Module: Genetics – Background, Case Study & Technology Highlights

- **Curriculum:** Science
- **Year:** 10*
- **Curriculum streams:**

  Science Understanding

  - **Year 10: Science Understanding: Biological Sciences**
    - **Biological Sciences:** *explain the role of meiosis and mitosis and the function of chromosomes, DNA and genes in heredity and predict patterns of Mendelian inheritance.*
    - **CD Code & Link** AC9S10U01

  Science as a Human Endeavour (Year 10)

  - **Year 10: Science as a Human Endeavour: Nature & development of science**
    - **Nature & development of science:** *investigate how advances in technologies enable advances in science, and how science has contributed to developments in technologies and engineering.*
    - CD Code & Link AC9S10H02

* Opportunity for content to be utilised across **years 9 & 10** ***Science as a Human Endeavour: Nature & development of science*** **streams**. These case studies showcase advancements in technologies & their research applications that lead to developments in various science fields.

  - **Science as a Human Endeavour: Nature & development of science**
    - **Year 9*: Nature & development of science:** *investigate how advances in technologies enable advances in science, and how science has contributed to developments in technologies and engineering.*
    - CD Code & Link AC9S9H02

Overview of Module & Case Study Examples

This year 10* genetics module & case study highlights aspects of the science of contemporary technologies, drawing upon examples from biology, genetics, agricultural science and technology.

This showcases direct applications of genetic research for the development of crops for feeding growing populations in changing climates, as well as broader applications of the technology for society.

Senior secondary: Learning concepts can also be applied within senior secondary subjects, including Biology.

Compiled for TIA's Professional Learning Workshops *Engaging Students in STEM using Agriculture (2023).*
Contact bianca.deans@utas.edu.au for further information.

Below is an **overview of the genetics module**:

Genetics Module: Background, Case Study & Technology Highlights

Part 1: Background

- **Mitosis**
  - Key principles
  - Example activities for engagement & inquiry
- **Meiosis**
  - Key principles
  - Examples & impacts
- **Genetics to physical traits**
  - Genotype & phenotype
  - Examples: inheritance

Part 2: Technologies that enable genetics research

- **Crop Research –** spotlight & aims
  - Background
  - Adaptive strategies & influencing factors
  - Traits
- **Technology highlights:**
  - DNA extraction
  - Polymerase Chain Reaction (PCR)
  - Gel electrophoresis
  - HRM (high-resolution melt)
    - Genotype analysis
    - How it works – includes chemistry & biology principles
    - What is the wild type?
- **Opportunities for engagement**

Part 1: Background (cell division, genotypes, phenotypes, inheritance)

Part 2: Technologies & processes that enable crop genetics research

Purpose & aims: From plant crop research examples, this section highlights the sciences & technologies behind the genetics research. Sciences of key steps within this genetics research are outlined, with room for extension and adaptation.

Overall, this module unpacks concepts of plant genetics & physical traits, and how understanding these can inform the development of new crop varieties, including via selective breeding. Ultimately, this can assist food production in different environmental conditions, and improve food security.

This work includes examples as a case study of current genetics research relating to crop development, including research focussed on specific legume crops (belonging to the pea family, Fabaceae).

Part 1: Background

- This section includes examples for key background content for a lesson sequence for mitosis & meiosis. Content and sequence can be adapted at the educator's discretion for class size, student understanding and pace of progression through content.
- **Suggested resources** to supplement educator/ student learning:
  - YourGenome – meiosis & mitosis, genetics and genomics information resources.
- **Review process as class:**
  - **Example:** overall class discussion, small group work, definitions check, quiz or bingo
  - Include review of definitions & understanding of:
    - a cell & its nucleus (membrane-enclosed organelle that contains the cell's genetic information);
    - chromosome;
    - genetic material (types: DNA and RNA) – their similarities & differences.
    - Mutation (definition, examples)
    - "Ploidy"- Diploid (2N) and haploid (N)- the number of sets of chromosomes.
- **Suggested sequence:** Cell division lesson/s to introduce an initial focus upon mitosis, with a latter part/ or second lesson to be conducted on meiosis. **Example content flow:**
  - **Mitosis: Key principles of mitosis**
    - **Overall:** A parent cell duplicates itself to form two identical daughter cells.
    - **General process overview:** Outline the key steps of mitosis to class. Outcome: equal division of the cell's content into two daughter cells that have identical genomes (genetic blueprints).
    - **Daughter cells have the same total number of chromosomes as the parent cell.** Therefore this is also known as 'equational division'.
  - **Where does mitosis occur?** Discuss locations, example types of cells. Where does it not occur? Note that animal cells and plant cells have specific processes & components involved in mitosis that differ between them. General approach of concepts can be taken, or specifying these (at educator's discretion).
  - **Core purposes of mitosis:** Discuss the core reasons why organisms need mitosis.
    - The major purpose of mitosis is for growth and to replace old or dead cells. Mitosis is fundamental for growth of organisms.
  - **Mutations**: Discuss how these can occur in the DNA strands. (E.g., point mutations: swapping a nucleotide base pair for another base pair, resulting in another change in the sequence). This change in genetic sequence may result in a phenotype (physical trait/ attribute) change in the organism.
  - **Mutations:** Video/visual examples:
    - SciToons video (Brown University) of mutations (human example)
    - See short video & overview visual from the mutation section of the Genetic Science Learning Centre (US). Further down the webpage are summary visuals of other areas of genetics that may be useful revision.
  - **Main phases of mitosis** (educator outlines with class):
    - Outline main phases of mitosis; cover the key elements of each phase, connecting the name of the stage to what occurs at each phase.
    - Include: interphase, prophase, metaphase, anaphase, telophase & cytokinesis.

- Student activity example –Mitosis phases:
  - Outline & aim: Students to organise a logical sequence the steps within mitosis, firstly visually organising the phases together based on the description of phases provided earlier in summary. (e.g., use prepared cell cue cards as visual prompts).
  - Allow time for students to consider the options & correct logical sequence of steps and processes, discussing amongst small groups. Then bring together as a full class to share their sequence. Educator & students collaboratively organise the sequence, explaining the key processes.
  - Vocabulary suggestion: encourage vocabulary growth via connecting the names of the phases to the stages. For example, provide students with labels and define the prefixes for each stage. See if they can match the name of the stages. Connect the term of the phase to what is happening within that phase (key details). Examples:
    - Inter = "between"
    - Pro = "advance"/ "before"
    - Meta = "along with"
    - Ana = "up or apart"
    - Telo = "final or distant"

- Additional mitosis themed activity ideas:
  - Following background theory/ concepts and class discussions of the principles of mitosis, students can undertake a variety of activities:

  - Short small group activity (short activity, 10-15 min): Each group is provided with a stage of mitosis to summarise the key stages and present to the wider group. E.g., Written as dot points, drawing of the key parts of the division.

