to their tiny size they should be harmed? Do they even produce them? Perhaps they have an internal biochemical mechanism that either prevents production of these free radicals or removes them safely. Researchers are eager to uncover the secret to this long life (longevity), to isolate the gene sequence or sequences responsible and to compare them to other mammals, which are not so lucky with regard to life span. Could this research give doctors better insight into the ageing process, could it lead to genetic based medicine that prolongs a better quality of life?

These are of the many questions driving the UCD bat lab scientists.

1.8 Protein Synthesis - How are proteins made from a gene?

Important facts to consider:

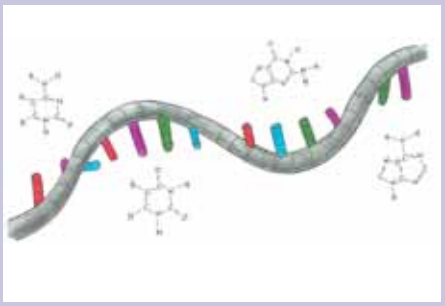
- DNA contains genes-sequences of bases within the double helix that give instructions on how to assemble particular proteins-so these sequences cause the production of a protein when expressed-when switched on.
- Protein synthesis takes place in ribosomes.
- The gene sequence must be transported to the ribosomes.
- DNA in eukaryotic cells (all plant and animal cells) is contained in the nucleus (chromosomes) and in the mitochondria (circular strand) of animal and plant cells, not forgetting that plant cells also have chloroplasts and these contain a circular strand of DNA.
- A particular type of RNA (ribonucleic acid) called messenger RNA (mRNA) transcribes the gene sequence in the nuclear DNA and brings it to the ribosomes for assembly.
- RNA is single stranded and so can easily fit through the nuclear pores.

DNA compared with RNA

DNA is double stranded.

The sugar in DNA is deoxyribose.

The bases in DNA are A T G C



RNA is single stranded.

The sugar in RNA is ribose.

The bases in RNA are A U G C

4 steps in Protein Synthesis
Initiation, Transcription, Translation, Protein Folding.

1. Initiation. (Nucleus)

The DNA double helix unwinds at the gene-the section that codes for the protein that is to be produced.

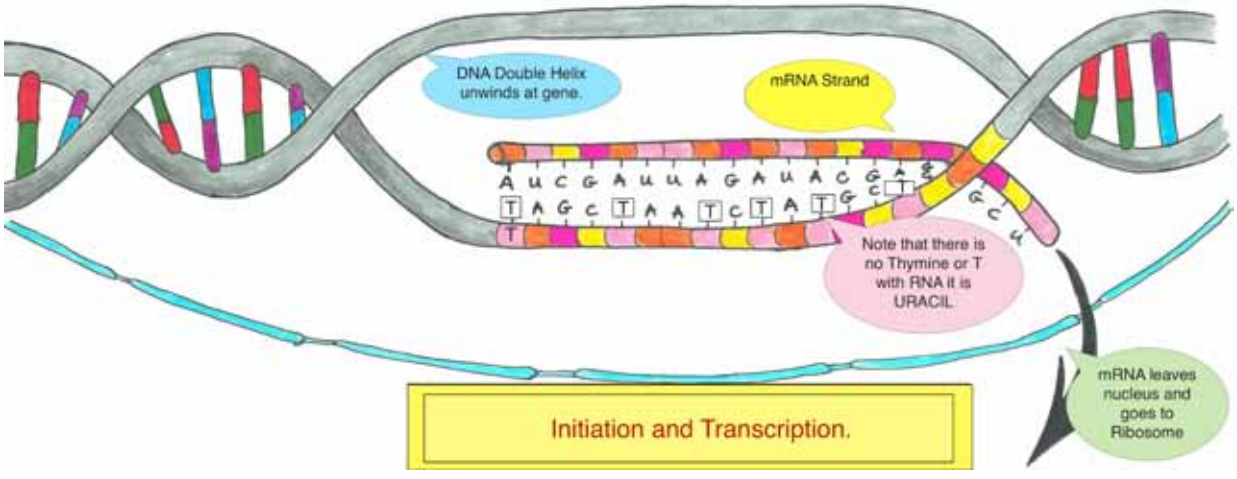
.....

2. Transcription. (Nucleus)

- The enzyme RNA polymerase pries the two strands of DNA apart exposing the bases.
- Free RNA nucleotides in the nucleus attach to the exposed bases of the gene using complementary base pairing, to create an mRNA strand.
- The enzyme RNA Polymerase joins the RNA nucleotides together to form a single mRNA strand (messenger RNA).
- The code was Transcribed from the DNA strand onto mRNA. The DNA strand acted as a template for the production of the mRNA strand. This is a simplistic account of what is actually occurring, in eukaryotes, the process is more complicated with additional steps in the process.
- The DNA double helix rewinds and the mRNA strand leaves the nucleus through the nuclear pores.

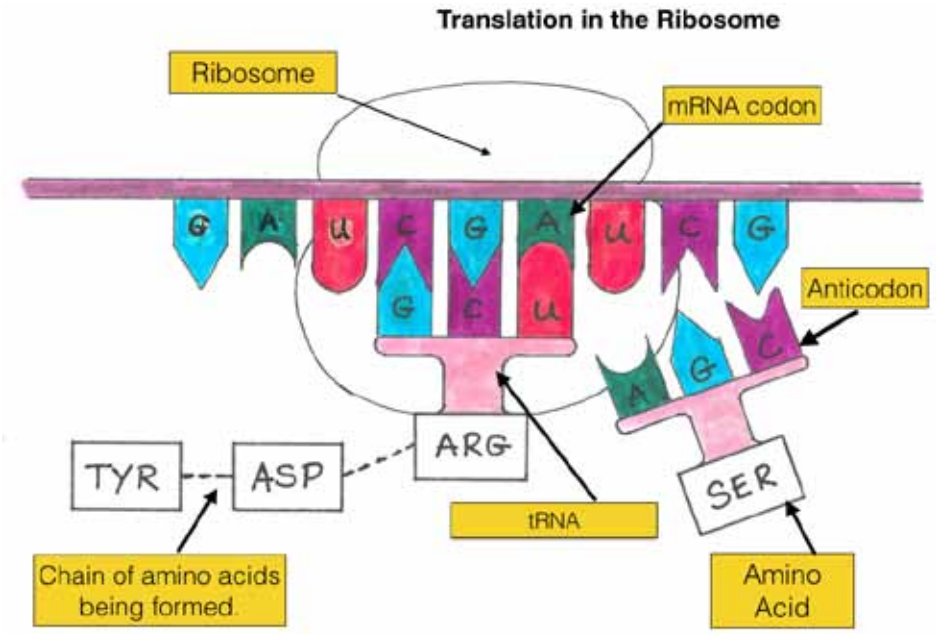
Note: 3 is that magic number-always remember that when we talk about genetic code we are talking about groups of 3 bases called codons and the same message will be transcribed into mRNA.

Very Important: there is No Thymine (T) in RNA-Uracil (U) replaces the T, so wherever there is Adenine (A) on DNA coding strand the complementary RNA strand will have Uracil (U).

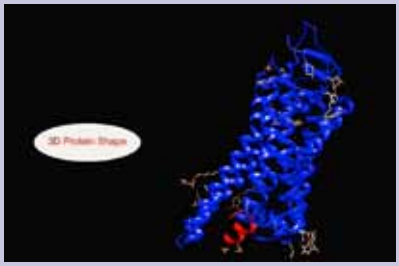


3. Translation. (Ribosomes)

- The mRNA arrives at the ribosome-a ribosome is made up of two parts or units stacked one on top of the other and ribosomes are made of RNA-specifically called ribosomal RNA (rRNA) and proteins.
- The mRNA enters the ribosome between the two subunits. Moving along through the ribosome other RNA nucleotides called Transfer RNA (tRNA) arrive from cytoplasm with an amino acid that corresponds to each codon on the mRNA Strand.
- As the mRNA strand moves through the ribosome tRNA molecules with the corresponding Anti-Codon deliver the correct amino acid, which joins to the amino acid just delivered, this process of delivering and attachment of amino acids continues until the STOP codon on the mRNA is reached.
  (Hint: tRNA=Transfer meaning to Transport)



4. Protein Folding.



- This occurs after synthesis and is needed to make the protein more functional.
- As the chain of amino acids emerges from the ribosome it begins to coil and folds into a specific shape.
- Folding is a crucial part in protein production-if not done correctly it can result in a non-functioning protein.
- Folding can involve removal of part of the chain and other alterations but you don't need to know these now.

Need a break?

Protein Synthesis Animation

This is a very simple yet informative video which covers the whole process. Watch it a few times-it seriously helps you understand protein synthesis. Perhaps it will inspire you to create your own version!

http://youtu.be/QY9LZ5tt-QE

Journey Inside The Cell
A great overview of Protein synthesis to build upon your knowledge gained.
http://youtu.be/1fiJupfbSpg

Greatest Genetic Discoveries
A superb documentary presented by Bill Nye.
http://youtu.be/vIrrtM47Wio

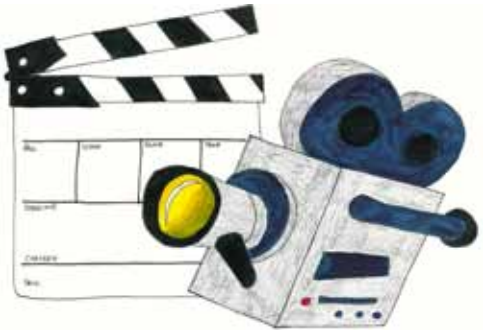
The Central Dogma of Molecular biology.

This term appears frequently when you study genetics and you will hear it often but don't worry you already know it! Dogma means a view or set of views that is accepted by peers or members of a group without being questioned or doubted.

The Central Dogma of Molecular Biology forms the backbone of this area of science. Simply put, it means that once the information contained in DNA gets into a protein, it cannot flow back from protein to nucleic acid.

Class project - YouTube is filled with thousands of Stop Motion movies on every topic of biology. They are not specific to the Irish curriculum and so are not ideal when you need a recap.

We need Irish produced biology videos on YouTube.



It's easy and not expensive. You most likely have all the materials you need.

Materials:

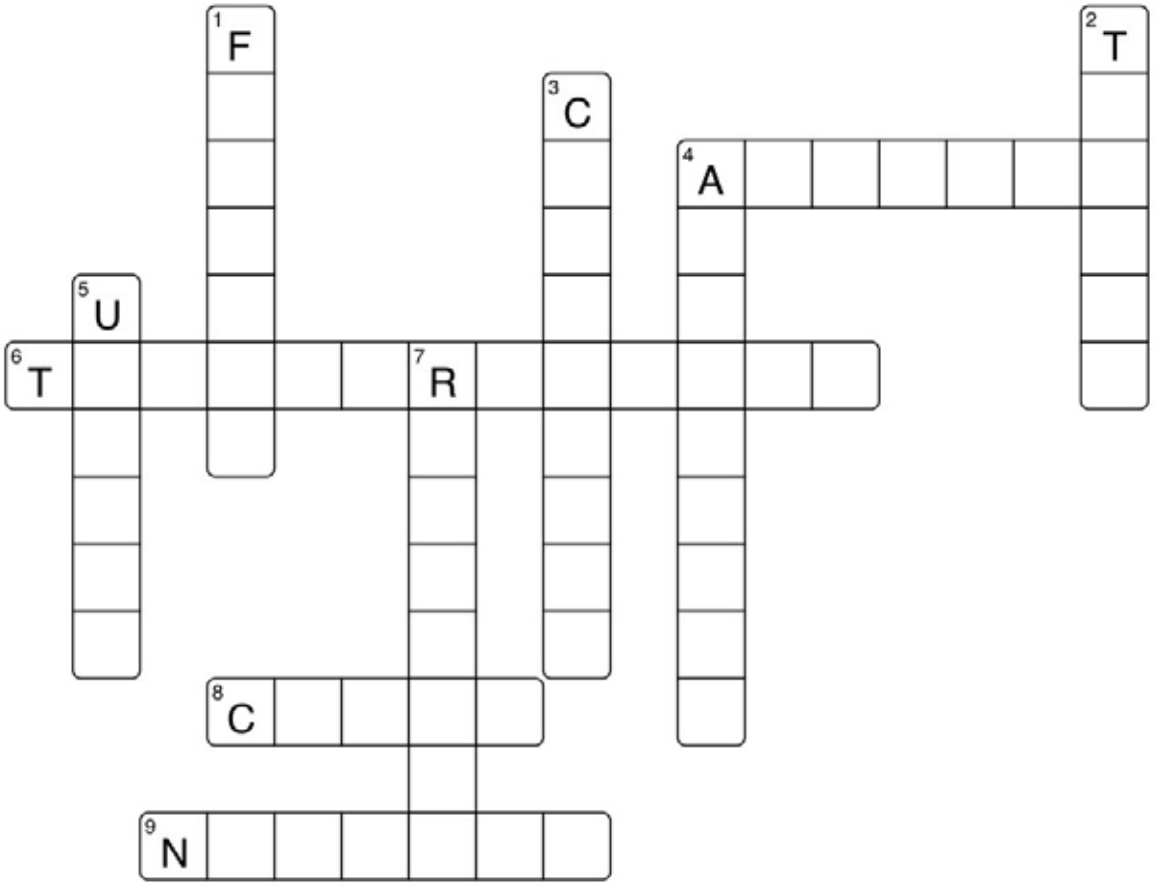
- A camera-the one you take on holidays, or any smart phone camera or any tablet camera.
- Software to put your video together-Windows Movie Maker is free and if you are using Mac, iMovie works really well.
- You need musicians-they need to create their own music-you don't want copyright issues!
- The rest is down to you-small white boards that you write or draw on and wipe off, Lego pieces that you move around, drawings-these work really well, play dough, you and your friends performing-it is up to you.

