Control of Gene Expression

The vast majority of a cell's DNA is not translated. Imagine – tongue cells expressing hair genes!

The Basics

- → **Only some genes are switched on in each cell. They are controlled by multiple systems.**
- → **Changes in the DNA are called mutations, some are harmful and other aren't.**
- → **RNAi, transcription factors and epigenetics regulate expression.**

Gene mutation

- → Gene sequences are specific for the product they code for.
- → If the sequence changes the product can also change.
- → Changes in the sequence of DNA are called **mutations**.
- → They occur regularly in the DNA and their rate can be increased by **mutagens**.

Mutagens – *Most commonly they are: toxic chemicals, ionising radiation and high-energy radiation.*

Types of mutation

- → **Addition** – base pairs added, causing **frame shift** and affecting all amino acids after the mutation.
- → **Deletion –** base pairs removed, again causing **frame shift**.
- → **Substitution** – one base is swapped for another, which may or may not cause change, depending on the code.
- → **Inversion** – the DNA molecule is cut on both strands and flipped, causing widespread sequence changes.
- → **Duplication** – large sections of the gene are copied to a different part of the sequence, essentially coding twice.
- → **Translocation –** one part of a gene breaks off, and inserts somewhere else.

Mutation	Before	After
Addition	CTGG**A**G	CTGGG
Deletion	C**TG**GAG	CGAG**TG**
Substitution	CTGGAG	CTG**G**GAG
Inversion	CTGG**A**G	CTGG**G**G
Duplication	CTGGAG	CTGGAG**CTGGAG**
Translocation	CTG**GAG** GAC**CTC**	CTG**CTC** GAC**GAG**

Frame shift

Consider a sentence:
The dog was fat
Remove one 'base'
hed ogw asf at
The entire sentence loses its meaning.

Remember:

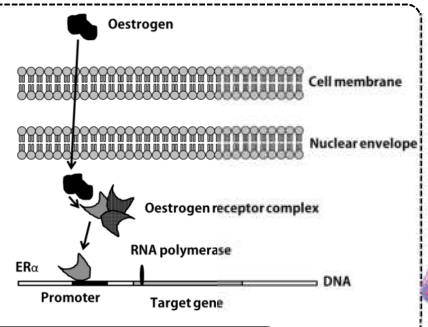
Not all mutations cause change in the amino acid sequence.
Also, not all mutations are bad – evolution is driven by mutations!

Stem cells

- → **Totipotent** cells can divide and produce all cell types, but during development, only some of the DNA is translated, so the cell becomes specialised. They only exist in embryos.
- → **Pluripotent cells** are also only found in embryos, and can form any cell type, besides extra-embryonic tissues, such as the placenta and umbilicus.
- → Adults have some stem cells – they are **multipotent** and **unipotent** cells. Only multipotent can become multiple cell types, though they are more restricted. They only make cells like their neighbours.
- → **Unipotent** cells, such as cardiomyocytes, are restricted to forming only one cell type.
- → New technology is allowing the production of **pluripotent** cells from unipotent sources, called **induced pluripotent stem cells** (iPS cells). They offer great hope to medicine, as they come from the patient and won't be rejected.

Controlling transcription

A transcription factor is a protein that can move into the nucleus and 'turn on' DNA so that genes are transcribed to mRNA.



Oestrogen and transcriptional control

Oestrogen is not a transcription factor.

However, it can enter the cell nucleus, where it binds to its receptor.

Binding oestrogen causes the receptor (**ERα**) to change shape and detach from a protein complex.

Once loose, the oestrogen receptor can act as a **transcription factor**. It binds to the promoter and causes RNA polymerase to transcribe the target gene.

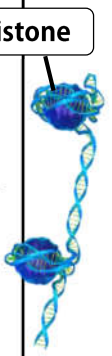
Oestrogen plays a role in some **breast cancers** as the oestrogen receptor becomes **overactive** and stimulates abnormal growth of breast tissue.