  - Alternative longer activity: small group activity where students summarise the key stages of mitosis (e.g., drawing, annotating key terms and noting the stages in their books). Write 1-2 sentences per stage for alongside the visual representation.

  - Development of material or card/paper plate diagrams of each phase, with students pairing the stages to the specific cell division images. Get creative & use a variety of materials (e.g., coloured card cut-outs, plasticine for 3D elements).

  - Option: Compare & contrast by outlining mitosis & meiosis steps *side-by-side*. It may be easier for students to consolidate and apply their learning by using mitosis as the foundation activity to demonstrate cell division, then follow this by unpacking meiosis (same lesson/ following lesson). Punnet square of genetics (centre) can be also used to illustrate genetic crossing. Examples of workings include:

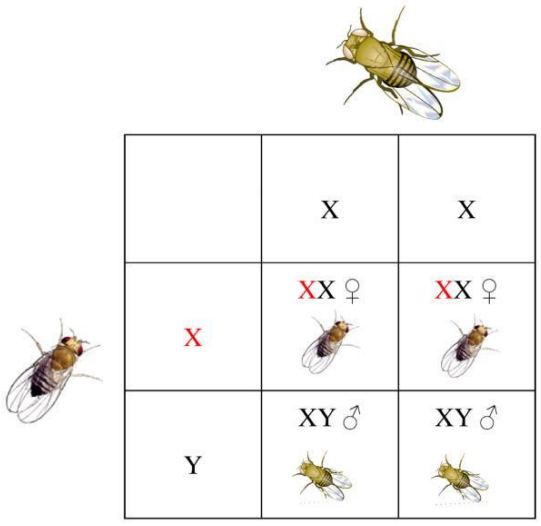
    - Make a poster or visual display that can be an attractive and engaging feature for the classroom/ learning areas.
    - Can also use this as for inspiring peer science communication within the school/ community – making this in a session (tactile, visual), or explaining this verbally (visual, verbal).

- **Building upon these core principles of mitosis, introduce students to meiosis.** Allows comparing & contrasting of the different cell divisions.
    - **Meiosis: Key principles of meiosis:**
        - Single parent cell (2n) divides twice to form 4 daughter cells. The daughter cells contain half the number of chromosomes as the parent cell (n = haploid).
        - Recap: Key phase names – apply this knowledge to cover the meiosis phases. (Two cycles means that each phase is labelled I or II).
    - Formation of a similar material/ card/ paper plate diagram as for mitosis may be developed for communication of key stages and create a class display.
    - **Example diagram** outlines the key phases and two cycles of meiosis (Source: YourGenome).

- **Meiosis – use & impacts**: Meiosis produces sex cells (gametes, N) which are required for sexual reproduction to occur. Gametes are haploid (N, one set of chromosomes). Meiosis occurs in reproductive tissues & organs.
        - Briefly discuss sexual reproduction (from gametes fusing in fertilisation), and its impacts on genetic diversity (increase genetic diversity vs. asexual reproduction/cloning) and survival in changing conditions.
        - Highlight examples of where gametes in sexual reproduction occur: plants, animals, fungi.

Genetics to physical traits: Genotype & phenotype

- **Genotype & phenotype:** define & provide examples of each, including:
    - Genotype (genetic sequence/ make-up)
    - Phenotype (physical traits) that the results of genetic instructions (genotype)
- **Genotype**: Introduce alleles; dominant & recessive traits; Punnett square crosses.
- **Selective breeding**: examples from Mendelian inheritance (classical pea experiments).
- **Introduce:** Sex chromosomes (i.e., XX = female; XY = male). Features (e.g., relative size).
- **Example of sex-cell linked traits**: fruit flies and their eye colours.
    - **The eye colour gene is located on the fruit fly's X chromosome** (one of the sex determining chromosomes of *Drosophila* fruit flies*)*. White eye colour is a recessive trait.
    - **Sex cell genetics (genotype) influence upon the phenotype (physical trait):** when a red eyed male mates with white eyed females, their daughters will have red eyes, but their sons will have white eyes.
    - **See below diagram to show the genetic cross** (sex chromosomes X and Y shown in their respective colours to illustrate the linked traits).
    - **Outcome:** There can be no red-eyed males in the 'first generation' (Punnett square outcome shown), but they will appear in the second and later generations.
    - **Further generations:** Explore further generation outcomes with students completing a new Punnett square using the offspring from the first generation (below inner Punnett square).



Part 2: Technologies that enable genetics research

Case study: Science behind crop research for food security

- **Australian Curriculum 9.0 Alignment:** Science, Years 9-10
- **Science as a Human Endeavour:**
  - **Nature & development of science:** *investigate how advances in technologies enable advances in science, and how science has contributed to developments in technologies and engineering.*

Module flow & content note: This case study part of the module can be undertaken as the full module, or in parts. Extension of depth of this content and associated activities can be adapted to apply to senior secondary/ college units.

Aim: Highlight the sciences and technologies behind plant genetics and agricultural research, plus illustrate the importance of this science for food security.

This includes by:

- Drawing upon technology examples from a case study of current genetics research where towards the development of legume crop varieties.
- Learning about how understanding the connection between genetics and a plant's physical traits can lead to the development of new plant varieties by selective breeding processes.
- Highlighting that this research can assist the growing of food crops in changing environmental conditions, plus enhance food security and economic stability.

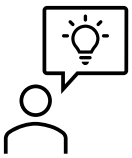
Background: Genetics Research for Improved Crops

The following background & case study leads students through research being conducted in Tasmania to tackle key issues with feeding a growing global population amidst a world that us impacted by the challenges of climate change.

This section of the module provides students with context to connect to research into real-world issues, plus insights of the technologies that enable research and progress.