Hints & Tips: the most important part to making a movie is a steady camera fixed in one position. Use the retort stands in the lab and tape your cameras. Avoid shadows, so ensure you have a well lit room. Have a background colour if you are using drawings or play dough-it looks better on screen.

The iPhone and iPad have very good microphones-sound recordings are crisp in comparison to other devices where sound can echo or sounds distant.

How to do it-
- Make a story
- Create the props
- Start taking pictures
- Take a picture move your prop slightly, take another picture, move your prop, take another picture.
- When you have done with photography upload the pictures to the computer and open Movie maker.
- Record your music and add it to Movie maker.
- Create your YouTube channel and upload.
- Stuck-there are great instruction videos and tutorials on YouTube-they do help.

Do You Know Protein Synthesis?



Across Hints

4. The name of the base which is complementary to Uracil on RNA.
6. The process where the genetic code gets transferred from one nucleic acid to another.
8. A group of 3 nucleotides-Hint: START & STOP
9. The site where initiation and translation occur.

Down Hints

1. The last stage in protein synthesis.
2. The number of amino acids.
3. The place in the cell where you find tRNA.
4. The opposite to codon.
5. The base unique to RNA.
7. An organelle which is made up of two units.

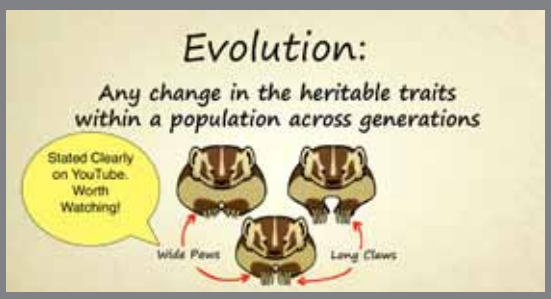
1.9. Genes, Variation, Speciation & Evolution.

Molecular evolution forms a large part of the many research projects ongoing in the Bat Lab. Scientists are looking inside the cells and their biochemical mechanisms to uncover how new species evolve and what is occurring at a cellular level, so genetics is crucial.

In the past fossils and carbon dating were used to map how organisms had evolved, however with regard to bats there is no great library of remains, perhaps because the tiny bat skeletons are very delicate and are more easily broken down. Today scientists are using a combination of morphology (studying the physical structure) with molecular science to study speciation (how new species are formed) and evolution. They are a bit like the super sleuths of science- tracing back to see why one group of bats changed and became so different that a new species was born.

What is Evolution?

Change happens in DNA due to imperfect replication/division. Some of these changes are beneficial or adaptive i.e. individuals can live longer, have more babies (offspring) or are more attractive (more successful at finding mates) and so leave more babies. As these organisms reproduce and leave more offspring it ensures that the beneficial traits are more prevalent in a population.



Stated Clearly is a science education channel on YouTube. There are a number of really excellent short videos that cover Natural Selection, DNA, Genes and Evolution.

http://www.youtube.com/user/sciencestatedclearly

What are Species?

Species are groups of similar organisms that can successfully reproduce and these offspring will also be capable of successful reproduction (they will be fertile). There are over 1200 species of bat in the world (bats make up 1/5 of all living mammals). All of these species came from a common bat ancestor but how did they separate into this number of species. Are they evolving at present? Question: Are humans still evolving?

Why study evolution?

Evolution helps us to understand the history of life, knowing the past can enable us to better study and understand the present and predict the future. To understand evolution you must embrace the "Tree of Life" concept. Life on this planet came from common ancestors, some tiny microorganisms that over billions of years changed, genetically altered and branched off into new different forms of life, this process of slow genetic change continued, still continues today and can be presented as a "Tree of Life".

The steps involved in evolution.

Genetic Variation.
Evolution will not occur unless there is genetic variation. Variations in genes or their regulation that result in differences in physical traits, traits which can be passed on to future generations are a prerequisite for evolution. An organisms genetic makeup is called its **Genotype** and the physical characteristics of an organism is called its' **Phenotype** (how the organism looks).

You are 99.9% genetically identical to your neighbour, you're both human but you look nothing like each other, unless of course unless you are identical twins (genetically identical). This difference in your phenotype (physical appearance: eye colour, skin colour, blood type, hitchhikers thumb etc.) is caused by that 0.1% difference in your genome and that of your neighbour. Important: when you talk about traits that can be passed from one generation to the next you are talking about hereditary traits. The **Gene** is the **unit of heredity**. You are genetically similar but different to your parents. Evolution cannot occur without genetic variation.

Sources of Genetic Variation.
- **Sexual Reproduction** (half of your genes are from mother half from father).
- **Gene flow** - movement of genes from one population to another. Example: A bat from one population could mix with those from another population due to migration (must be same species). When gene flow results in the introduction of new gene versions into a population, this is a great source of genetic variation.
- **Mutations**-changes in the DNA (insertion of extra, deletion or alteration of nucleotides) which mostly occur randomly due to mistakes at DNA replication. Other mutations can be due to exposure to substances that cause mutations (mutagens) examples: cigarette smoke, chemicals, ionising radiation). Only mutations that occur in the DNA of the egg or sperm matter in evolution. Why is this the case?

Speciation-the formation of new species.

It is possible to catch two bats that look similar but when you analyse their DNA you determine that they are actually different species. In the wild it would be unlikely that these bats would ever mate but if they did they would not produce any offspring that could reproduce.

What causes speciation?

1.Geographic isolation (Allopatric Isolation) - bats migrate, perhaps a group cannot return with the rest of its population, stranded they form their own little colony. Each of these bats would have some degree of genetic variation, only those with traits (features such as colour, sight, hearing etc features controlled by genes) making them better suited or adapted to their environment will survive. These survivors reproduce passing on those genes which gave them the more useful traits. **Darwin's Theory of Natural Selection**.

Time passes and the isolated population of bats continues to breed amongst themselves, the reduction in gene flow, variation and environment all results in this population changing slowly over time. Perhaps it was the ability to echolocate at a slightly higher frequency that gave the surviving bats the edge-perhaps they found more insects! Millions of years pass and the isolated bats are now so genetically different to their original ancestors that they are unable to breed with them. Perhaps they cannot hear each other as they are echolocating at different frequencies. A new species has formed-Evolution.

Recommended Viewing-Darwin's Dangerous idea PBS. Available on YouTube.
https://www.youtube.com/playlist?list=PLSvL9i5v5LaLSd7_z1TSxJrFlrBVcjK-G

2. Reproductive Isolation (Sympatric Isolation) - a population of bats sharing the same habitat become unable to reproduce together. Something occurs that makes it impossible for different members of the same species to mate-a magic trait of some kind! Scientists are still trying to work out how this can occur.

Evolution would not happen without genetic variation. Genetic variation gives rise to traits-those traits which enable the organism to best adapt to its environment are selected by nature and passed on. The offspring with the better suited trait lives to reproduce and the genome of that population alters slowly.

Genetic changes can occur in populations to produce a new species slowly over millions of years.

Bats and Evolution

Taxonomy is the classification or grouping of organisms, it was a system designed by Carl Linnaeus in 1735. It was once based on morphology (e.g. bones, skull shape, teeth shape, leaf shape etc) to group organisms. Today classification can be based on a molecular phylogeny which uses DNA analysis to group together related organisms based on genetic similarities and differences.

Bats are grouped into an order called Chiroptera meaning "hand-wing" and this order further splits into two sub ordinal groups which have been proposed based on molecular data: Yinpterochiroptera and Yangochiroptera. There are two types of bats: megabats which are non-echolocating and eat mostly fruits and the microbats which do echolocate and eat mainly insects. Ireland has nine species of microbats and no megabat species (refer to section 4.2 on bat identification).

Bat evolution is still a puzzle with missing pieces and the lack of fossils makes it difficult to map the evolutionary pathway of bats. They have been around for approximately 64 million years and the oldest bat fossil can be dated to 50 million years ago approximately. Based on bat fossils, scientists think that that flight evolved first before echolocation. How and when bats developed and evolved echolocation is still unknown. The creation of phylogenetic trees. which can be made with both molecular and morphological data, aims to answer these questions (see sections 3.2 & 3.3).

Bats Hunting Their Prey

Great YouTube clip from the BBC. Watch microbats in action and learn how crucial echolocation is to their survival.

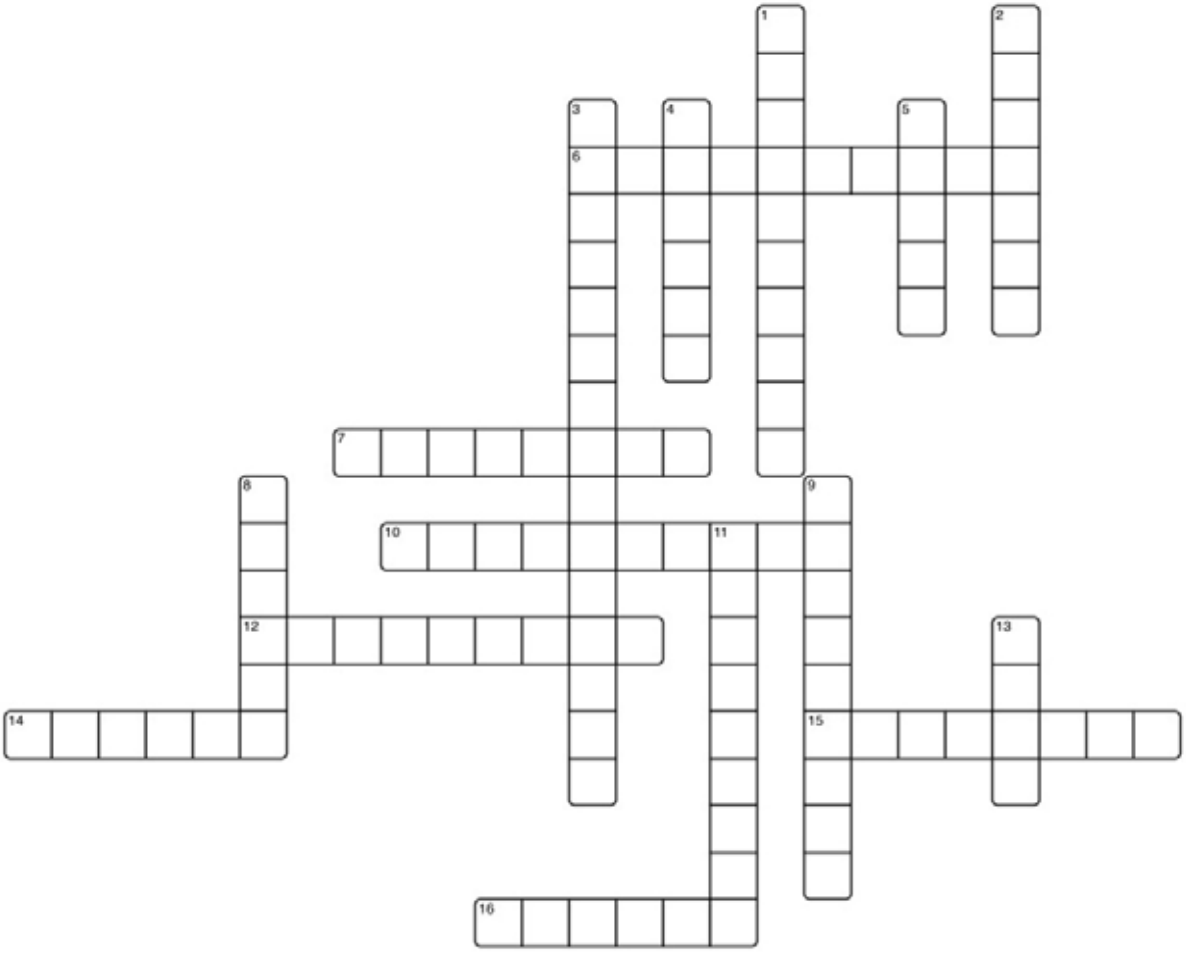
http://youtu.be/p08Y0oRAX3g

Bats and Their Evolution.

