Berg and Laws Survival Guide to Genetics

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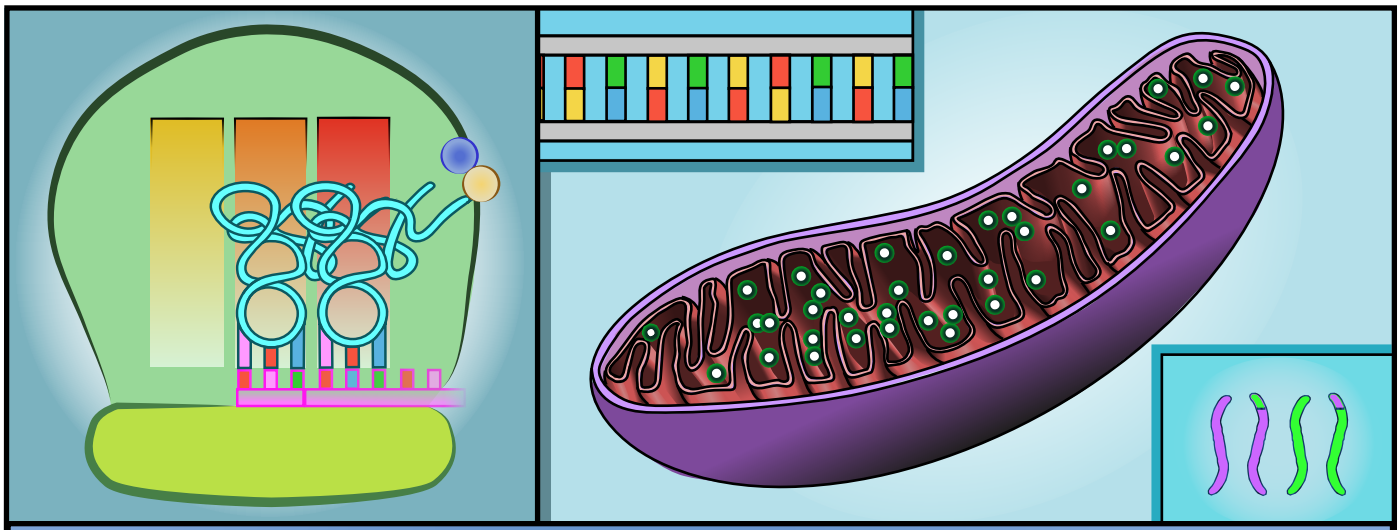
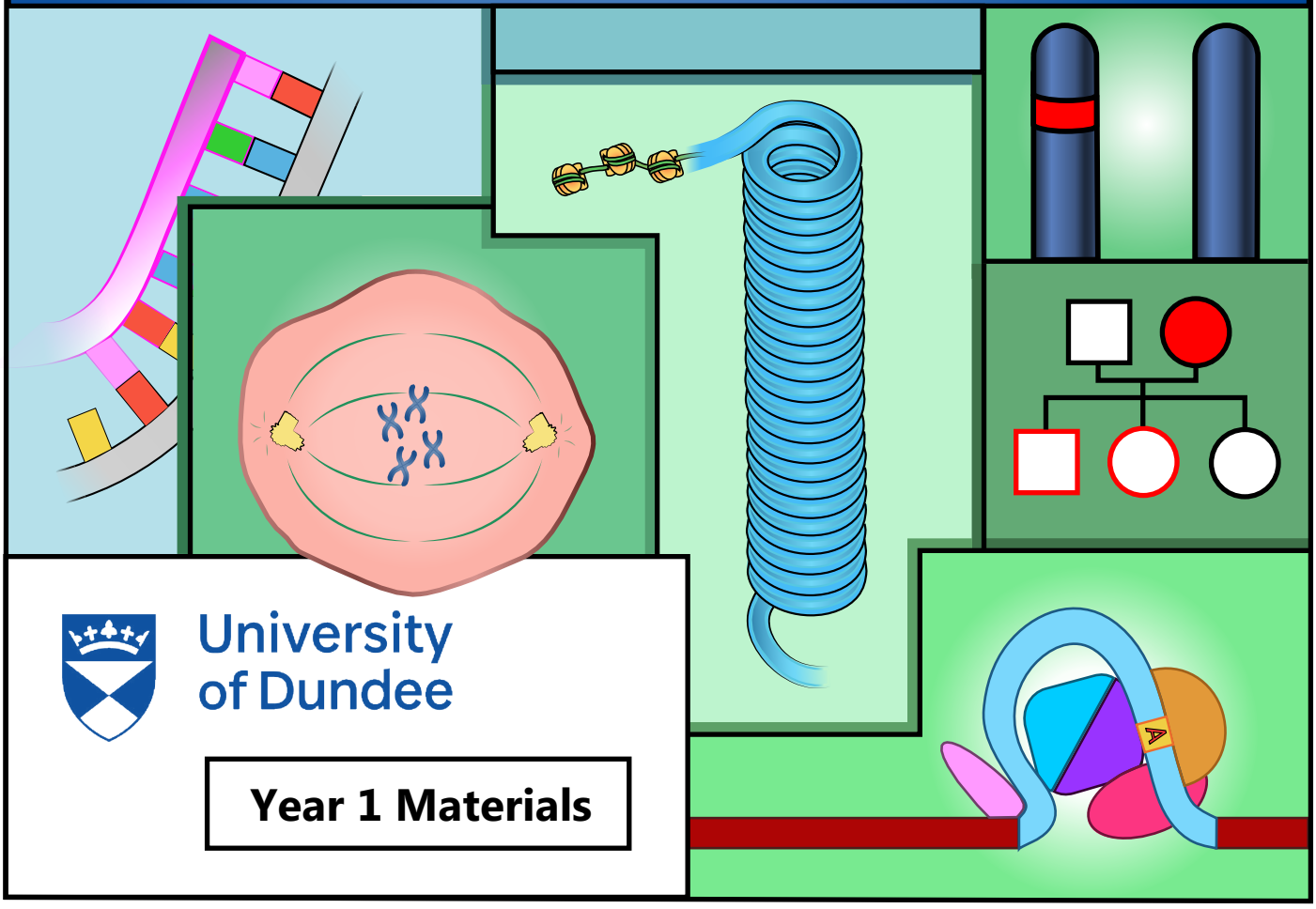