- The **University of Tasmania** is part of the Australian Research Council's Centre of Excellence for Plant Success in Nature and Agriculture in a collaborative network of research institutions across Australia. This brings together science from many disciplines to better understand, predict and improve plant crops in diverse environments.
- The **Centre for Plant Success** are developing innovative tools and strategies to:
  - improve the productivity of plants (e.g., yields, growth) and plant resilience to environmental conditions (e.g., heat and drought).
- **Resource: Introductory Video (3 min) - Centre of Excellence for Plant Success in Nature & Agriculture**: https://youtu.be/rlMpaZURt1Q. This short video highlights the interdisciplinary nature of science, including the combined expertise to help solve world food security issues. Agricultural crop development is a major focus of this work. This includes:

- By looking to nature, scientists are discovering the diverse adaptive strategies that plants use to cope with heat and drought.
- Adaptive strategies: Because plants are not mobile in the way that animals are (they simply cannot relocate themselves at a whim in changing conditions), plants must make "trade-offs" between where and how to allocate resources. (E.g., Resources: water, sunlight, energy, nutrients).
  - Adaptive strategies- example: Allocation of energy and nutrients to growth, reproduction, or maintenance of the plant, they respond to their environment by adopting strategies that allow them to persist, survive, and reproduce.
  - Plants may have multiple strategies to survive at different life-stages (e.g., juvenile vs. mature adult form) and therefore be subject to multiple "trade-offs" throughout their life cycle.



Activity suggestion: Adaptive Strategies & Questions

- Format:
  The activity & its discussions could be undertaken in several ways, including:
  - Whole class/ small group format
  - Individually- worksheets provided for individuals to complete, concurrently as the class discussion is active.

- Resources: Student can use the physical print out, create their own diagrams on large paper/in their book, or in their devices in a digital template. Resources that could be used include:
  - Graphical organisers:
    - Tabulated worksheet (key theme = adaptive strategies, with place for a definition & key terms/related themes). Rows below this can be populated with factors & descriptions as the discussions develop. Alternatively, each row could relate to a prompt question or theme.
    - Central-theme diagram, with off-shoot bubbles for students to connect to the central theme. These off-shoots can contain factors (and examples that relate to these to illustrate them) as they arise in the discussions.

- Prompting questions for students
  - 'Adaptive strategies': What do you think are 'adaptive strategies'? *(Think: link to evolution concepts previously covered in curriculum).* Define this as class/ group/pairs. Think-pair-share example.
    - Meaning: adaptive strategies represent an an evolved change in the genetic make-up of the plant (genotype) in response to influences from their changing environment.
  - Why do you think plants adapt with these strategies? What drives or promotes this adaptation?
  - What sorts of environmental factors may impact a plant?

- Disturbances & stresses. Plants can be influenced by a range of disturbances and stresses, evolving traits that are adapted to survive these conditions.

Ask: Can you think of some examples of what these influences might be? Label them as either abiotic (non-living), or biotic (living).

Think: environmental influences upon a plant - climate change, drought (water stress), extreme weather, salinity (e.g., high salt conditions) etc.

Examples include:

- **Disturbances:** availability of water, nutrients, light, temperature, presence of toxins (e.g., in soils) etc.
- **Stresses:** herbivory, pathogens (causing disease), human-influenced factors, fire, wind.

- Ask: How might scientists discover these adaptive traits or strategies? Observation is a key component and the building a collection of data from these observations.
  - **Observation** of a plant at across a range of scales– e.g., at the whole organism level, cellular (cells), molecular and genetic level.
  - **Geographic distribution** of plants: observation of where plants grow where, and not others.
- Ask: How might a global effort or knowledge of different locations worldwide benefit the program? E.g., in different climates there will be species and varieties of plants that grow in these areas. They would be adapted to environmental conditions.

Activity flow & beyond: Whole class or small groups to discuss each question prompts, sharing examples. Any unanswered questions that arise could form inquiry learning investigations (extension opportunity), or invitation for a scientist guest speaker (e.g., from TIA/UTAS).

Introducing traits & modern genetic technologies

Researchers use new technologies to better understand traits of organisms (both genetic & physical traits).

- Rapid advances in genetic technology **genome sequencing** (sequencing of DNA & RNA, including whole-genome sequencing), **mathematical modelling**, and **computational technologies** have enabled researchers to join the dots between desirable whole-plant adaptive traits (phenotypes) and the genetics that underpin them.
- Scientists can discover more about the complex interplay of the genetic and physiological networks (within the plant). It's a complex puzzle that they scientists are working to learn more about!
- By **using knowledge from modern technologies and combining multiple sciences together** (including biology, agriculture, mathematics, physics, chemistry), scientists are developing **predictive tools for plant breeding** to improve the agricultural industry.
  - In other words, developing ways to predict the results of plant breeding with the aim to develop improved crops and those more appropriate for environmental conditions.

- Traits
  - **Physical traits (phenotype)** are key to this research: how a plant physically appears (e.g., from a molecular level in its chemistry and physiology, all the way up to the whole organism level that you can see with your eyes). Understanding the genetics underpinning these physical traits are important to assist predictive plant breeding (mentioned in the background).

- Traits can relate to function, growth, reproduction, maintenance & persistence of a plant in an environment.

Activity/ Discussion: Crop physical traits. Prompting questions & discussion for class/small groups

- **Task: Identify some physical traits (phenotypes) that you might observe when in species for food crops.**
  - ***Think:*** *link these to some of the adaptive traits to disturbances and stresses mentioned above.*
  - **Examples** for a crop species (could use a specific example such as a bean):
    - **Leaf shape (morphology)-** the physical structure & features of the leaves. (e.g., size of leaf; physical structure of leaf may be more resistant to water loss).
    - Plant nutrition (nutritional content – e.g., high protein),
    - Tolerance or resistance to environmental conditions: drought (water stress), salinity (salt levels), poor soils, toxins etc.
    - Resistance to herbivores or pests
    - Flowering timing & duration of when the plant it flowers (as well as the conditions/ cues needed for this to occur, e.g., sunlight, temperature etc.), seed production (e.g., yield, nutritional content of seed).

- **Task: For each of the above physical traits, list at least one type of plant crop that you can think of, describing the trait in that crop.**

  - **Task: For each trait above- explain why these traits are important.** For any of these traits mentioned, ask students to consider what benefits these traits might mean for a crop, and why it might be useful to have these traits when breeding a new crop.

- **Example format:** Students could develop a list of example traits that may be desirable for a crop in Australia, considering changing climate conditions and drought stresses. (Table/ mind-map).