RNA interference (RNAi)

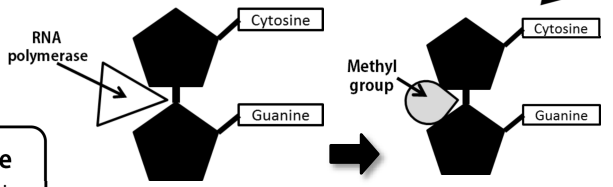
- → An additional way of controlling gene expression.
- → In these cases, genes may be transcribed to mRNA by the cell, but in the cytoplasm, the **cell degrades the mRNA** before the protein is made.
- → The mechanism for breaking these molecules down depends on a double-stranded RNA molecule called **siRNA (short interfering RNA)** becoming processed to a single strand and binding to the target mRNA and to a protein complex, which then breaks down the mRNA.

Epigenetics

- → **Epigenetic** changes to DNA can be inherited, but do not affect the actual DNA code.
- → They alter the way DNA is **structured** in the nucleus.
- → DNA wraps around proteins called **histones**. They can be changed by adding **acetyl** groups.
- → By adding acetyl groups, the DNA winds **less tightly** around the histone, and can more easily be transcribed by **RNA polymerase**.
- → If **very few** acetyl groups are added, the DNA winds **very tightly** – the RNA polymerase cannot bind.
- → Similarly, **methyl groups** bind to the DNA at sites where **cytosine and guanines** are next to each other.
- → By **methylating** the DNA it is essentially turned off as the RNA polymerase struggles to bind to it.



Remember: **Increased** methylation and **decreased** acetylation have the same results – they turn **off** genes.



Epigenetics and disease

- → **Proto-oncogenes** control cell division, and **tumour suppressor** genes stop it.
- → Epigenetics can affect these genes.
- → A tumour suppressor might normally be only slightly methylated.
- → If it is **more methylated** then it is turned off, and does not inhibit cell division, **leading to cancer**.

Cancer

- → Tumour growth; either malignant or benign.
- → **Metastatic** tumours can **invade** multiple tissues and **spread** through the body. **Benign** tumours do not and **stay** in the same body area.
- → Oncogenes are mutated proto-oncogenes. They are responsible for controlling cell growth and division.
- → Mutations in the oncogene, or abnormal methylation of it, can drive cancer development.

Gene Technology 1 – Fragmentation and Amplification

The Basics

- → **Recombinant technology is the process of combining DNA from various organisms.**
- → **Enzymes called endonucleases cut DNA and ligases recombine.**
- → **PCR is a method for amplifying DNA in vitro.**
- → **In vivo, plasmids can be inserted into bacteria.**
- → **Marker genes are used to detect inserted genes.**

Recombinant DNA technology

Recombinant is based on the word 'recombine' – it is technology that takes DNA from one organism and recombines it with the DNA of another to produce a transgenic organism.
To introduce a piece of DNA into another organism, the donor DNA must first be turned into **fragments**.

Fragmentation

Reverse transcription

Remember transcription? It involves coding DNA into RNA.

Reverse transcription does the opposite.

mRNA exists as much smaller fragments and each mRNA normally codes for a specific protein.

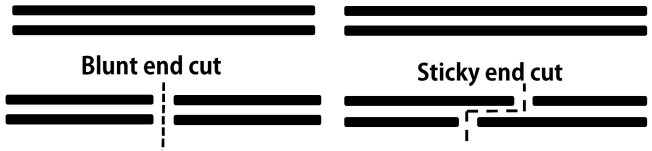
Reverse transcription uses an enzyme **(reverse transcriptase)** to convert mRNA molecules back to a form of DNA, called complementary DNA **(cDNA)**, which can then be inserted into the DNA of another organism.

Fragmentation

Restriction endonuclease

An endonuclease is an enzyme that cuts the DNA molecules at a specific point. Restriction endonucleases cut both strands of the DNA at a specific point. The specific point the nucleases cut is called the **recognition sequence**.

Restriction endonucleases cut in two ways, across the middle to produce **blunt ends** or staggered to produce **sticky ends**.