BERG AND LAWS

SURVIVAL GUIDE TO GENETICS



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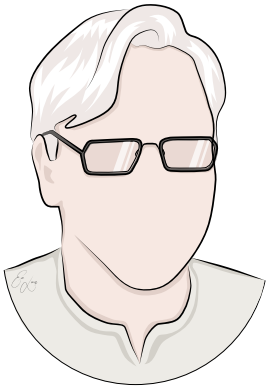
Year 1 Materials



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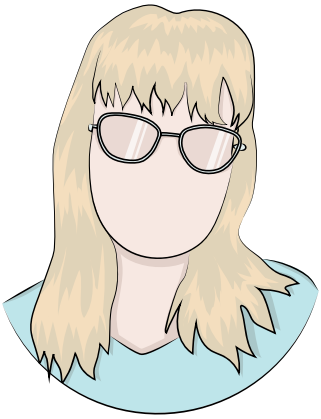

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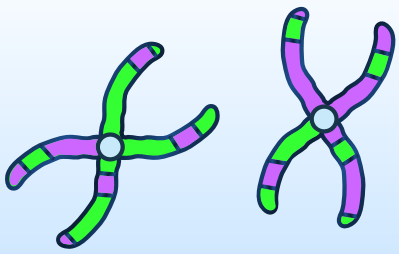
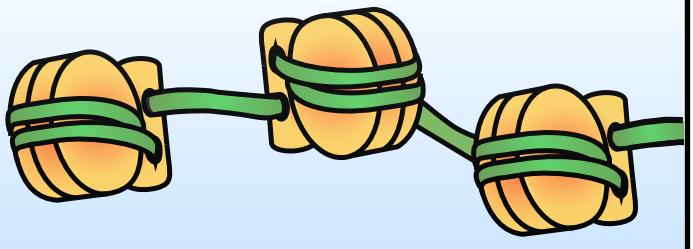
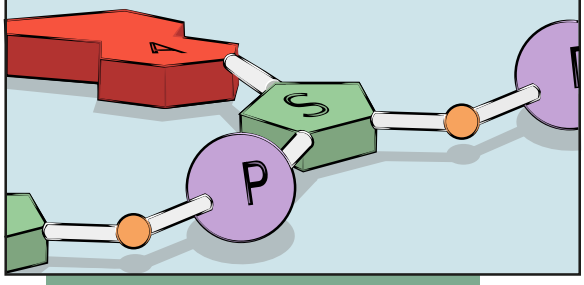
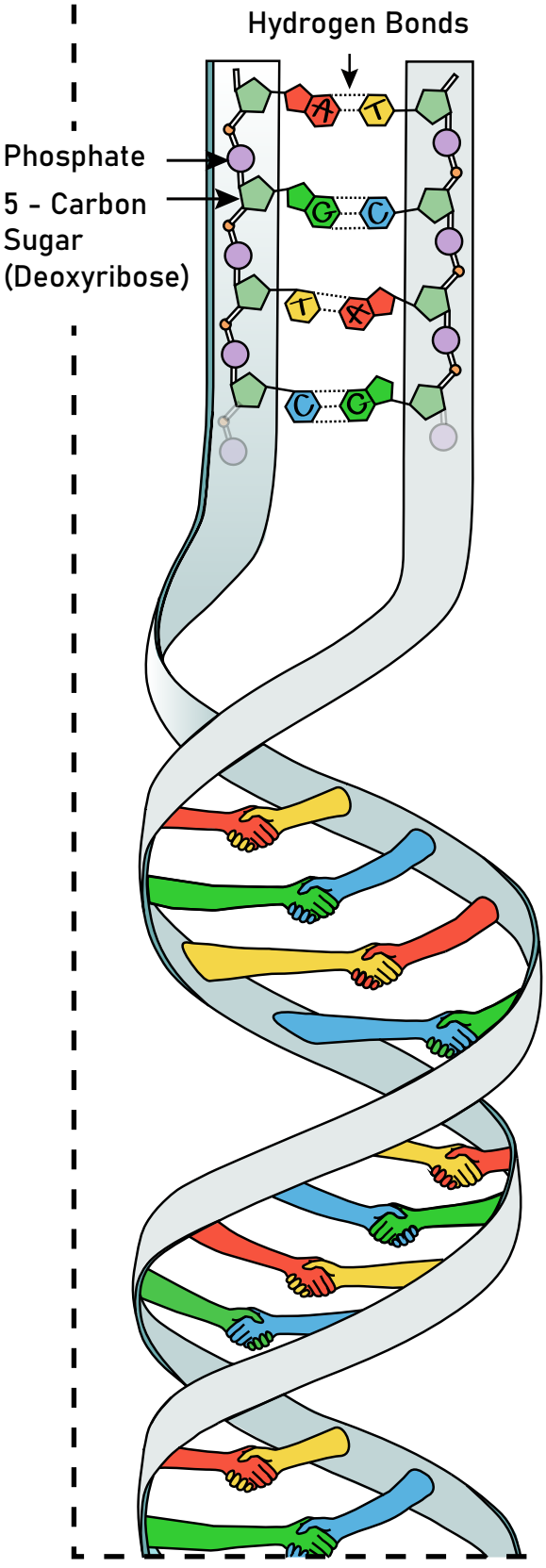




D N A

