Section 2. UCD Bat-Lab

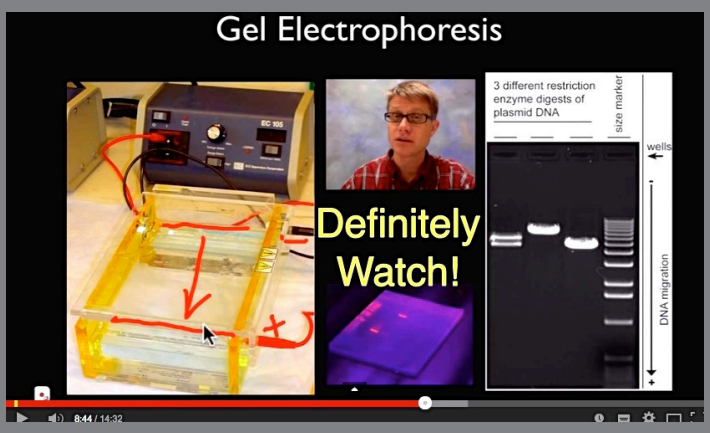
2.1 Introduction to Bat Lab.

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'

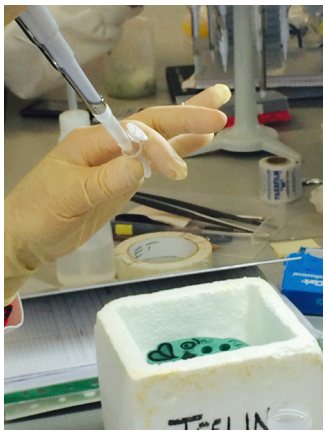
http://youtu.be/yYIZgS-L5Sc

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

2.2.1 Extraction and Purification of DNA.

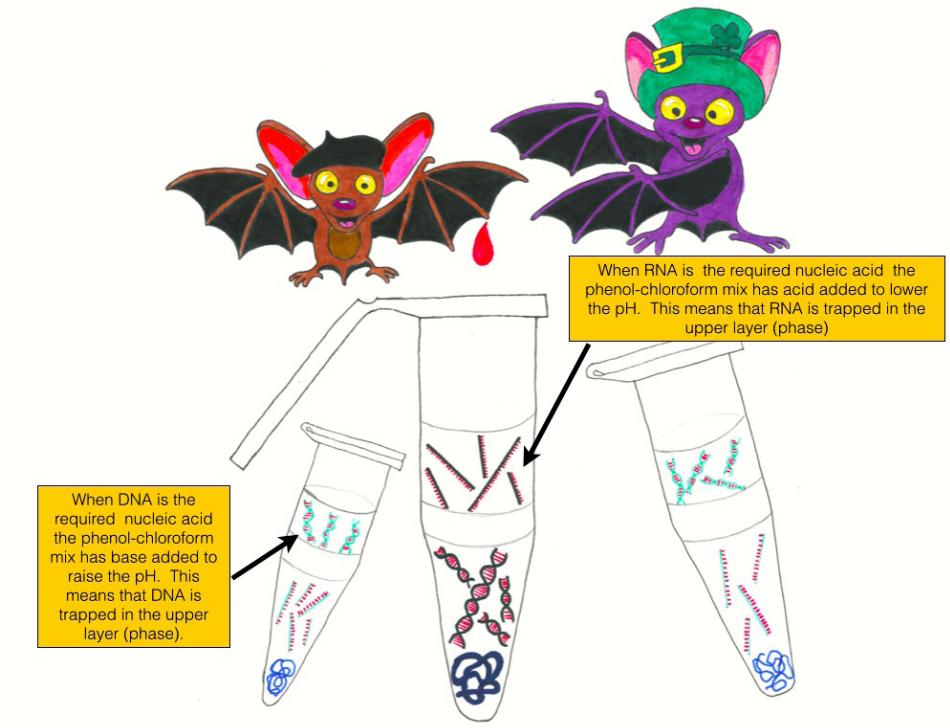
Extract the DNA-release it from the cells.
Lysis buffer is added to the tissue samples (e.g. wing clippings) and homogenised or mixed. The buffer contains a detergent which disrupts the membranes and also salt which causes the DNA to clump to together. (Lysis means to split). Remember your DNA extraction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