  - **Example:** a legume crop (pea family) may have several desirable traits that scientists may note when looking to improve cropping outcomes and food security, including:
    - higher yields of seeds compared to other varieties of the crop
    - drought tolerance or resistance
    - improved protein levels in their seed (nutritional content)
    - longer flowering time (e.g., potentially broaden the pollination window and/or production period)
    - tolerant of lower nutritional soils
    - salinity tolerant (able to tolerate higher levels of salt in soils).

Part 2- Technology Highlights: Processes & genetic technologies that underpin crop genetics research

This 'technology highlight' provides a taster of processes & modern technologies that are important to plant genetics research. Photos and examples from UTAS research facilities are provided, showing current genetics research into different food crop varieties.

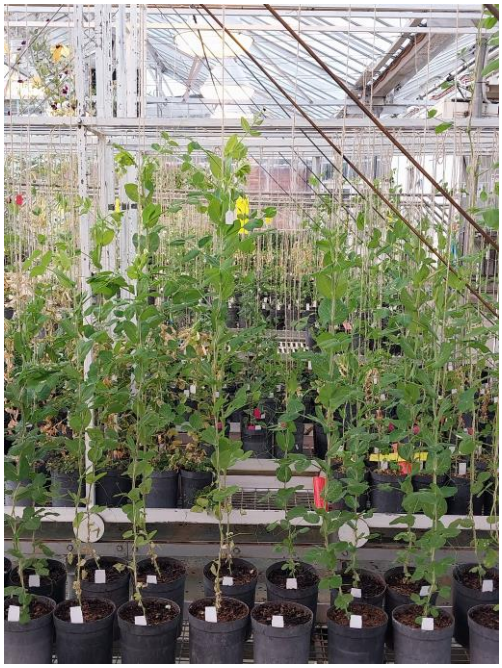
- Background information, discussion prompts, activities and online resources are outlined.

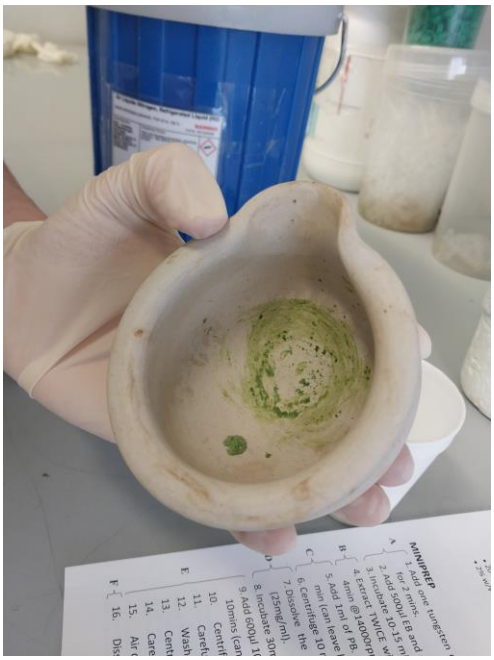
Each process & technology are introduced in the following sections:

- DNA extraction
- Polymerase Chain Reaction (PCR)- *additional supplementary material provided separately*
- Gel electrophoresis
- High-resolution melt (HRM) analysis – *additional supplementary material provided separately*

Process Highlight: DNA extraction

- Analysing genetic material is a key component of genetics research for developing improved crops.
- DNA extraction is the process of purifying DNA from a sample by using physical and/or chemical methods. The DNA genetic material needs to be separated as the other components can interfere with the analysis of the genetic material.
- The tissues of plants and their cells are complex mixtures of proteins, fats (including cell membranes), genetic material (DNA & RNA) in the cell nucleus, plus other cellular components in the cells.
- Below are some examples of preparation steps required for the extraction of DNA.
    - This example is from a researcher who is focussing on a variety of peas (legumes) that are an important nutritional food source (high protein content), and several other desirable traits.
    - Process outline: the scientist has picked a young leaf sample from a pea variety growing in a glass house research facility (below left), then whilst still fresh, the sample is finely ground (below right).





- Homogenization: grinding up the cells to break up the cell walls (*for plant cells*) and membranes is an important step in DNA extraction to ensure that we can extract the genetic material.
  - This can be achieved by a range of methods, including grinding the sample physically (e.g., mortar & pestle), or by vibrating a small sample very quickly with a metal ball-bearing to break up the material.
  - In this case, the scientist used a mortar & pestle physically with the addition of liquid nitrogen.
  - Ask: why might liquid nitrogen be used? (Hint: liquid nitrogen is extremely cold at-196 °C and requires safety precautions to avoid cold burns of the skin). *Answer points: the aim is to access the plant's nuclear material without degradation (i.e., the cold temperature freezes the cell contents, and helps to pulverize the cell walls of the plant tissue into a fine dust with grinding. The cold can also help in deactivating DNase (enzymes that digest DNA!)*
- Following this, multiple steps are conducted involving a range of chemicals, each with specific roles. These are mixed (centrifuged at high speeds!) and components are separated. The steps include lysis, separation, precipitation, and further purification.

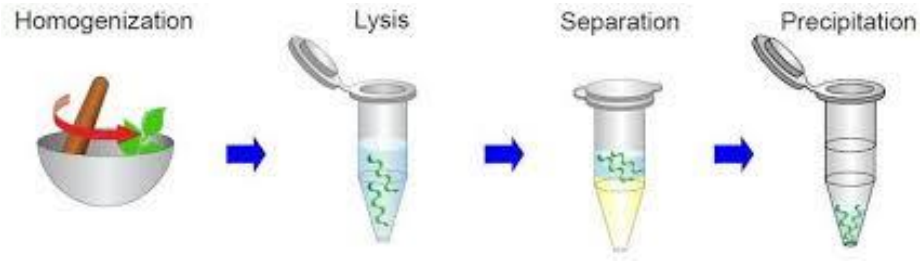
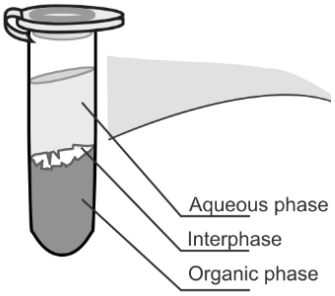


Diagram below: Both differences in density (mass per volume), and solubility (like-dissolves-like) allow the different layers to form. These layers include:

- Upper layer: A mixture of DNA & RNA can remain in the aqueous phase (water-containing later).
- Middle layer (interphase): Lipids and proteins are non-aqueous and separate out into the interphase.
- Bottom layer: The bottom layer contains organic solvent that binds to non-aqueous compounds in the 'organic phase' (e.g., chloroform).