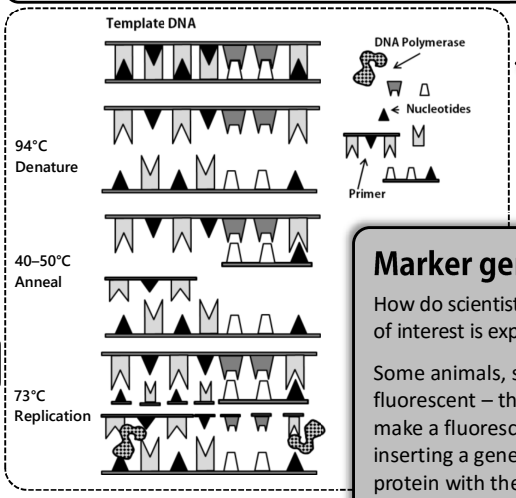


Amplification

Polymerase chain reaction

You can think of PCR as a DNA photocopier.
The DNA is put in a tube with a **primer**, which is a short fragment of DNA that is **complementary** to the sequence you require, free nucleotides (building blocks of DNA) and a DNA polymerase enzyme.
Using specific temperatures, scientists can manipulate the DNA molecule and the enzyme to undergo DNA replication time and time again.

1. Denature DNA at 94°C, breaking hydrogen bonds.
2. Cool to between 50 and 60°C.
   Hydrogen bonds reform and primers anneal with complementary pairs.
3. Heat again to 74 °C.
   This is the optimal temperature of the DNA polymerase – DNA is copied.
4. Raise to 94 °C again to repeat cycle.
5. Repeat approximately 25 times – produce over 50 million DNA copies.



Marker genes

How do scientists know if their gene of interest is expressed as a protein?

Some animals, such as jellyfish, are fluorescent – they must, therefore, make a fluorescent protein. By inserting a gene for fluorescent protein with the gene of interest, a scientist can assume that if there is fluorescence in a cell, then their **gene of interest is also expressed**.

'Gene Machine'

Machines that synthesise DNA are available, and will create any sequence you input into the machine.
This is especially useful if you already know the exact sequence you require.

Genome projects

- → Modern biologists are sequencing the genomes of many organisms.
- → By understanding the genome, diseases with genetic components can be better understood.
- → Additionally, sequencing lower organisms, such as viruses and bacteria, allows a better understanding of what proteins they make so that vaccines and drugs can be designed.

Word cloud

Recombinant
Fragment
Reverse transcriptase
Restriction endonuclease
PCR
Gene machine
Transformation
Terminator
Ligase
Marker
Sticky end
Blunt end
Primer
Recognition sequence

Amplification

Transformation

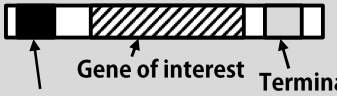
- → Is the name given to the insertion of foreign DNA into a cell.
- → Often transformation happens in bacterial cells, but can also happen to cultured cells in a lab.
- → Scientists adopt certain tricks to ensure their gene of interest is both **expressed** and **detectable**.

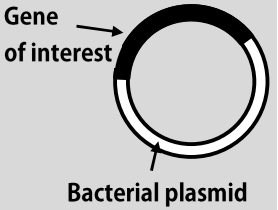
Promoters and terminators

- → Even though a gene is inserted into a host, it isn't necessarily expressed as a protein.
- → By adding a host-specific promoter and terminator, the scientist can be more assured that the host will express their sequence of interest.

Ligases

- → Bacteria are unique in that they have **plasmids** – circles of genetic material that they can translate proteins from.
- → Scientists can **insert** their gene of interest into a plasmid using **endonucleases** and 'glue' the DNA into the plasmid with enzymes called **ligases**.





Gene Technology 2

The Basics

- **DNA probes are sequences of DNA that are complementary to a region of interest.**
- **As they are complementary they hybridise to the DNA.**
- **This technique is used for disease diagnosis or detection.**
- **DNA fingerprinting uses VNTRs to identify changes between individuals.**
- **DNA technology will have many diverse uses** in the future.

Gel electrophoresis

- A method used to separate small fragments of DNA.
- Fragmented DNA is loaded on to an agarose gel, and placed in a tank filled with solvent.
- An electrical charge is passed across the gel.
- Due to the negative charge of DNA, it is attracted to the positive electrode.
- Lighter fragments of DNA move further than heavy bands, leaving a 'band' pattern on an agarose gel.