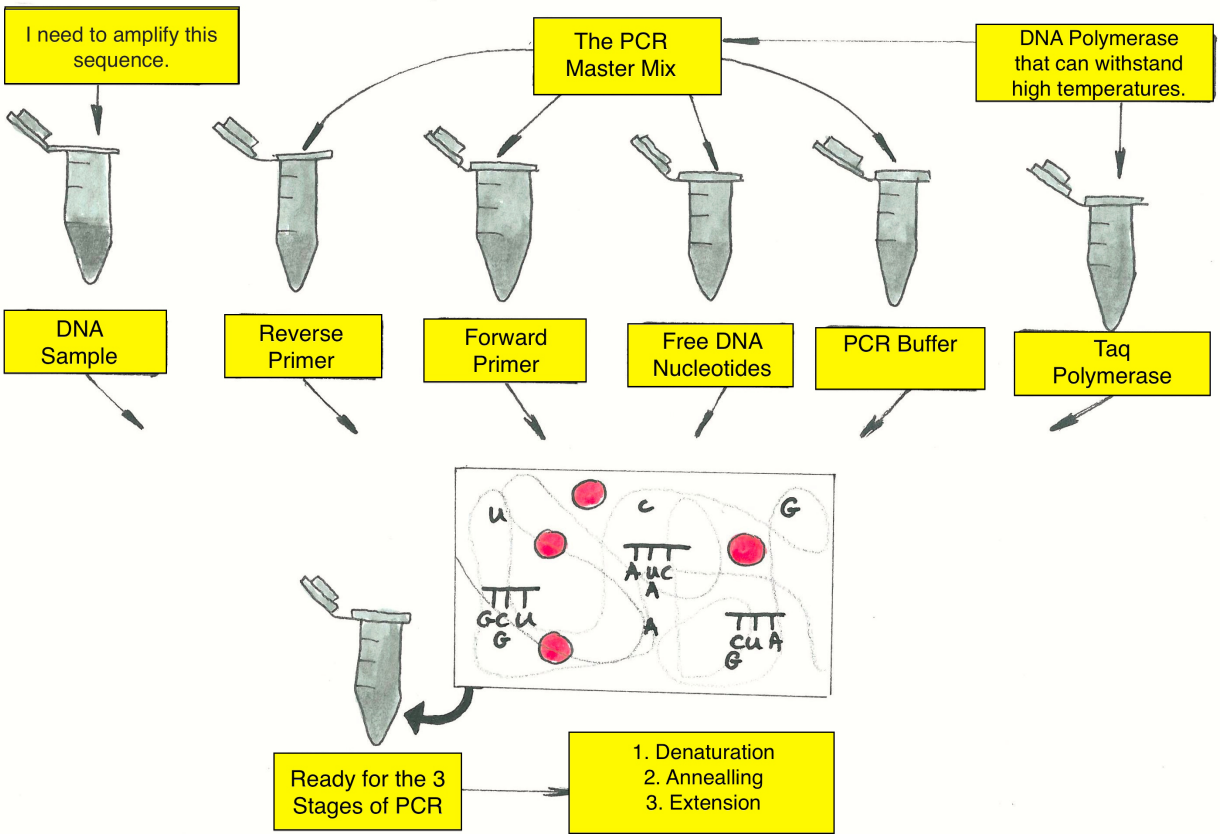
Add your DNA samples to the Master Mix - load into the PCR machine. The PCR machine will carry out the following steps:

- **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



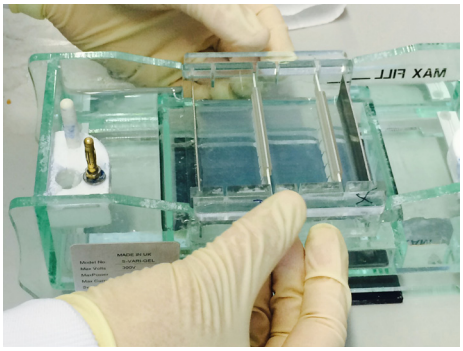
PCR

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.

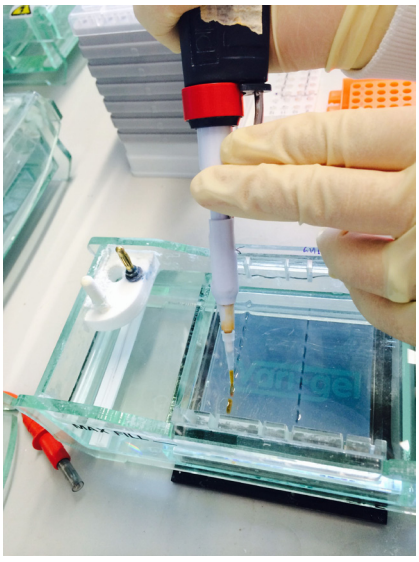
1. Make the gel-this is made with an agarose powder (extracted from seaweed), to this you add a special DNA detecting dye-this enables you to see your DNA under uv light. The gel is made so that there are a number of spaces (wells) where your DNA can be loaded. Leave to set and then place in the electrophoresis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