DNA as the target: This research focussed on DNA, so the aqueous layer (upper layer) containing the DNA is carefully separated for further steps.

Methodology- Separate samples: In this plant crop research, each sample is prepared separately with careful cleaning of equipment between samples (e.g., the mortar & pestle is cleaned; new tubes and pipettes are used across samples).

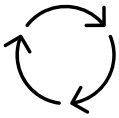
Ask: Why would it be important to keep individual samples separate? Why would scientists want to ensure they are not contaminating samples (even of the same species or variety of crop?) *Think: there can be genetic variation between individual specimens, within the same species or variety.*

- Suggested practical: Extraction of Strawberry DNA

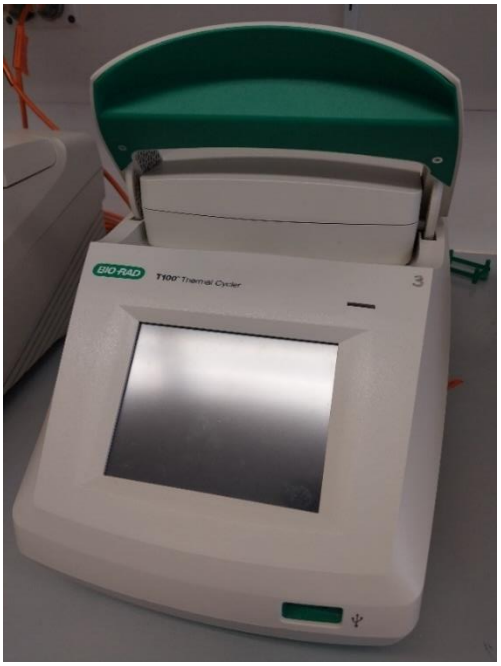
Extraction of strawberry DNA is an accessible practical that uses household materials & does not require specialised equipment. This allows students to extract the strawberry DNA from whole fresh strawberries, resulting in them observing the cloudy DNA at the conclusion of a lesson. Whilst conducting the process, prompt students to consider why different materials and processes are conducted (link to content covered above).

  - Example Resource Outlining Strawberry DNA Extraction:
    - Imperial College London (*Be Inspired* STEM Outreach resource): https://www.imperial.ac.uk/be-inspired/schools-outreach/secondary-schools/stem-in-action/the-human-body/strawberry-dna--activity/. Includes YouTube video outlining the process. Prompt questions for discussion and reflection after activity provided.

Technology Highlight: Polymerase Chain Reaction (PCR)



- Polymerase Chain Reaction (PCR) technology is a key process used in genetic research across diverse fields of science that use genetics, including many areas of agriculture, microbiology, plant science, marine science, medical research and forensics.
- Key introductory resources:
  - PCR introduction video – find at the top of this page (source: Genetics Learning Centre, US). Engaging & digestible content via animations.
    - Key concepts of PCR introduced, plus terms.
    - Note: Video with slide-deck format (needs to be prompted to play next slide).
    - Interactive components throughout (dragging/ clicking). Opportunity for engagement from class or in small groups.
- There are many touchpoints for this technology across science – a potential opportunity for educators and/or students to identify and investigate ways PCR links with different areas of a field. For example:
  - Sciences of Agriculture: microbiology (food safety: bacteria RNA), plant pathology (viruses and/or fungi affecting crops), food crop development (plant genetics: DNA analysis), soil science (microbes present in soil samples).
- Polymerase chain reaction (PCR) is a technique used in the lab to make millions of copies of a particular section of DNA (known as 'amplification'). It was first developed in the 1980s, and today is a core technology in genetics research.
- PCR rose to prominence in the public eye during the COVID pandemic as a rapid technique to amplify specific genetic material (in the case of the coronavirus, RNA genetic material) within samples to determine if someone was infected with the COVID.
  - COVID pandemic link: The coronavirus PCR test requires a sample of ribonucleic acid (RNA), which the technology 'amplifies' the genetic material. Amplifying RNA helps to make traces of the coronavirus detectable in a test sample, making the PCR technique is highly sensitive. Even a small trace of the genetic material of the virus in a body will be amplified and detected.
- Example photo from PCR work within a genetics laboratory that focuses on a range of molecular biology projects, including investigating traits of plants (e.g., legumes) to develop improved food crops:



Laboratory photos: Sample preparation post-DNA extraction (left), including mixing of the primers ready for the PCR thermocycling process (right).

- **Additional information for PCR**:
  - See **PCR Protocol & Information** document (provided in this TIA teaching resource download) for further details of the PCR stages, chemicals used and theory. This may be used for extension, or feed into senior secondary units (e.g., Biology, Chemistry, Science inquiry tasks).

Technology Highlight: Gel electrophoresis

This section highlights the technique **gel electrophoresis** as a fundamental process in genetic analysis & research in various scientific areas.

- **Gel electrophoresis is often used following PCR** to help scientists work out the length/s of DNA they have in a genetic sample.
- For example, when a scientist has 1. Extracted DNA from a sample (e.g., from plant's leaf), 2. Prepared the sample for PCR, and 3. Amplified the DNA using PCR, the next step is often **gel electrophoresis.**

This section will provide information & resources to cover the following aspects of **gel electrophoresis**:

- Key steps
- What is the science behind each step?
- What does gel electrophoresis tell us?
- Application examples

Example applications of gel electrophoresis can include:

- To **analyse results** of polymerase chain reaction (PCR).
- To **analyse genes** associated with a particular physical trait, response, illness or disease.
- To use in **DNA profiling for taxonomic studies (classification of species)** to distinguish different species.
- To **separate DNA fragments** for DNA fingerprinting (such as to investigate crime scenes in forensic science).

Resource for gel electrophoresis introduction:

An **interactive introduction** resource for gel electrophoresis is the gel electrophoresis virtual lab video/ slideshow from the *Genetic Science Learning Centre* (US). This online resource provides step-by-step information of the gel electrophoresis and why it's important.

Options for sharing this in class include:

- Educator could share the interactive video on a whole-class screen, seeking inputs from the class (like the PCR example above), or
- Divide into small groups where they undertake the laboratory process virtually, taking steps together.

Case study example: Pea traits for improved food crops

Understanding plant traits at the genetic level: Combining the technologies of PCR and gel electrophoresis, researchers can better understand traits of food crops to develop improved food sources.