Great YouTube clip as Professor Emma Teeling (UCD) gives some insight into bat evolution.

http://youtu.be/0nqYi7ydEzg

Do You Know Bat Evolution?



Across Hints

6. Another name for Geographic Isolation.
7. A cause of genetic variation.
10. Means 'Hand-Wing"
12. The only type of bats in Ireland.
14. Compressed dead plant and animal remains.
15. The study of classification.
16. The father of evolution.

Down Hints

1. Study of structure (bones, teeth etc).
2. A group of similar organisms capable of inter-breeding to form fertile offspring
3. MicroBats belong to this sub-order.
4. A trait which evolved before echolocation.
5. Megabats eat these.
8. Bats belong to this group.
9. genetic _____ essential for evolution.
11. This will not occur unless there is genetic variation.
13. The number of resident bat species in Ireland.

Genetic variation-Positive or Negative you Decide?

You now know that genetic variation is simply a difference in nucleotides where they were either deleted, extra nucleotides were inserted or the original has been replaced with a different nucleotide. This is only an issue when the variation results in a different amino acid being inserted into the protein, changing the intended protein. The outcome of this change can be positive or negative.

Are you a Super Taster?

Super tasters are those people that experience taste with far greater intensity than most, they account for about 1/4 of the population. Super tasters have more papillae (taste receptors) on their tongues. Super tasters are more sensitive to bitter tastes and fattiness in food and so generally they will not eat food with these traits. Foods such as broccoli, dark chocolate, coffee, cabbage, Brussels sprouts and green tea are avoided. Researchers from Yale University in the U.S. have found that there are more women (35%) then men (15%) that are super tasters. Why would this be the case?

BBC Science Club Super Taster Test.
http://www.bbc.co.uk/ science/0/22941835

Investigate how many super tasters are in your class.

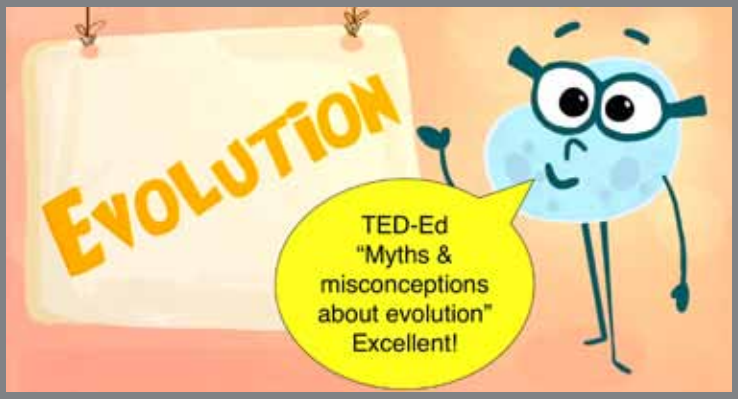
Materials:
Bottle of blue food colouring (ensure you are not allergic)
Cotton buds
Tweezers
Hole punch reinforcers or a square of grease proof paper with a single punch hole.
Magnifying glass & Mirror.

Instructions
Please ensure that hygienic procedures are adhered to.
Dip the cotton bud in the bottle of food dye and apply to a small area of the tongue.
Place the paper reinforcer over the area with the food dye using the tweezers.
Using the magnifying glass count the number of pink bumps-the Papillae-they should not be stained blue.

Results
If you have more 35 papillae you are a super taster
If you have between 15-35 papillae you are an average taster, about 1/2 of the population.
If you have fewer than 15 papillae you are a non taster..

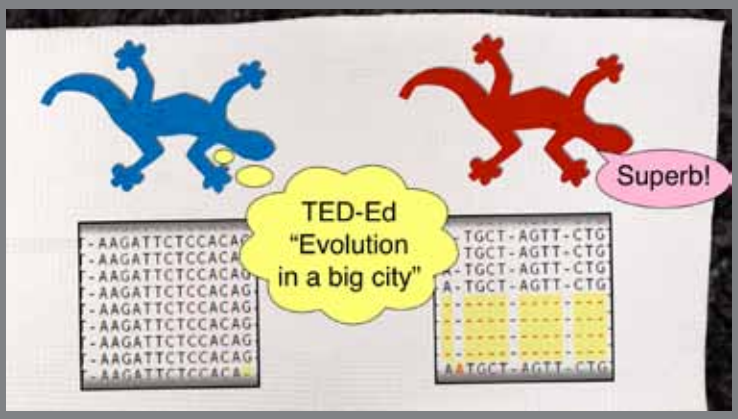
Discuss: Is it better to be a Super Taster or not? Any health implications? Would they make better chefs?

3 - Video Clips Which Serve to Educate and Entertain.



TED-Ed Myths and misconceptions about Evolution

http://youtu.be/mZt1Gn0R22Q



TED-Ed Evolution in a Big City

http://youtu.be/ckGB5lHyzME

The Epigenetics of Identical Twins

The University of Utah has some great videos on genetics. This is a great clip on why identical twins (genetically identical) change in their physical characteristics as they age.

http://youtu.be/AV8FM_d1Leo

Section 2. UCD Bat-Lab

2.1 Introduction to Bat Lab.

The UCD Bat lab-is a short name for the **Lab of Molecular Evolution & Mammalian Phylogenetics**. At any one time there are a number of research projects running simultaneously. Summer is a busy time for scientists involved in bat research, bats hibernate in winter and this rules out winter for any bat field studies. In July the bats as very active and scientists are often on field study at this time. Field study can involve just counting numbers and selection of bats, using bat sensors to record their calls and using harmless methods of tagging the bats (important to keep a check on population number). Genetics is the key to looking at how bats have evolved and how they have changed through time to develop these marvellous amazing feats of long life (longevity), resistance to harmful viruses such as HIV and Ebola and echolocation. Genetics is also used in distinguishing one species from another. To examine these traits DNA is needed and this forms a huge part of field studies. The research team in UCD go to Brittany in France (huge amount of bats) where part of their work involves collecting specimens from the bats for genetic analysis. These specimens can be a very small sample of blood or a tiny wing punch. Bat wings are made of a very thin light skin membrane, when the bats are caught a tiny sample of this skin is collected and immediately frozen in liquid nitrogen to preserve it. Please note that the scientists involved are specially trained and licensed to perform this and the bats are not harmed in the process. So samples are collected and frozen, then time to return to UCD, Dublin.

One project which is ongoing in the bat lab is a study to better understand the ageing mechanism in bats. A specific mitochondrial gene called Cytochrome c is examined in a number of bats year on year. The bats have been caught in France when the study began and marked, so that on return each summer the scientists can re-trap these bats, know who they are, take wing clippings, extract the DNA and sequence it. The aim of the study is examine the sequence of nucleotides (A,T,G,C) in this gene from each bat clipping. Scientists want to examine if as the bats get older, does the sequence of the gene change. (Mitochondrial DNA is frequently used in genetic analysis as it contains multiple copies of the same gene. The genes in mitochondrial DNA evolve more rapidly than those in DNA found in the nucleus and this means that any changes will be evident earlier than gene changes in the DNA found in the nucleus).

2.2 The Steps involved in DNA Analysis. Bat Lab UCD.

1. Collect tissue samples.
2. Extract and purify the DNA.
3. Use PCR (Polymerase Chain Reaction see below for full description of this key method) to produce multiple copies of the particular gene.
4. Check that the PCR has worked using gel electrophoresis.
5. Sequence the PCR product (DNA)— getting the A,T,G,Cs
6. Bioinformatics-use of computer programmes to identify the sequence, align the sequence with the same gene in other species and identify the any differences in terms of bases or amino acids. Phylogenetic trees can also be created (section 3.2 on phylogenetics)

Paul Anderson-an award winning biology teacher in the United States has an amazing science channel on YouTube - Bozeman Science.

This is the most amazing tutorial on many of the procedures used in the Bat Lab.

Highly Recommend that you watch 'Molecular Biology'

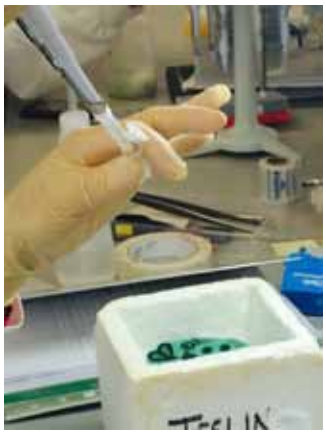
http://youtu.be/yYIZgS-L5Sc

A bat wing-made of a very thin skin membrane.

2.2.1 Extraction and Purification of DNA.

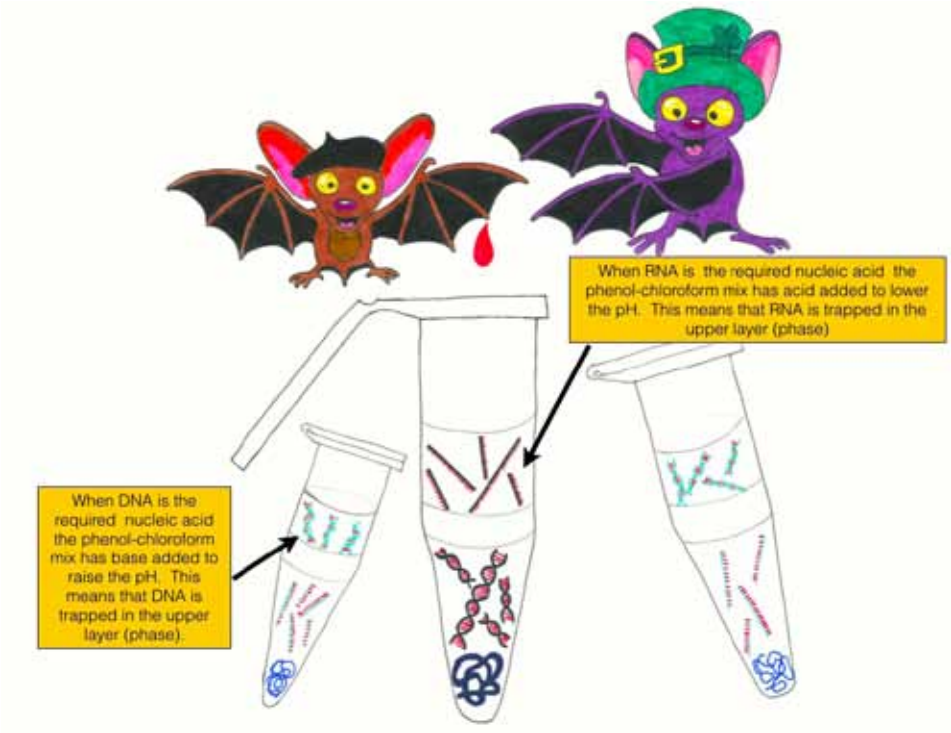
Extract the DNA-release it from the cells.
Lysis buffer is added to the tissue samples (e.g. wing clippings) and homogenised or mixed. The buffer contains a detergent which disrupts the membranes and also salt which causes the DNA to clump to together. (Lysis means to split). Remember your DNA extraction of plant cells!

Purification-The DNA extracted in school labs would not be of much use to research scientists as it contains too many impurities. Research labs use two chemicals phenol and chloroform to get of the impurities and to end up with a solid DNA pellet. The process is similar to a cell fractionation, add the phenol and chloroform and some base and centrifuge. The end result is that you end up with layers, a liquid top layer and a cloudy bottom layer .Using the micropipette the top layer containing the DNA is removed and the process is repeated. The phenol & chloroform is added and the tube is centrifuged and any impurities get trapped in the bottom layer leaving pure DNA in the liquid top layer. Pure alcohol is added to the tube and it is stored in the freezer overnight. The sample is centrifuged and the DNA forms a pellet at the end of the tube. Remove the alcohol-You have pure DNA.