Preparing the gel

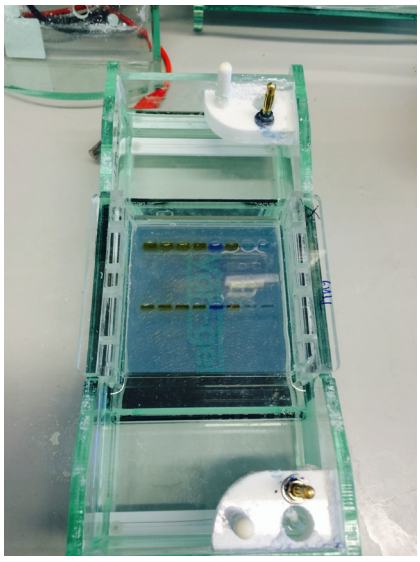
2. The PCR samples are then prepared, you use only a small amount of your PCR samples and save the rest for sequencing later.

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.

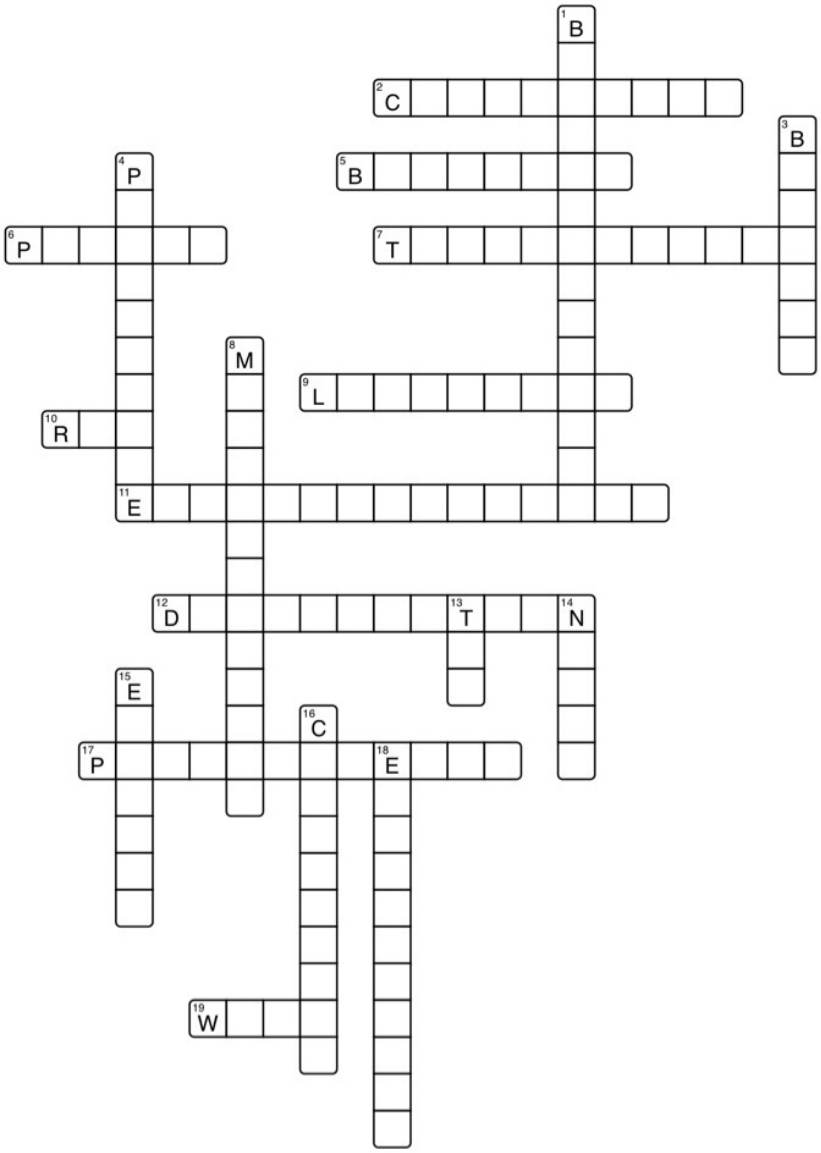
Note: Gel Electrophoresis is used in Bat Lab to check that the PCR worked-if you see bands in the correct positions and the band size is correct, then you know that it has worked. It is also used to identify which species of bat you have caught by sequencing the PCR band. In species identification you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