Flowering time traits (when a plant develops its flowers, and therefore impacting its production of seeds) are a group of traits are of interest seeking to understand and develop improved food crops. A **case study example** is shown below for a variety of pea:

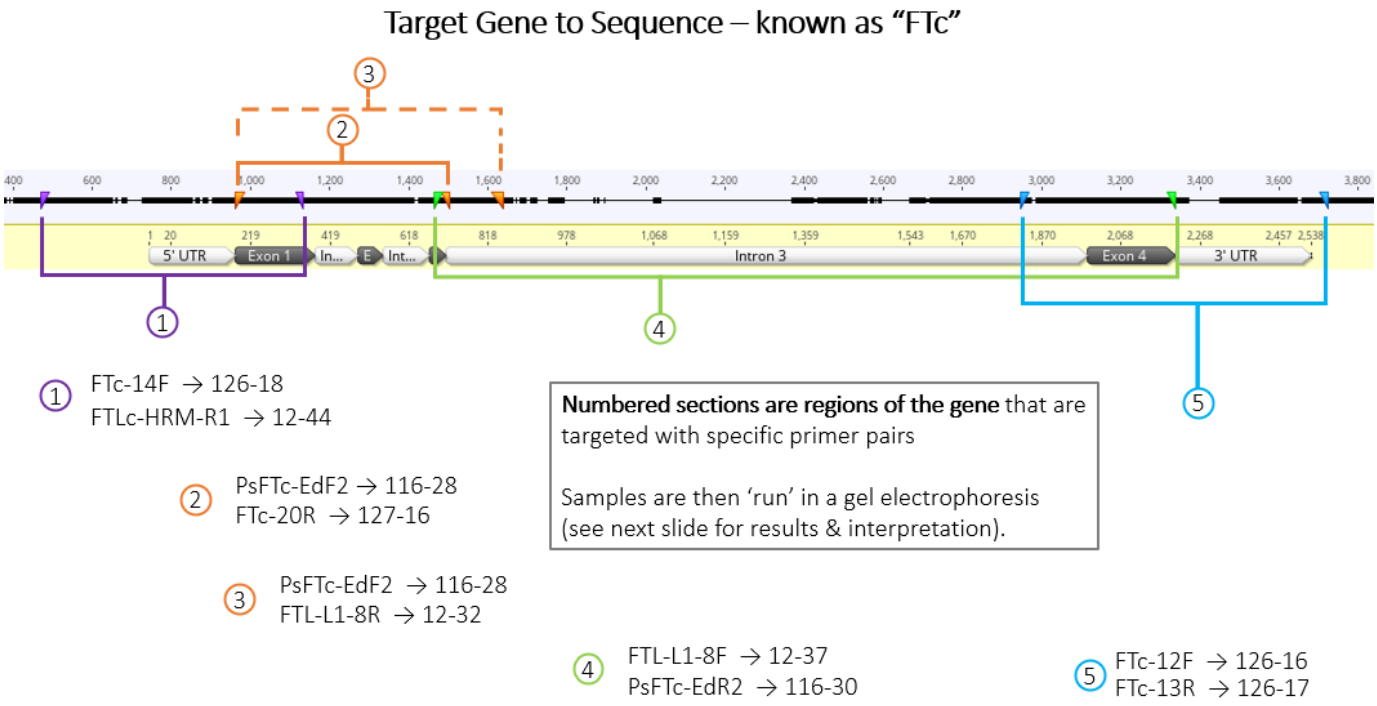
- **Processes:**
  - **Extraction:** DNA from pea leaves was extracted (several individuals of a variety),
  - **PCR:** This was followed by PCR for each individual sample to amplify the genetic material of interest.
  - **Gel electrophoresis**: Then, the researcher wanted to find out the lengths of the DNA fragments that they have from the PCR process using gel electrophoresis.

- **Gene Target: a 'flowering time' gene & the primers used:** The scientist wanted to target a gene that affects the flowering time of plants. This is the equivalent of 'zooming into' the gene to focus specifically on this genetic material

  .

  - **Aim:** This way they can investigate whether the genetic section is present in any of the pea specimens that they are testing. The gene sequence is known from prior understanding and research, so they can specifically target areas using **primers** in the PCR process.

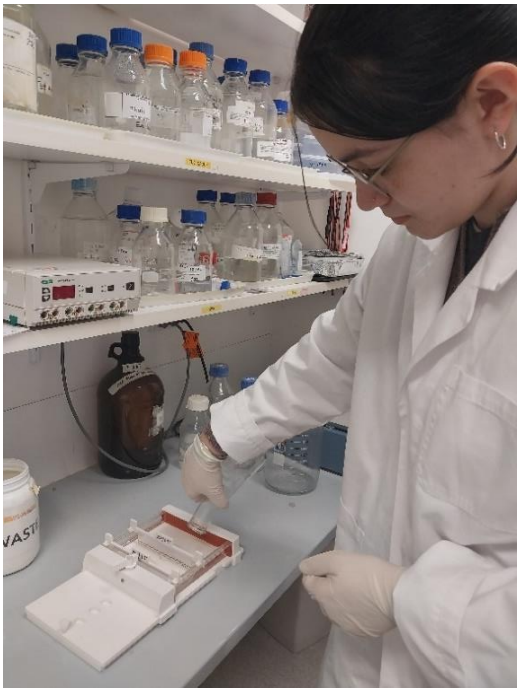
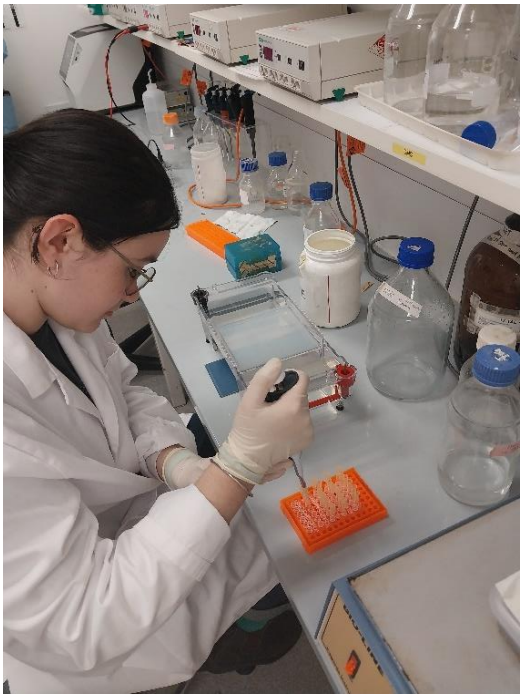
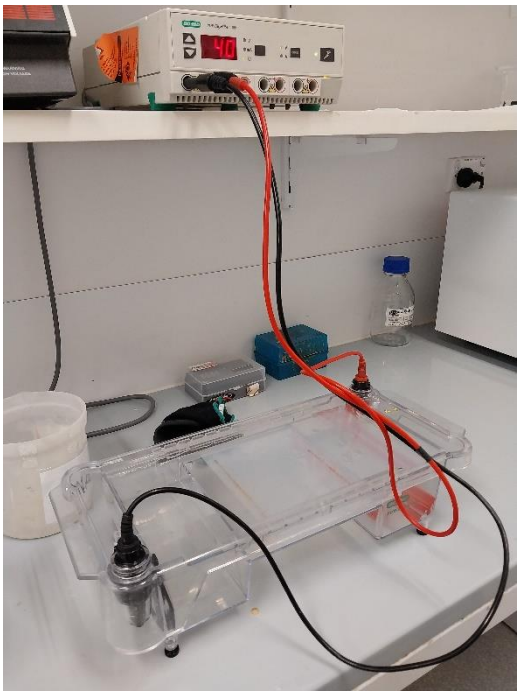
    The **target gene regions** in this case study are shown on the following page:



Preparing the gel electrophoresis 'gel' and samples. Steps involved to prepare the gel in a research laboratory are shown on the following page. This shows a scientist preparing the gel & commencing the experiment.

These steps include (pictured):

- Preparing the agarose gel (seaweed-derived gel) which is heated, dissolved, and then poured into a mould to set. This creates a gel medium for the DNA to move through and separate into different lengths of DNA (measured in nucleotide base pairs, bps) once a voltage is applied.
- Once the gel is set, the researcher pipettes small volumes of the genetic material (from the PCR stage) into small wells in the gel. Multiple samples pipetted (**5 individual pea plants** are in this example), with specific sections of the flowering time (FT) gene of focus.
- PCR has been performed on each of the five pea individuals' DNA, with the researcher interested in **five different sections of the gene (1-5 regions)** – as shown in the **primer information section** (above). This means the researcher carefully adds **25 separate pea DNA samples** into the gel.
- There is also a separate channel in the gel for a DNA standard to form a reference ladder of DNA base pairs. This helps scientists understand the length of the DNA segments of the experiment by allowing comparison of the five pea varieties to the reference lines.
- **Running the gel**: Once the samples are pipetted, the scientist pours a liquid over the gel (a buffer liquid containing charged particles called ions to carry the electrical charge) and connects the electrodes (black = negative; red = positive). **By applying the voltage from an external source (power pack), the movement of the particles is being forced**. The DNA is negatively charged (-), so moves from the negative black electrode to the positive (+) red electrode. (Note: Can relate this to the virtual lab video).
- *Note: small bubbles of gas will appear once the voltage is applied (this is water splitting into hydrogen gas & oxygen gas by **electrolysis**).*
- **Separation of DNA:** The DNA samples separate based on their **segment size** (i.e., the number of base pairs (bps) in the DNA section). Smaller lengths of DNA strands travel further in the gel, whereas longer strands travel a shorter distance.

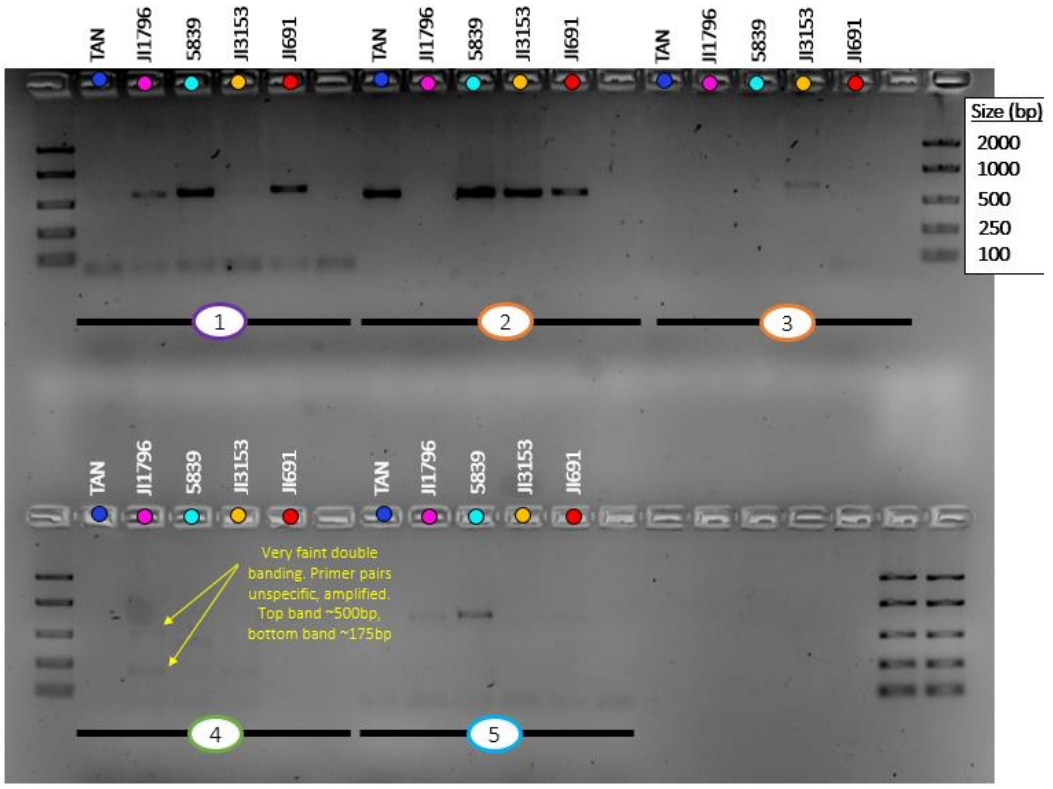

- **Finishing the gel 'run' & photographing the result:** Once the gel has had sufficient time for the DNA strands to pass through and separate in the gel medium, the researcher turns off the power, unplugs the electrodes, and the gently removes the gel (containing the now separated strands of DNA). A photograph of the resulting gel can be taken under UV light (see above image), with a stain in the gel allowing the strands to be visible). The resulting ladder-like lines of DNA strands are then compared to the reference ladder to understand their lengths.