Adding the Phenol-Chloroform

Loading the centrifuge



Note:

RNA is also frequently analysed-extraction and purification is exactly the same-the only difference is acid instead of base is added to the phenol-chloroform. The acidic pH traps the RNA in the liquid top layer.

2.2.2 PCR - To amplify a gene or DNA sequence.

Polymerase Chain Reaction

This technique was developed by Kary Mullis in 1980, for which he was awarded a Nobel Prize.

Polymerase chain reaction (PCR) is a lab technique where a tiny sample of DNA with a sequence of interest can be amplified (multiple copies) and vast amounts of that same sequence can be produced within 2 hours.
PCR is used in forensics when blood or hair samples are collected and the DNA is extracted from these samples, however one hair or tiny blood spot may not have enough DNA with which to work and so PCR is an essential process for the molecular biologist. PCR amplifies the DNA sample, thus ensuring that there is a workable amount for any further tests.
PCR is also used in medicine, one application is the detection of HIV or Ebola, another is in cancer treatment where PCR can be used to analyse tumours and predict if they will respond to chemotherapy and also to identify a virus you might have.

What is needed for PCR? The Master Mix-a solution which contains everything that is needed for the reaction. Think of what is needed for DNA replication!

- Primers-these are small sections of DNA which will bind to opposite strands of the DNA double helix when it unwinds and separates. They mark where the nucleotides should be placed in the building process.
- Nucleotides-these are the separate building blocks for the new DNA strands-A,T,G,C
- DNA polymerase-this is the enzyme needed to connect all the nucleotides together and form the new DNA. A very specific type of DNA polymerase is used in PCR, it's called Taq Polymerase. Usually enzymes are denatured with very high temps and PCR reaches 95°C, Taq Polymerase is used as it is not denatured (broken down) by high temps. Taq Polymerase was originally isolated in a bacterium found in the hot springs of Yellow Stone Park in the U.S. It is now produced using genetic engineering in labs.

The PCR Process

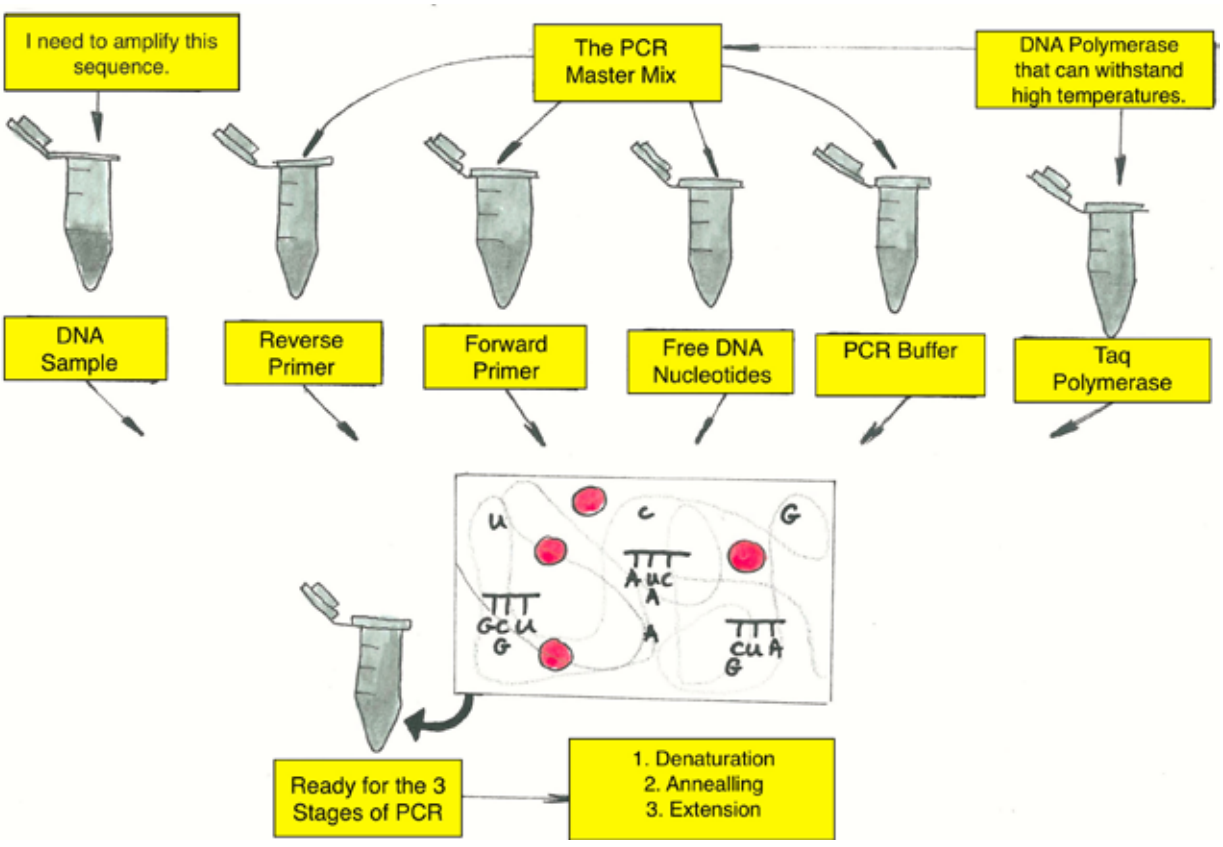
Add your DNA samples to the Master Mix - load into the PCR machine. The PCR machine will carry out the following steps:

- **Denaturation**-The DNA is heated to 95°C- this unwinds and separates the strands in the double Helix.
- **Annealing**-The temperature is lowered to approximately 50-60°C and the Primers attach to each strand of the DNA.
- **Extension**-The enzyme Taq Polymerase adds the new nucleotides to the end of the primers on both strands-the new DNA rewinds into the double helix, occurs at approximately 70°C.

This process is repeated about 30-40 times, at the end of this you will have very large quantities of the DNA sequence you wanted. (see Molecular Biology by Bozeman Science, link given at start of section)

2.2.2. PCR

The PCR Machine



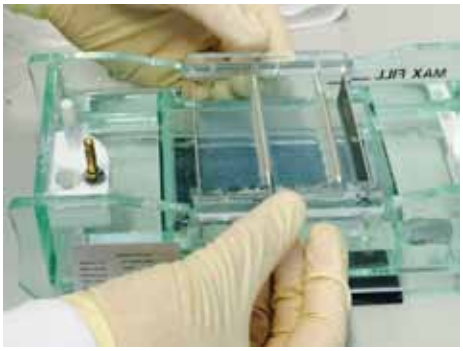
PCR

2.2.3 Gel Electrophoresis.

PCR is used frequently in the Bat Lab-it's very useful for identifying species of bat which look very similar but have different genomes. You will have performed all the steps and PCR has amplified the DNA sequences that you selected. The next step is to use gel electrophoresis to separate the DNA fragments amplified and create a pattern for comparison.

Gel electrophoresis - using electricity to separate fragments of DNA according to size.

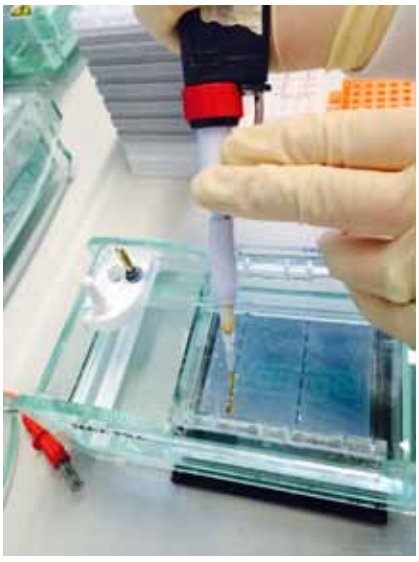
1. Make the gel-this is made with an agarose powder (extracted from seaweed), to this you add a special DNA detecting dye-this enables you to see your DNA under uv light. The gel is made so that there are a number of spaces (wells) where your DNA can be loaded. Leave to set and then place in the electrophoresis machine, which connects to a power pack.



Preparing the gel

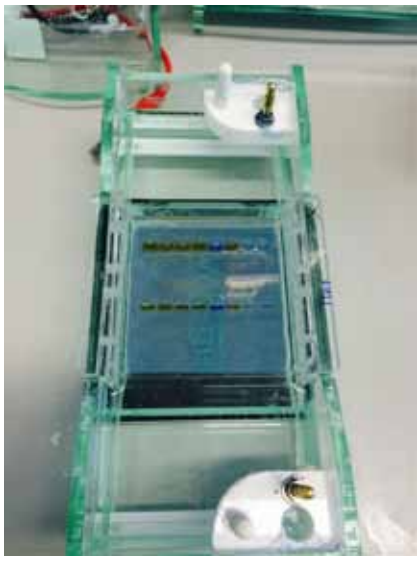
2. The PCR samples are then prepared, you use only a small amount of your PCR samples and save the rest for sequencing later.

3. The samples are loaded into separate wells in the gel and the electricity switched on. Electricity causes the strands of DNA to move down the gel. The gel acts like a sieve the higher the % agarose gel the smaller the holes. The smaller sized DNA fragments move more quickly across the gel from negative to positive poles. DNA is negatively charged so moves towards a positive charge. The DNA is separated on the basis of size. (cont...)



Loading Samples

Lanes loaded and gel ready to run



4. After about 20 minutes the process is complete, remove the gel and place in the u.v, light chamber. This is connected to a computer that takes a photograph.

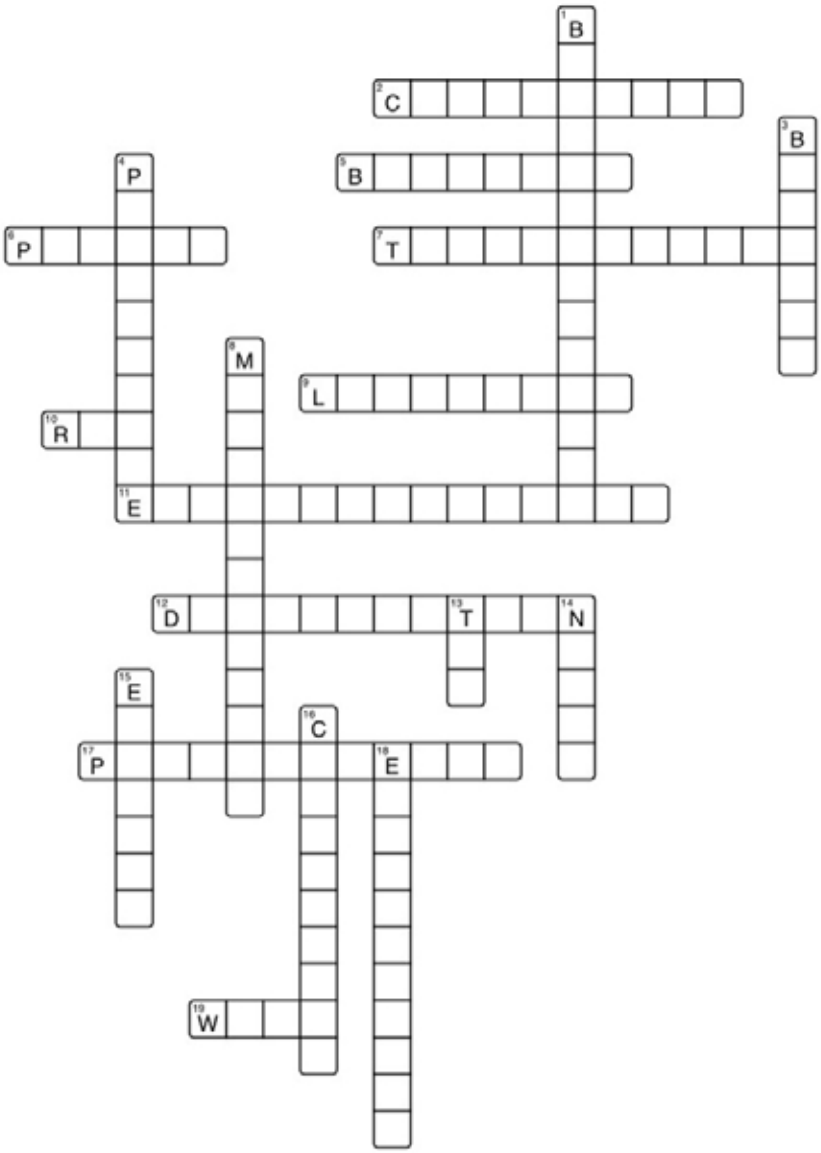
Note: Gel Electrophoresis is used in Bat Lab to check that the PCR worked-if you see bands in the correct positions and the band size is correct, then you know that it has worked. It is also used to identify which species of bat you have caught by sequencing the PCR band. In species identification you can compare bands produced with those of a definitely identified bat.