DNA fingerprinting

Restriction endonucleases cut at regular internals across the entire genome. When cutting across the genome, fragments of varying size are produced. The size of each of these fragments differs considerably between individuals – not even siblings have the same pattern of fragments, meaning individuals can be identified with high certainty.

VNTRs

- The non-coding regions of a person's genome contain long, repeated nucleotide sequences.
- These are referred to as **variable number tandem repeats (VNTR)**.
- Two individuals will have different lengths of repeating units between restriction endonuclease sites.
- As a result, when the DNA is cut, the lengths between the cut sites will differ due to the length of the VNTR.
- Only identical twins, who are born with the exact same DNA sequence, have the same VNTR pattern.

Personalised medicine and genetic counselling

- Tumours are unique to each patient.
- Different tumours respond differently to each treatment. One breast cancer drug doesn't necessarily work for all breast cancer, and the dose will not be the same for every patient either.
- By sequencing each patient, or the DNA from their tumours, a unique drug regime can be designed for each patient.
- Additionally, genetic counsellors combine the scientific method of DNA hybridisation as well as probabilities, with emotional and medical support to support people making very difficult decisions.

Heritable conditions

Some diseases are heritable – they have a genetic component. As a result, the DNA sequences associated with many diseases are known. If the sequences are known, then probes can be designed.

Example

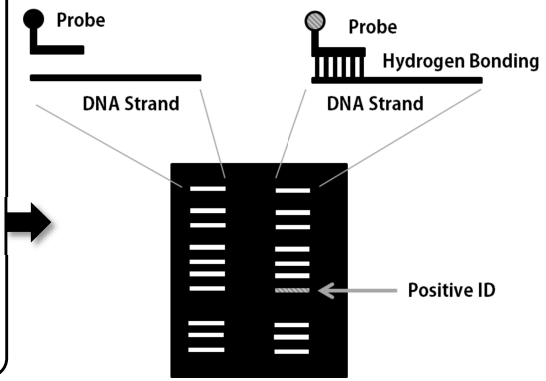
The end of the Huntington gene contains 6–35 repeats of CAG, coding for the amino acid glutamine. Patients with over 35 repeats of CAG are more likely to suffer from the neurological condition Huntington's disease. A probe can be designed with long GTC repeats. This hybridises with the CAG repeats and in positive situations can identify Huntington's disease risk.

DNA probes

The DNA sequences of some harmful alleles are known.
Probes designed for these genes identify their presence or absence.

DNA hybridisation

- Gel electrophoresis is used to run a whole genome DNA sample across an agarose gel, and transferred to a membrane.
- The specially designed probe 'hybridises' to the DNA at the region of interest.
- The probe only identifies the complementary region, and is indicated on the membrane.
- Without the sequence of interest being present, the probe will not bind.
- If the sequence is present, the probe binds and is detected.



Paternity

DNA contains alleles from both the mother and the father.
In case of VNTRs, some will be inherited from each parent, giving the child a unique pattern of bands on a gel electrophoresis trace, reflecting the loci of both parents. This can be used to determine the parents of a child.

Uses of DNA fingerprinting

Animal breeding

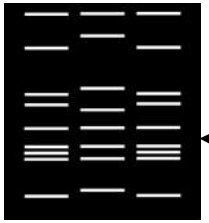
Identify optimal breeding stock

Diagnosis

Identify infection based on DNA

Forensics

Identify criminals from their DNA



Probability

The probability of having the same VNTR sequence as another person is 1 in 9 billion. This can be presented as 1/9,000,000,000 or 1.11×10^{-10}.
Current estimates give the population of the earth as 7.4 billion.

Drug responses

- Drugs are metabolised by the body – many are not in an active form in a pill, and many are toxic if not broken down.
- The enzymes to break down the drug are not effective in some patients, due to a normally harmless mutation in the enzymes.
- DNA hybridisation can identify the mutations so that harmful drugs aren't given to someone that can't metabolise them.

Word cloud

Probe
Hybridisation
Personalised medicine
VNTR
DNA fingerprint
Electrophoresis
Genetic counselling