Explaining Cards
For Science Uncovered
Mechanical Grinding Specimens

For specimens composed of tough material such as fibrous plants or insects with a hard carapace the first step in obtaining DNA is to grind the specimen into smaller pieces, releasing the soft cellular components.

Sand is added to increase the abrasiveness of the process. The sand is sterilised so that it does not contaminate the samples.
Micro-Pestle and Tube

For small samples like mosquitoes or when only a leg from a larger insect is being used, a micro-pestle which fits tightly into a tube keeps the working volume small so the specimen isn’t diluted.

A small amount of lysis buffer is added and the micro-pestle is twisted to macerate the specimen.
Manually grinding is time consuming so if there are a lot of samples to process we use a machine that agitates ball-bearings with the sample in a tube. The machine vibrates at 6000 time a minute and can process lots of samples at once.
Chemical Digestion (I)

Soft specimens can be digested whole – we routinely digest beetles overnight at 56°C in a special chemical solution and replace the carapace in the collection for morphological study.

Samples which have been mechanically broken down can then be chemically digested to break open the cells to release the DNA [Lysis] – we pulverised plant material for this purpose.
Chemical Digestion (II)

Resuspended

Detergent and alkali are added to help break cells – sample is frothy.
Neutralisation buffer added to precipitate proteins and fats from cells; DNA stays in solution.

Using a high speed centrifuge
Cell debris is pelleted.
Centrifugation

Tubes are placed in a centrifuge and the rotor spins at up to 12,000 rpm.

This ensures the contents of the tube are forced to the base with the heaviest material tightly packed in the bottom.
Column Isolation and Purification of DNA

This is an alternative to manual DNA extraction.

Columns are a special type of filter which enables us to extract clean DNA quickly, but is more expensive than manually processing the sample.

DNA will bind to the filter column in high salt buffer, the impurities can then be washed off and the DNA finally eluted in water.
PCR to identify our specimens.

After we have extracted the DNA we analyse it with PCR.

The Polymerase Chain Reaction, or PCR, is an enzymatic reaction that makes lots of copies of a small part of the whole DNA chain.

This is important because the small DNA region of interest often exists in quantities too small to detect. For example, some of our specimens are very small (0.3mm) or we may only be able to use a leg from a mosquito.

PCR targets a small region of DNA from the entire genome that is specific to identifying the organism.

This amplified region is further analysed to determine its exact DNA sequence. The sequence is compared to existing data so that an identification can be made.
The PCR Reaction

1. **Denaturation**: Heat briefly to separate DNA strands
2. **Annealing**: Cool to allow primers to form hydrogen bond with ends of target sequence
3. **Extension**: DNA polymerase adds nucleotides to the 3’ end of each primer

**Cycle 1** yields 2 molecules

**Cycle 2** yields 4 molecules

**Cycle 3** yields 8 molecules; 2 molecules (in white boxes) match target sequence
PCR tubes.

Special tubes are used for the PCR reactions. These have very thin plastic walls to allow rapid and uniform heat transfer.

This is important to ensure an efficient reaction occurs to give us the best results possible.
Our laboratories have over 50 PCR machines.

Tubes or plates are inserted into a heating block and the machine rapidly runs repeated cycles of predetermined temperatures in a programme which is tailored to each specific experiment.

Typically 30 cycles of 3 temperatures in an experiment takes approximately 2 hours.
DNA analysis

After the PCR we need to visually check the reaction has been successful. To do this we look for an amplification product using a technique called gel electrophoresis.

Casting an Agarose Gel

A gel is made by pouring a molten agarose gel solution into a mould.

A comb forms wells in the gel when it is removed after the agarose has set.
Loading Samples onto an Agarose Gel

A small amount of the PCR product is loaded into a well, dye is mixed with the PCR product so we can see where the sample is.

A heavy compound such as sucrose is also added to the PCR product to ensure the sample sinks into the well within the gel.
Running an Agarose Gel

DNA molecules have a small negative charge so within an electric field they migrate through the gel towards the positive electrode.

The dye is only to see how far the sample has gone – it is NOT the DNA.
Visualising DNA

A Fluorescent Dye is added to the sample before loading which binds to DNA.

Using UV light the fluorescence can be seen and illuminates the DNA. A standard marker of known DNA sizes is also loaded on the gel. Our DNA is compared to this so we can confirm that our PCR product is correct.

Sometimes the PCR products are the wrong size and we need to optimise the experiment. Larger molecules will move slower than smaller ones and migrates less far.
SEQUENCING

Once we are satisfied that the PCR product is correct the final steps of analysis are performed. The actual DNA sequence is determined using a technique similar to PCR but using different colours of fluorescent dyes for each base (letter) of the DNA.

Computer software analyses the colours and predicts the sequence

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T     C     C     A     A     G     A     T     A     C     A
20  30
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By comparing the sequences from lots of specimens we can create a database of unique sequences to help us identify the specimens.

Alternatively we can also search a global database to compare our sequences against those produced by other laboratories around the world so that we can share the data and help each other identify our organisms.