Next step is to send your PCR samples away for sequencing. Specialised labs perform this and you receive back a file with a list of the nucleotides (ATGC) in your sample.

DNA Barcoding.

This is a technique used to identify species. It can even be used to identify what an animal may have consumed! Barcoding uses a short genetic sequence (approximately 648 base pairs long), which is unique to each species, taken from mitochondrial DNA, specifically the cytochrome oxidase 1 (CO1) gene. This mitochondrial gene is used as all animals have it, it is easy to extract, and it is different in every species. Most importantly this CO1 gene is well conserved through evolutionary history so that it can be identified as being the CO1 gene in many organisms (animals, a different gene is used in plants) but it also accumulates small changes – mutations- as species diverge or differentiate-perfect for identifying new species. This gene sequence is extracted, amplified using PCR and sequenced (A,T,G,C), the information is then added to a database and compared with others, in this way new species can be identified. DNA barcoding is commonly used to check that food sold is as per its label, remember the horse meat scandal in Ireland in 2013! It should also be noted that less than 2 million of the estimated 5-50 million plant and animal species have been identified to date, species risk extinction without formal identification. **The International Barcode of Life (iBOL)** is an amazing project in which perhaps your class could become involved. Check out their website http://www.ibol.org and the barcode of life site http://www.barcodeoflife.org.

Test Your Bat Lab Knowledge



Across Hints

2. Chemical used in DNA purification-used as anaesthesia in bygone times.
5. An area of France where there are many bats.
6. What DNA looks like when centrifuged with ethanol.
7. Loves high temperatures.
9. A trait which bats have and we long for.
10. Single stranded nucleic acid.
11. Separating DNA fragments using electricity and gel.
12. The first stage in PCR.
17. Another name for a gene tree.
19. A part of bat where tissue samples are taken.

Down Hints

1. Computer analysis of genetic sequences.
3. DNA_____, created using part of the CO 1 gene sequence to identify species.
4. A type of enzyme used in PCR.
8. Organelle with DNA-useful in examining DNA changes.
13. Enzyme used in stage step 3 of PCR.
14. The prize the inventor of PCR was awarded.
15. DNA is insoluble in this.
16. Machine which spins samples, used in separation.
18. Causes the DNA to move up the gel in electrophoresis.

Section 3 Comparative Genomics and Phylogenetics

At the end of this section you should be able to:

- Describe what is meant by DNA sequencing.
- Explain what is meant by Bioinformatics and Comparative Genetics.
- Carry out a task using Bioinformatics
- Complete your own Phylogenetic tree using on-line software.

3.1 DNA Sequencing

So what is the next step after Gel Electrophoresis?
Well, if your samples are of good quality with a sufficient concentration of DNA, the samples can undergo DNA sequencing, a technique by which the exact order of nucleotides within a DNA molecule can be determined. The process has come a long way since the initial manual 2-D chromatography methods were developed and today, automated procedures are used which have the advantage of speeding up turnaround times. When you send your sample of DNA away for analysis, the sequencing result that you get back may look something like this:

ATGACCAACATTCGAAAATCTCACCCCTTAATAAAAATTATTAACAGCTCATTTATTGACCTCCCTGCCCCATCAAACATTTCATCTTGATGAAACTTTGGATCTCTCCTAG
GAATTTGCTTAGCACTACAAATTTTAACAGGACTATTTCTAGCTATACACTACACATCAGACACCACAACAGCTTTTAACTCTGTCACCCATATTTGCCGAGATGTAAACTAT
GGTTGAGTTCTACGCTACTTGCATGCAAATGGAGCCTCCATATTTTTTATCTGCCTATATCTCCATGTAGGACGGGGCCTTTACTATGGGTCCTATATATATACAGAAACCTG
AAATATCGGAGTTATTCTATTATTTGCTGTAATAGCAACAGCCTTTATAGGATATGTACTTCCATGAGGACAAATGTCTTTCTGAGGAGCAACAGTAATTACCAACCTGCTCT
CTGCAATTCCGTACATTGGAACAGACCTTGTAGAATGAATCTGAGGCGGCTTCTCTGTTGACAAAGCTACTTTGACCCGATTCTTTGCCTTTCACTTTTTACTCCCATTTA
TTATTGCAGCCATAGTCATAGTCCACCTCCTATTTCTTCACGAAACCGGATCCAATAACCCAACAGGAATCCCCTCCAACGCTGATATAATCCCCTTCCACCCCTACTATAC
AATTAAAGACATTCTTGGCCTGCTATTAATAATTACAGTCCTACTCATACTAGTACTATTCTCCCCCGACCTGCTAGGAGACCCTGACAACTACACACCAGCGAACCCACTAA
ACACCCCTCCCCATATCAAACCGGAATGGTACTTTTTATTCGCATATGCAATTCTACGATCAATTCCAAACAAACTAGGAGGAGTGTTAGCCCTAGTACTATCAATCCTTATT
CTAATTATCATTCCCCTACTCCACACCTCCAAACAACGCAGCATAACTTTTCGTCCCTTAAGCCAGTGCCTATTCTGACTATTAACAGCAGATCTATTCACTCTAACATGAAT
CGGAGGACAGCCCGTCGAACATCCATATGTCATCATTGGCCAACTAGCATCAATTCTTTATTTTTCTATTATCATTATCCTAATACCACTTATTAGCCTGATAGAGAACCACCT
ACTAAAATGAAGA

So what do all these letters mean and what can we do with this information?

All of the genes that have been sequenced are sent to a central database which acts like a huge library. Therefore, when you get your sequencing result like the one above, you can compare it against known results in the database and look for similarities and differences.

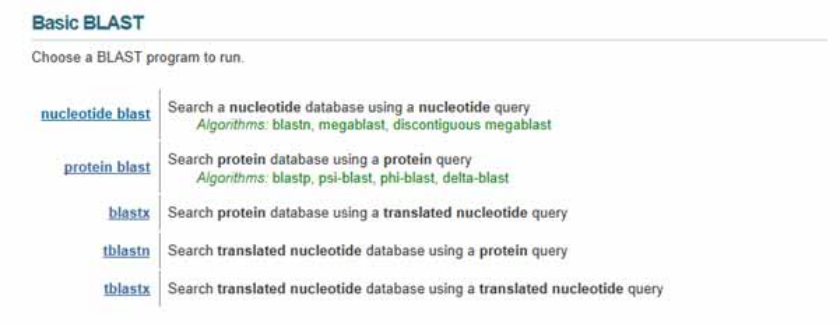
To do this manually would take a very long time and a lot of patience. Thankfully, we can use Bioinformatics. By using software known as BLAST (Basic Local Alignment Search Tool), the unknown or query sequence can be compared and contrasted against a database of known sequences in a matter of seconds.

Please note that the following tasks do not require any software to be downloaded onto your computer. All of the programmes are available online. To find out what species of bat the above unknown sequence (in blue) belongs to, you can use BLAST as follows:

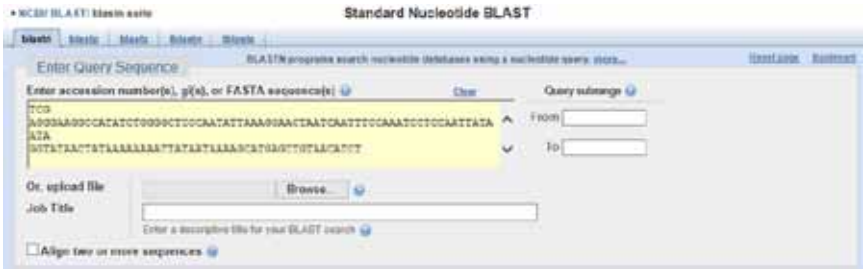
Log on to: http://blast.ncbi.nlm.nih.gov/Blast.cgi

(cont...)

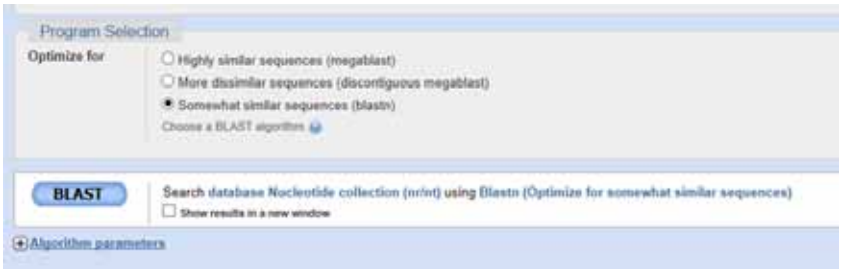
Scroll down the page and click on nucleotide blast.



Copy and paste the sequencing results (provided by your teacher) into the space provided in 'Enter Query Sequence'

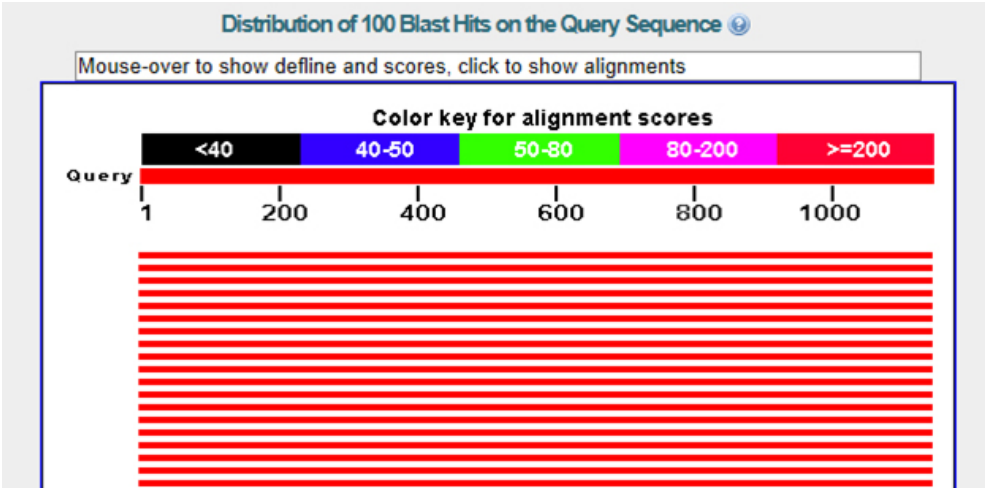


Scroll down the page and click 'Somewhat similar sequences'. Then click BLAST.



You'll need to wait for a few minutes while the programme is running and it is important not to refresh the page or hit the back arrow during this time.

The results will look something like this:



3 1 2

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Myotis brandtii cytochrome b (cytb) gene, complete cds: mitochondrial gene for mitochondrial produ	2057	2057	100%	0.0	100%	AF376844.1
☐	Myotis brandtii isolate 21 cytochrome b (cytb) gene, complete cds: mitochondrial	2051	2051	100%	0.0	99%	AY065139.1
☐	Myotis brandtii mitochondrial cytb gene for cytochrome b, specimen voucher NMP P8 916 (Nat. Mu	2042	2042	100%	0.0	99%	AM261886.1
☐	Myotis keaysi voucher JAG 286 cytochrome b (cytb) gene, complete cds: mitochondrial	1492	1492	100%	0.0	89%	JX130525.1
☐	Myotis keaysi voucher TK 13526 cytochrome b (cytb) gene, complete cds: mitochondrial	1483	1483	100%	0.0	89%	JX130449.1
☐	Myotis keaysi cytochrome b (cytb) gene, complete cds: mitochondrial gene for mitochondrial produc	1480	1480	100%	0.0	89%	AF376852.1
☐	Myotis mystacinus mitochondrial cytb gene for cytochrome b, complete cds, specimen voucher: OC	1471	1471	100%	0.0	89%	AB106605.1
☐	Myotis sp. KKD005 mitochondrial cytb gene for cytochrome b, complete cds, specimen voucher: KK	1462	1462	100%	0.0	88%	AB106609.1
☐	Myotis cf. albescens voucher TK 101479 cytochrome b (cytb) gene, complete cds: mitochondrial	1452	1452	100%	0.0	88%	JX130480.1
☐	Myotis cf. albescens voucher TK 101723 cytochrome b (cytb) gene, complete cds: mitochondrial	1452	1452	100%	0.0	88%	JX130479.1
☐	Myotis atacamensis mitochondrial cytb gene for cytochrome b, specimen voucher MVZ 168933 (Mu	1447	1447	100%	0.0	88%	AM261882.1
☐	Myotis cf. vivesi voucher TK 49533 cytochrome b (cytb) gene, complete cds: mitochondrial	1443	1443	100%	0.0	88%	JX130483.1

So what do these results mean?