- **Results of the gel electrophoresis:** An example interpretation of the gel electrophoresis results are shown in the below image. Consider linking these results back to the 'virtual lab' gel electrophoresis resource as this can be useful to reinforce both the practical steps and understanding the separation of the DNA samples.

Gel Electrophoresis Example
(UV photograph image of the gel)

This is an example gel electrophoresis result from a pea variety, with the genetic material of 5 individuals tested after PCR (each named along the top).

- Numbered sections on the gel refer to **primer pairs** (see previous page for what section of the gene each is to target).
- Outcome: All primers pairs worked successfully in at least 1 of the 5 individuals. On average product size ~750bp
- In the individual samples with **no amplification** (blank result), this could be due to various reasons:
  - **Difference in sequence**: The DNA sequence that the primers target is significantly different, or missing in those individuals
  - **PCR set up errors**, such as a component missing in the PCR mix, or the conditions (such as temperature or ratio of components in the mix) are unsuitable
- In primer pair 1 (and to a lesser extent 5) **'primer dimer'** is observed, where the primers bind to themselves instead of the target DNA. This creates their own band.
- Interestingly, primer pair 2 & 3 had the same forward primer but a different reverse primer. Since **3 amplified less**, the region reverse primer 3 is located must be significantly **more variable in its DNA sequence** compared to the region where reverse primer 2 is located.

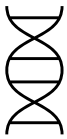


Activity: Identify key processes of conducting science from this example (pea genetics)

- Ask students to identify components of the case study that illustrate the process of conducting science, inquiry and solving problems. This can also consider many stages from prior to conducting the experiment, conducting the research, analysis and communicating these results & knowledge.
- Format: class discussion, small group collaborative discussion, or individual. Written exercise utilising graphical organisers can be effective for this – see below.
- Additional resources: use of graphic organisers- clearly organising and outlining these elements (e.g., divide between different stages of the research; flow chart, concept onions or other templates may be useful. Can refer to templates mentioned in educator PD session.

- Example elements (factors to consider):
  - Background: Where might the research begin/originate from? This includes identifying an issue/problem that guides the research, gaining background understanding of the topic, prior research & reading, who to collaborate with in my field or outside of my field of science, will this involve industry (e.g., commercial grower of crops, small or larger business)
  - Preparation & conducting the research: Planning of the experiment, breeding of the pea varieties (i.e., from seed, growing the plants so fresh genetic material can be

obtained), gaining practical skills for specific techniques in the laboratory and field, conducting the experiment, collection of data, as well as analysis & interpretation).
- Communication of the results and outcomes- who to (audiences), how, where, why? What more questions might we have? How might we approach these?



Activity: Ask students to **identify ways that the genetics case study illustrates scientific practice.**

This case study & interpretation of the results illustrates key parts of the scientific process.

Format: Discussion or short-answer writing task. Examples include:

- **Trying to understand & interpret the results** with what data was collected/ provided. This could also include comparisons to known information (from published scientific literature, or own personal research that led to this experiment).
- **The importance of both expected** and **unexpected results** – both are importance and can help us better understand science.
- **If an unexpected result was received**, what might this mean?
- **Thinking of ways that to potentially improve processes for the future** (e.g., change methods/processes; more practice at a particular technique).

Activity: Inquiry task to extend from PCR and/or gel electrophoresis

- **Aims:** Improved awareness and understanding of technologies in genetics for real-world applications.
- **Task:** Students investigate an example of where PCR and/or gel electrophoresis could be used to answer scientific issues, identifying, and describing how they link to society. Example are provided below:

Examples: Agricultural science abounds with example applications from food safety & microbiology, plant science for the development of improved plants (see attached gel electrophoresis results example), to understanding diseases affecting food crops. Examples could include:

- **Food safety- identification of harmful microbes in prepared food samples:** PCR can be used to help identify what types of microbial organisms are growing in a sample of food, and therefore its potential risks to consumers (Examples: packaged meats). This can help scientists understand what biology is happening in the food product, and if the food is safe to eat. After identifying the microbes, they can then find strategies to minimise or prevent the risks from the microbes in food samples. This is a key consideration when processed & packaged food is prepared & stored (e.g., processing temperatures, moisture content, pH levels etc.).

- **Plant pathology – health of crops & diagnosing disease**: identification of particular pathogens (harmful organisms) that may be present in a leaf sample from a diseased crop. Combining the visual cues (e.g., observations of the leaf health, roots, stem etc.), genetics from PCR can help with understanding if any organisms such as a fungus or bacteria are causing the disease (e.g., the presence of these organisms in an infected leaf sample).

- **Genetic analysis of plant varieties to develop improved food crops-** see case study example on legume varieties.

Technology Highlight: HRM (High-Resolution Melt) Analysis

This section highlights **HRM (High-Resolution Melt) analysis** as another technology used within genetic research, plus its applications. This example of technology can help better understand genetic material, specifically **genotypes & alleles**, plus their relationship to phenotypes (physical traits).

See the **supplementary material** for this module in a separate document (HRM Analysis 'spotlight'). Areas covered in this section, and followed into the supplementary resources include:

- What is HRM analysis?
- What can HRM tell us?
- How does it work?
- Additional technologies (e.g., robotics) involved for its preparations
- Applications & opportunities for engagement

- **High Resolution Melt (HRM)** is a relatively new, powerful technique used for detecting differences in genetic material, often conducted after PCR.
  - **Terminology check**: at the start of this HRM section, it may be useful to have a brief term-check & refresher of the following definitions (as a class or small groups):
  - **DNA –** definition, its role, structure, components- including **nucleotide bases** (types (A,T,G,C).
  - **Mutation –** definition, types of changes in the genetic sequence of an organism (can be in DNA or RNA material). Resource example: short video & genetics overview visual below on this page from the Genetic Science Learning Centre (US)
  - **Polymorphism-** the presence of two or more variant forms of a specific DNA sequence that can occur among different individuals or populations. The most common type of polymorphism involves variation at a single nucleotide base (also called a single-nucleotide polymorphism, or 'SNP').
  - **Epigenetics** – the process where the environment & behaviours can cause changes that affect the way that genes operate. Unlike genetic changes, *epigenetic changes are reversible and do not change the DNA sequence, but they can change how an organism reads a DNA sequence.*

See the **HRM Analysis supplementary material** for further specific information on the HRM method & suggested activities (available with TIA educator resources).