There are three important parameters that you need to look at:

1. The E value: This is a mathematical calculation and gives the probability of the result being a false match. What you are looking for here is a result of zero.
2. Ident: This is the percentage of nucleotides matching between your query sequence and the results in the database. Here you are looking for 100% for an exact match to an unkown species. If you do not know what your gene does, even Ident of 70% or above can still help you figure out your gene's function.
3. Query cover: This tells you how much of the sequence length has matched you query. Again, ideally you are looking for 100%.

Note: The accession number is the unique identifier assigned to each gene in the database.

FUN FACT: There are nine species of bat resident in Ireland. They are all insectivorous.

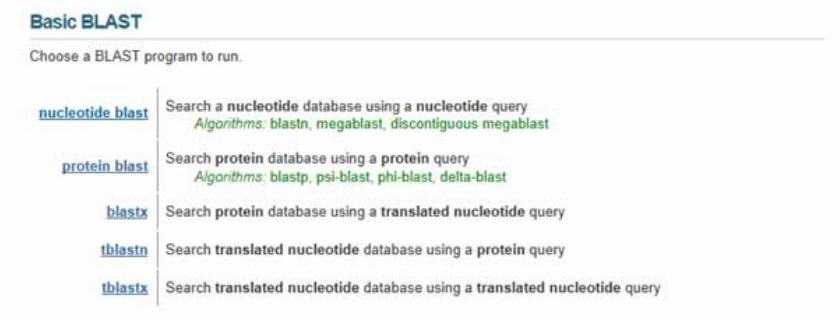
So from the above table it can be deduced that our unknown bat gene has been identified as belonging to the ***Myotis brandtii*** species.

STUDENT TASK:

While the bats native to Ireland eat only insects, some bats in other parts of the world eat fruit or other animals such as frogs. It is possible to extract DNA from bat faeces (e.g. poop, droppings) to help figure out what the bat's diet consists of. Your task is to use the software tools to analyse bat faeces. By doing this you should be able to find out what these bats ate for dinner. Good Luck!

A sample of bat droppings which can be used for analysis of bat diet

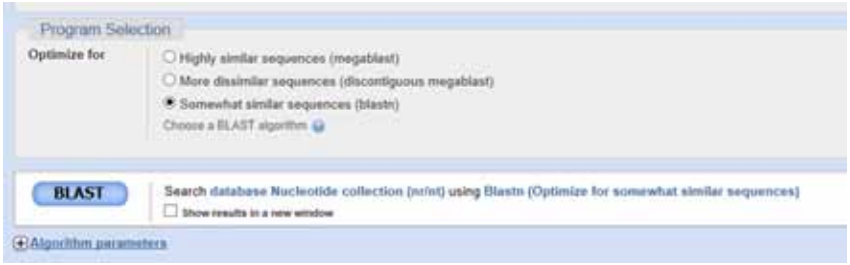
Let's get started - log on to:
http://blast.ncbi.nlm.nih.gov/Blast.cgi
Scroll down the page and click on nucleotide blast.



Cut and paste the **gene for unknown bat food 1** into the space under 'Enter Query Sequence' (your teacher will provide this).

STUDENT TASK (cont...):

Scroll down the page and click 'Somewhat similar sequences'. Then click BLAST.



You'll need to wait for a few minutes while the programme is running and it is important not to refresh the page or hit the back arrow during this time.

The results will look something like this:

And if you scroll to the bottom of the page you will see the following table:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Musa acuminata subsp. zebrina ribosomal protein S16 (rps16) gene, intron, chloroplast	1480	1480	100%	0.0	100%	FJ428139.1
Musa rubra ribosomal protein S16 (rps16) gene, intron, chloroplast	1460	1460	100%	0.0	99%	FJ428132.1
Musa acuminata subsp. malaccensis chloroplast complete genome, biomaterial CIRAD 930	1449	1449	100%	0.0	99%	HF677508.1
Musa rosea ribosomal protein S16 (rps16) gene, intron, chloroplast	1449	1449	100%	0.0	99%	FJ428131.1
Musa textilis plastid, complete genome	1433	1433	100%	0.0	99%	KF601567.1
Musa yunnanensis ribosomal protein S16 (rps16) gene, intron, chloroplast	1425	1425	100%	0.0	99%	FJ428143.1
Musa laterita ribosomal protein S16 (rps16) gene, intron, chloroplast	1424	1424	97%	0.0	99%	FJ428136.1
Musa banksii ribosomal protein S16 (rps16) gene, intron, chloroplast	1411	1411	97%	0.0	99%	FJ428138.1
Musa acuminata subsp. burmannica ribosomal protein S16 (rps16) gene, intron, chloroplast	1409	1409	97%	0.0	99%	FJ428135.1
Musa siamensis ribosomal protein S16 (rps16) gene, intron, chloroplast	1409	1409	96%	0.0	99%	FJ428134.1

STUDENT TASK (cont...):

Now use the internet to find out the common name for *Musa acuminata*. This will give you some information about the diet of this bat.

Question: Is this an Irish bat?

Repeat the exercise using **gene for unknown bat food 2** and complete the table.

Question: Is this an Irish bat?

	gene for unknown bat food 1	gene for unknown bat food 2
Scientific name for food found in bat faeces	*Musa acuminata*	
Common name for food found in bat faeces		
This bat has a diet that consists of:		

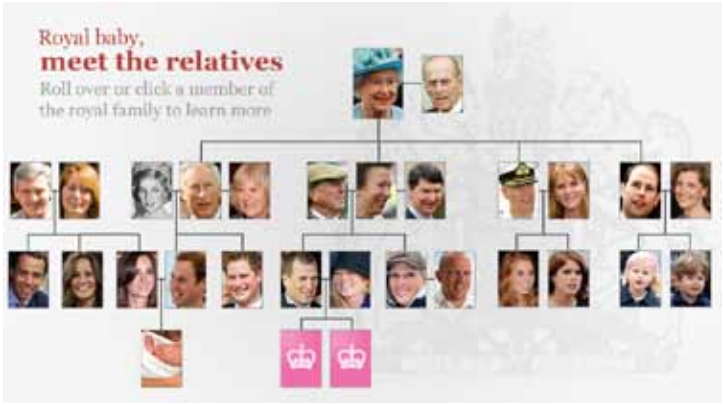
FUN FACT: The smallest bat is the Kitti's Hog-nosed bat which is also known as the Bumblebee Bat. It can be found in parts of Thailand and Burma. This species is considered as the smallest mammal in the world.

3.2 Phylogenetics

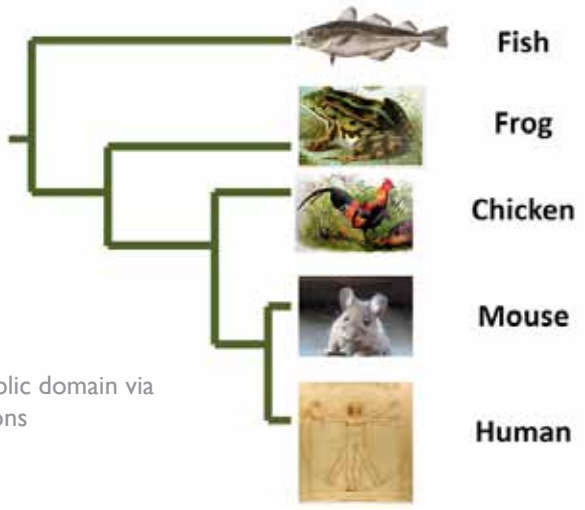
Phylogenetics is the study of how different species are related. We can say that phylogenetics is looking back in the evolutionary time to trace the history of species or a group of species. This evolutionary history of a group of organisms can be represented in a diagram called a phylogenetic tree.

Let's plant a phylogenetic tree

What is a phylogenetic tree? Well, have you ever seen a family tree? Or perhaps you had to prepare a genealogical tree of your family for some school project before? Actually, a phylogenetic tree is a quite similar. Family tree shows how family members are related to each other – it includes both really close relatives (like your mum and dad) and more distant ones (like this far cousin living in Australia, who you've never met). A phylogenetic tree, on the other hand, presents how different species are related to each other (who is our closer relative: monkey or a horse?).



This family tree presents the history of British royal family.
Screenshot from http://edition.cnn.com



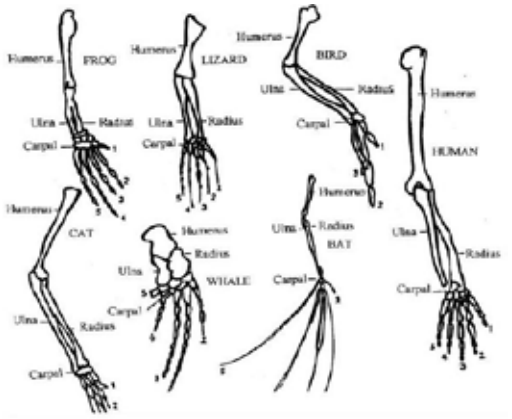
All images used: public domain via Wikimedia Commons

Phylogenetic tree! It shows how different organisms are related.
Are humans more closely related to fish or frog?

When you prepare a family tree you can use different historical documents (like marriage and birth certificates). You can also use genetic tools (if you have, for example, a blood sample) to determine if two people are closely related. When scientists construct phylogenetic trees they can also use different sources of information. They can look at how similar animals are or use a more modern approach – look how similar are certain genes (or other DNA segments) between the species. So both MORPHOLOGY and GENES are useful for constructing a phylogenetic tree!

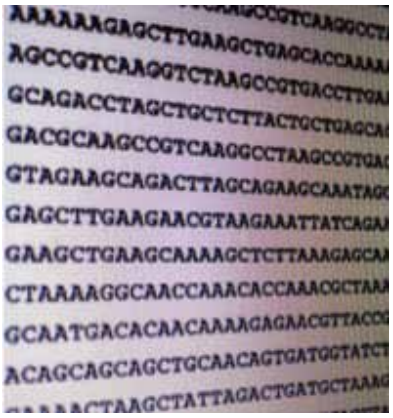
When Constructing a Phylogenetic Tree - you can use both:

MORPHOLOGY



"Anatomy and physiology of animals Various vertebrate limbs:"
Licensed under CC BY 3.0 via Wikimedia Commons

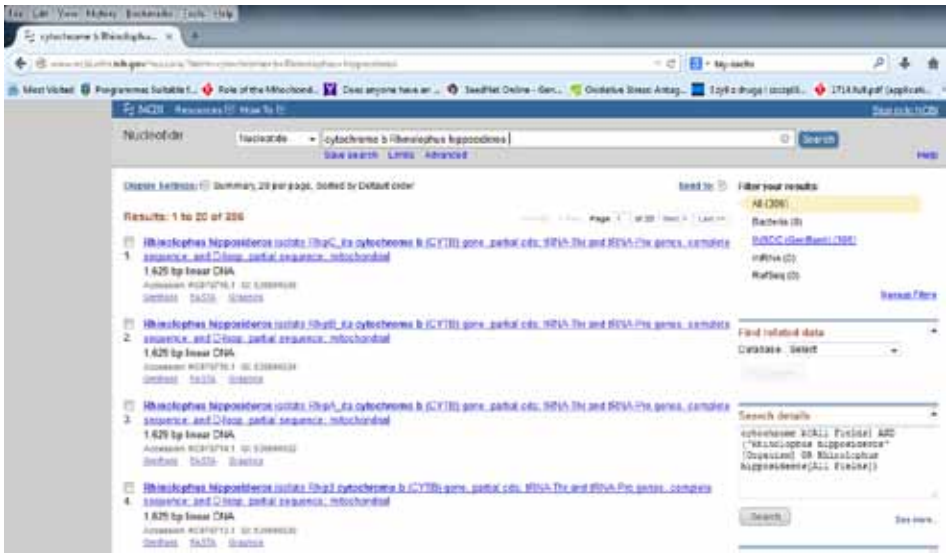
GENES



BAT CASE STUDY:
Let's take a look at a gene called cytochrome b, which is found in the mitochondria. We are going to use this gene to construct a phylogenetic tree to study the evolutionary history between several different species of bat (Lesser Horse-shoe Bat, Black Flying Fox, Bumblebee Bat, Little Brown Bat, Flying Fox, Common Pipistrelle, Brown Long-Eared Bat and Leisler's Bat).

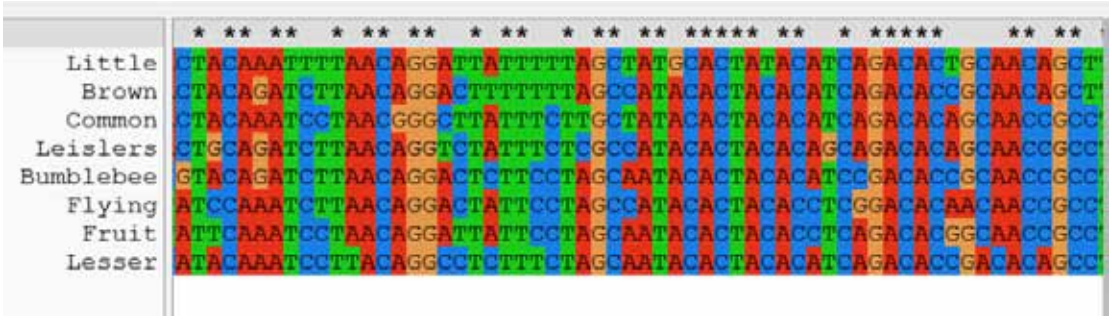
1. First we need to find the cytochrome b gene sequences for all these bat species.
The gene sequences can be obtained using NCBI database. NCBI is a great source of biological information. You can search the available nucleotide sequences at:
http://www.ncbi.nlm.nih.gov/nuccore

Note that usually the scientific names of species are used in such databases – always good to know both common and scientific name of the species you want to study! For example, the Latin name of Lesser Horseshoe Bat is *Rhinolophus hipposideros*. That's how I found the cytochrome b gene sequence of the Lesser Horseshoe Bat. The search query I used was 'cytochrome b *Rhinolophus hipposideros*'. ***Why don't you try to find seqeunces from the other species mentioned above!*** **Remember you should find out what the latin scientfic name is first before searching for the cytochrome b sequence.**
The correct cytochrome b seqeunces will be provided by your teacher (they are in the teacher's pack).



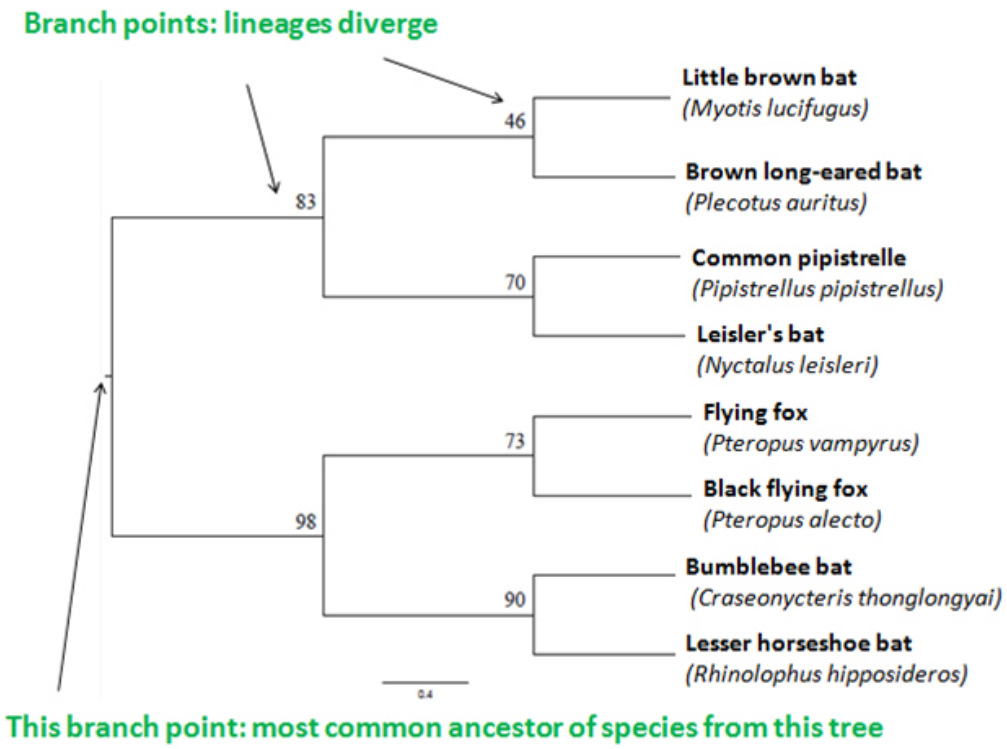
2. Now let's compare these sequences

We can use an online software (such as Clustal: http://www.ebi.ac.uk/Tools/msa/clustalo/) to carry out a process known as alignment. Cut and paste your seqeunces into Clustal, then hit submit (see below for more detail). The cytochrome b genes from the 8 bat species will be arranged in a way that allows for similar regions to be identified. Similarity between the cytochrome b regions may be due to evolutionary relatedness. The more similar the sequences are – probably the more closely related are two species.



3. Construct a phylogenetic tree based on the cyt b sequences

From the above data it is possible to create a phylogenetic tree using a programme called MEGA 6 (http://www.megasoftware.net/). MEGA offers many different methods for construction of a phylogenetic tree from the aligned sequences. See an example tree overleaf.



How can we read this tree?

At the right hand side, you can see all the names of the species that have been analysed. These are the 'leaves' of the tree. Each leaf has its own 'branch'. Branch points (tree nodes) tell us where two lineages diverged, explaining the evolutionary history of species presented on this tree. From this tree, you can for example, read that the Bumblebee bat and Lesser Horseshoe bat shared an immediate common ancestor. We don't know who this ancestor was, but at some time in the past it branched off into two separate evolutionary paths resulting in the Bumblebee bat and the Lesser Horseshoe bat.

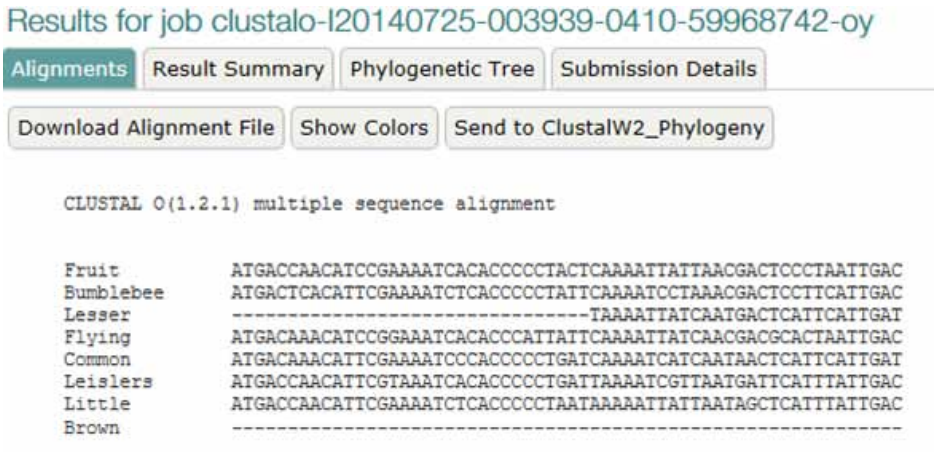
Do I trust this tree? Let's BOOTSTRAP!

A phylogenetic tree is a model – it presents the relatedness of species based on available data. Different trees can be prepared for the same group of species. There are different statistical methods to assess how good our tree is. Did you notice the numbers displayed at each branch point of this tree? They are the results of a test called Bootstrap. These values tell us how certain we are of each connection, the higher number - the better. For example, there is a number '90' displayed at the branch point at which Bumblebee Bat and Lesser Horseshoe bat diverged. It means that 90 out of 100 times we prepare this tree this node will be the same. Take a look at the tree yourself and use the Bootstrap values to assess different nodes of this tree. Which one you would trust the most? Which one is the least reliable?

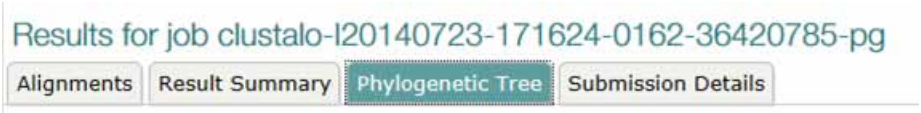
STUDENT TASK – CREATE YOUR OWN PHYLOGENETIC TREE

1. Log on to: http://www.ebi.ac.uk/Tools/msa/clustalo/
2. Copy and paste all genes from the 8 species of bat into the space provided.

3. Scroll down the page and click submit. Our results should look like this:



4. Now click on Phylogenetic Tree



5. Scroll down the page to see your tree that you have created. Does it look the same as the tree created above? Why do you think that there might be differences?

You have probably noticed that the phylogenetic tree produced by Clustal online tool is quite different than the one we generated using programme called MEGA. The phylogenetic tree generator tool offered by Clustal will never produce an accurate phylogenetic tree. The major function of Clustal is to align the sequences and the tree is used to guide this alignment. More specialized programmes, like MEGA, should be used to build (and evaluate) more accurate phylogenetic trees.

3.3. Comparitive Biology and Its Uses

Due to all the advancements in modern molecular technologies and the software available, it is now possible for us to sequence large DNA fragments or even your whole genome (all of your DNA in your cell). The process is getting faster and cheaper, which means we can build a huge databases containing DNA sequences from different species.

PGenome 10k Project – Unveiling animal diversity

A project aimed at sequencing 10 000 vertebrate genomes to understand how the complex animal life evolved through changes in DNA. Watch a video presenting this genetic Noah's Ark:

https://genome10k.soe.ucsc.edu/

Mind that DNA sequences alone are only beginning of the story – the big challenge is to figure out how genomes work. Comparative genomics is a field of biological research in which the genomic features of different organisms are compared. Comparative genomics allows the construction of the evolutionary tree of all living organisms (Tree of Life) – with more detail and accuracy than ever before!

By making comparisons between similar DNA sequences from human and from different species, we can also learn a lot about how our own genome works.

Comparative genomics and medicine

Comparative genomics is an amazing tool to predict if a single nucleotide variation, SNV (a change in a single nucleotide), in certain site in the genome is harmful, not-harmful or perhaps beneficial. Each of us have thousands of SNVs in our genomes, could this make us different from each other?.

Think about your colleagues from school. What if I tell you that at certain site in the genome all of your school friends have a nucleotide Guanine (G), and you are the only person with a Thymine (T) instead? Is it good or is it bad? It's hard to say if we don't know what this region in the genome does!

T Instead of G: Good or Bad?

How would you feel if I tell you that this site lies within the gene needed for vision? Let's sequence this gene in other mammals and investigate if they have a G or a T at this site? What can that tell us?.

SNP in vision related region

Humans: G
Giraffes: G
Gorillas G
Elephants: G
Dogs: G

Bats: T

Bats can do plenty of amazing things – but some don't see very well. Knowing that of all mammals, the ones having T in this particular site do not have the greatest vision may suggest that that this change in humans (from G – like in most of human population to T – like bat) can indicate increased risk of a vision related disease. We can make predictions like that thanks to the power of comparative genomics!

Perhaps in the future, comparative genomics will be used on regular basis in medicine.

Section 4. Ecology and Ecosystems

FUN FACT: The scientific name for bats is Chiroptera. What does this mean?

In this section you will learn:

- What the typical year for an Irish Bat is like.
- How to identify different bat species through morphology.
- Methods used to record and capture bats
- The implications of green energies for bats and their environment.

4.1 All About Bats

Bats are mammals. They are warm blooded and covered in fur and give birth to one pup a year. There are more than 1,200 species of bat worldwide. They can vary greatly in size from the tiny Bumblebee Bat (Kitti's hog-nosed bat) found in Thailand and Burma to the larger fruit bats found in the Tropics. Nine species of bat are resident in Ireland.

FUN FACT: The Royal Botanical gardens in Sydney Australia is home to huge number of Flying Foxes

Bats are amazingly important to our Ecosystems. There are a vast number of plants that depend on bats for pollination and seed dispersal. All of the Irish bats are insectivores i.e. their diet consists only of insects. Amazingly, one tiny bat can eat over 1000 insects per night making them very important for pest control.

A group of bats is called a colony and they live in roosts. When choosing a roost, they will want to be comfortable, safe, and warm and near a food source. Although many bats in Ireland will live near a river/canal lined with deciduous trees, a good number will also live in man-made structures such as buildings and bridges. It is thought that they hibernate through the colder winter months so they seek hibernation roots where they will not be disturbed.

A Year in the Life of an Irish Bat	
January/February/March	Bats are in hibernation. Their metabolism slows down to conserve energy. They are living off fat reserves.
April/May/June	As the weather gets warmer the bats start to emerge from their hibernation roots at night to forage for food. Female bats will begin their search for a suitable nursery roost in which they will give birth. Males roots either in small groups or on their own.
Late June/July	Females give birth to one pup which they suckle. They can recognize their pup by smell and sound.
August	The pups are now 6-7 weeks old and can echolocate and feed independently. Females leave the maternity roosts . Bats will move to mating roots.
September	The start of mating season. The bats are also consuming a large amount of insects to build up fat stores for winter
October	Bats are seeking suitable hibernation roosts
November/December	Bats are in hibernation.

FUN FACT: The French for bat is chauve-souris – bald mouse!.

9 species of bat resident in Ireland

Common Pipistrelle

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Soprano Pipistrelle

Photo by Evgeniy Yakhontov - Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons.

Brown Long Eared

Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons

Daubenton's

"Myotis daubentoni01" by Gilles San Martin from Namur, Belgium - Myotis daubentoni. Licensed under Creative Commons Attribution-Share Alike 2.0 via Wikimedia Commons

Natterer's

Photo by Armin Kübelbeck. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons.

Lesser Horseshoe

Photo by Lylambda. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons

Whiskered

icensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons

Leisler's

Photo by Manuel Werner. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons

Nathusius's Pipistrelle

Photo by Mnolf. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons.

4.2 Identification Of Bat Species Through Morphology

Scientists will use keys to identify species but they can actually gather a lot of information from an initial visual inspection. For example;

- Horseshoe Bats have a very distinctive horseshoe shaped nose so this helps to rule out other species.
- The Brown Long Eared Bat is the only Irish bat to have ears joined over the head.
- The tragus shape is particularly useful for identifying Myotis species.

It is also very helpful to determine how big the bat is by using callipers to measure the head and body in mm and also to measure the forearm (elbow to wrist) in mm. Using keys and combining the morphology of bats with their echolocation calls help to identify unknown species by a process of elimination .

Callipers for taking measurements

There are some excellent keys available of the following websites which give a fantastic amount of information about bat identification:

http://www.batconservationireland.org/pubs/reports/Leaflet_1_BatsinIreland.pdf

http://www.batconservationireland.org/ Irish bats in Flight

http://www.bats.org.uk Identification of British bats – Michael Walker, South Nottinghamshire Bat Group.

STUDENT TASK

Below are some pictures of Bats. You would use this simple key provided, to identify who is who ?

1	Are my ears joined over my head?	Yes No	Brown Long Eared Bat Go to question 2
2	Does my wingspan range start at 190mm?	Yes No	Soprano Pipistrelle Go to question 3
3	Do I have a distinctive horseshoe shaped nose?	Yes	Lesser Horseshoe

Here are some measurements we have already made from collected bat specimens. Use the key above to work out who is who!

	Ears joined over head Head and body length 37-48mm Wingspan 230-285 Answer _____

STUDENT TASK (cont...)

	Ears not joined over head Head and body length 35 -45mm Wingspan 200-250 Answer _____
	Ears not joined over head Head and body length 35-45mm Wingspan 190-250 Answer _____

4.3 Methods Used To Record Bats.

Bats have a very unique ability. They can echolocate. They build up an image of their surroundings using high frequency calls. Echolocation is individual to each species and although some of the social calls that bats make can be picked up by the human ear, their echolocation calls are completely inaudible to human hearing. Therefore, it is necessary to use bat detectors to pick up their calls. There are several types of detector available, ranging from basic and inexpensive through highly technical and very expensive. An amateur bat enthusiast is likely to use a Heterodyne Tuneable Detector. The detectors have an ultrasonic microscope and by adjusting the tuning frequency, different calls become audible. Bats will sweep through frequencies so if the detector is set at 45 kHz, it will pick up the calls from many bat species.

Many scientists are trying to understand how bats use sound to 'see' their enivornment. Below is a link to a research labortory at the university of Bristol. Here you can find what a bat sounds like through a bat detector. Echolocation calls are mostly used by bats to find food and to find a roost. It is possible to tell most different species of bat apart by listening to and analysing their echolocation calls? Can you find and listen to all of our Irish bat species? Can you hear the difference?

University of Bristol: Echolocation calls of British bats
http://www.bristol.ac.uk/biology/research/behaviour/batlab/downloads/echolocation/

Other detectors that are used are the Frequency Division Detector, Time Expansion detectors and the Full Spectrum Real Time Sampling Detectors. The calls can be recorded and are analysed by software to help identify species. Check out the following website for more information:

Bat Conservation Trust: Time expansion detectors
http://bats.org.uk/pages/bat_detectors.html#TE

4.4 What To Expect From A Bat Walk

Safety First! Bats are nocturnal. This means that a 'bat walk' will take place at night. A responsible adult should be present at all times. There are often guided bat walks that take place so check out the local information in your area. Bats like to forage near slow running water where there will be lots of insects. A canal or a slow running river lined with deciduous trees is the perfect spot for bats at dinner time.

FUN FACT: The Daubenton's Bat is also called the Water Bat. It skims the water in search of prey which it catches with its large feet.

Although it will be possible to see the bats without equipment, in order to hear them you will need a detector (the basic models retail at around €50). A torch is also a good asset.

By tuning the detector to different frequencies, it is possible to pick up different calls. At 25kHz, you can hear a Leisler's Bat. This is the biggest Irish Bat and is usually seen just after sunset. As the night progresses, adjusting the detector to a frequency of 45kHz will pick up the Common Pipistrelle at 55kHz, the Soprano Pipistrelle. These are Ireland's two smallest bats and have very fast and convoluted flight patterns. The Daubenton's Bat, also known as the Water Bat, can also be picked up at 45kHz but it will be spotted skimming the water looking for insects.

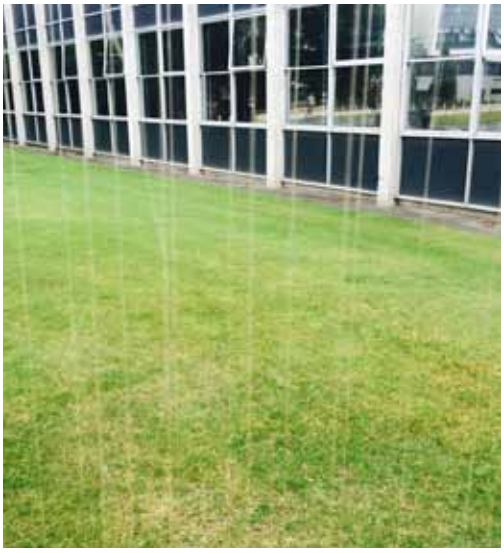
FUN FACT: Bats can navigate in complete darkness using Echolocation.

The following websites are packed full of information and it is also possible to hear recorded bat calls:

http://www.bats.org.uk
http://www.batconservationireland.org/

4.5 Methods For Capturing Bats

Sometimes, scientists have to capture bats. It is important that this is done with minimal distress for the bats. These tiny creatures are a protected species in Ireland so a special licence is required to catch and handle them.

	
MIST NET: This is a mesh of nylon that is suspended between two poles. The net is very delicate and care must be taken when unfolding and folding the net to avoid a mass of tangles occurring.	HARP TRAP: This consists of layers of 'harp strings' suspended from poles

4.6 The Implications Of Green Energies For Bats And Their Environment.

In Ireland, bats mainly live in man-made structures and deciduous woodlands. Loss of habitat is a huge and ongoing issue and loss of roosts may be as a result of timber treatment, tree felling, disturbances by other animals (such as cats) and water pollution, to name but a few.

FUN FACT: Bats can fly. While other mammals glide, bats are capable of true and continued flight.

In more recent times, the use of green energy, most notably wind-farms, has had an impact on bats. Below is a website that describes some of the problems that bats face in relation to wind-farms. This is a start for you to explore this idea more.

USGS: Bat Fatalities at Wind Turbines
https://www.fort.usgs.gov/science-feature/96

STUDENT TASK

In pairs/groups, discuss how wind-farms could affect bats and write your ideas in the box below:

With regards to the turbines, it is thought that bats may die or become injured in 2 ways. Firstly, by direct collision of the bat with the blades of the turbine. Secondly, there is a pocket of low pressure just behind the blade. If the bat gets caught in this pocket, it is thought that the change in pressure will result in haemorrhaging (bleeding heavily) of the lungs, similar to what divers may experience with decompression sickness (the bends).

Currently in Ireland, scientists are studying bats to find out where and when they roost. The data will be used to identify areas of the country that bats do not frequent making them suitable for windfarms.

Student Task Suggestions

- Create a poster based on some aspect of this chapter and present your poster to the class.
- Create a presentation or on the affects of windfarms on bats in Ireland to present to your class.

Conclusions

To truly understand how the world works it is important to study our world using all of the scientific tools available. By completing this transition year syllabus you have learnt, cell biology, genetics, field biology, ecology, bioinformatics and phylogenetics. You have also learnt about the diversity, uniqueness and wonder of the most fascinating mammals the bats. By integrating all of these different scientific approaches you are now in a position to delve deeper into the mysteries of life on this planet. We hope to see you all in UCD first year where you will learn more skills to explore these mysterys further. Well done!

Olivia Derwin, Clare Lamont, Joanna Kacprzyk, Emma Teeling and all members of BatLab.

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Oliva Derwin and Clare Lamont.

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"The status of the cryptic bat species Myotis Mystacinus and Myotis Brandii in Ireland", E.Boson *et al* "Genome analysis reveals insights into the Brandt's Bat Myotis Brandii" (Nature Communications) "Bat Conservation Ireland Leaflet No. 1"- Bats in Ireland

"Irish Bats in Flight"-Bat Conservation Ireland

"Identification of British Bats" - Michael Walker, South Nottinghamshire Bat Group "Phylogenetic Trees Made Easy" Barry G. Hall

"Campbell Biology" Pearson.

"Phylogeny for the faint heart: A Tutorial"-Sandra L Baidauf.

"DNA for Real: Learning about PCR in science workshops" Anna Joliffe.

"Phenol-Chloroform Extraction" P. Zumbo, Weill Cornell Medical College, Laboratory of Christopher E. Mason PH.D

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Useful weblinks

Genetics and Evolution.

http://www.yourgenome.org
http://ed.ted.com/lessons/how-to-sequence-the-human-genome-mark-j-kiel
http://learn.genetics.utah.edu
http://ed.ted.com/lessons/the-twisting-tale-of-dna-judith-hauck
http://unlockinglifescode.org
http://www.youtube.com/watch?v=QY9LZ5tt-QE&sns=em
http://www.statedclearly.com
http://evolution.berkeley.edu
http://www.bozemanscience.com
http://m.youtube.com/watch?v=H6IrUUDboZo
http://www.bbc.co.uk/darwi
http://www.bbc.co.uk/science/0/22941835

Bat Material

http://youtu.be/3BtbS9JC8x8 (great irish bat clip)
http://www.eurobats.org
http://www.csiro.au/Outcomes/Environment/Biodiversity/Spectacled-Flying-Fox/Bat-facts.aspx
http://news.nationalgeographic.com/news/2005/01/0127_050127_bats_2.html
http://www.csiro.au/Portals/Media/2011/Bat-immunity-key-to-controlling-deadly-viruses.aspx
http://www.batconservationireland.org/
http://www.thewildclassroom.com/bats/videos.html
http://www.arkive.org/daubentons-bat/myotis-daubentonii/video-00.html
http://www.nhm.ac.uk/nature-online/life/mammals/bats/session2/index.html
http://www.rte.ie/radio/mooneygoeswild/factsheets/bat/
http://www.bats.org.uk

PCR

http://m.youtube.com/watch?v=NYIT3f-MZ5o&sns=em
http://m.youtube.com/watch?sns=em&v=2KoLnIwoZKU
http://www.ncbi.nlm.nih.gov/genome/probe/doc/TechPCR.shtml
http://www.abpischools.org.uk/page/modules/pcr/index.cfm (PCR for Schools)

Sequencing

http://blast.ncbi.nlm.nih.gov/Blast.cgi
http://www.ebi.ac.uk/Tools/msa/clustalo/