DEOXYRIBONUCLEIC ACID AND BASE PAIRING





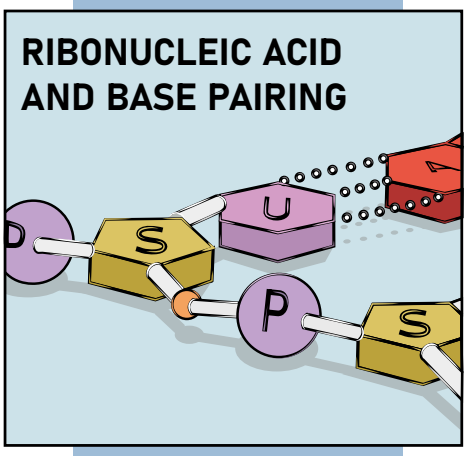
Deoxyribonucleic acid has a sugar-phosphate backbone, with information contained in the sequence of bases.

The double helix of DNA consists of two strands, running in the opposite direction to each other.

Hydrogen bonds between the bases link the strands, Adenine pairing with Thymine, and Guanine pairing with Cytosine.

Purines	Pyrimidines
A Adenine	T Thymine
G Guanine	C Cytosine

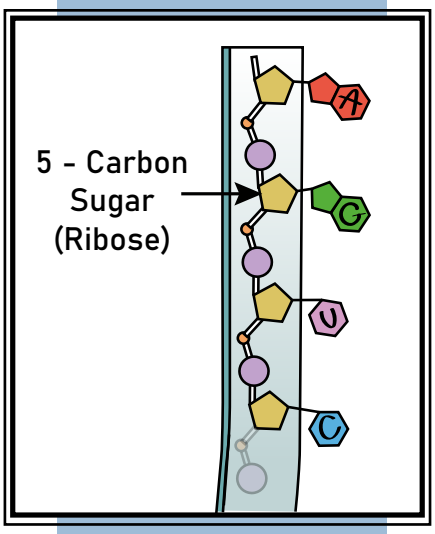
RNA

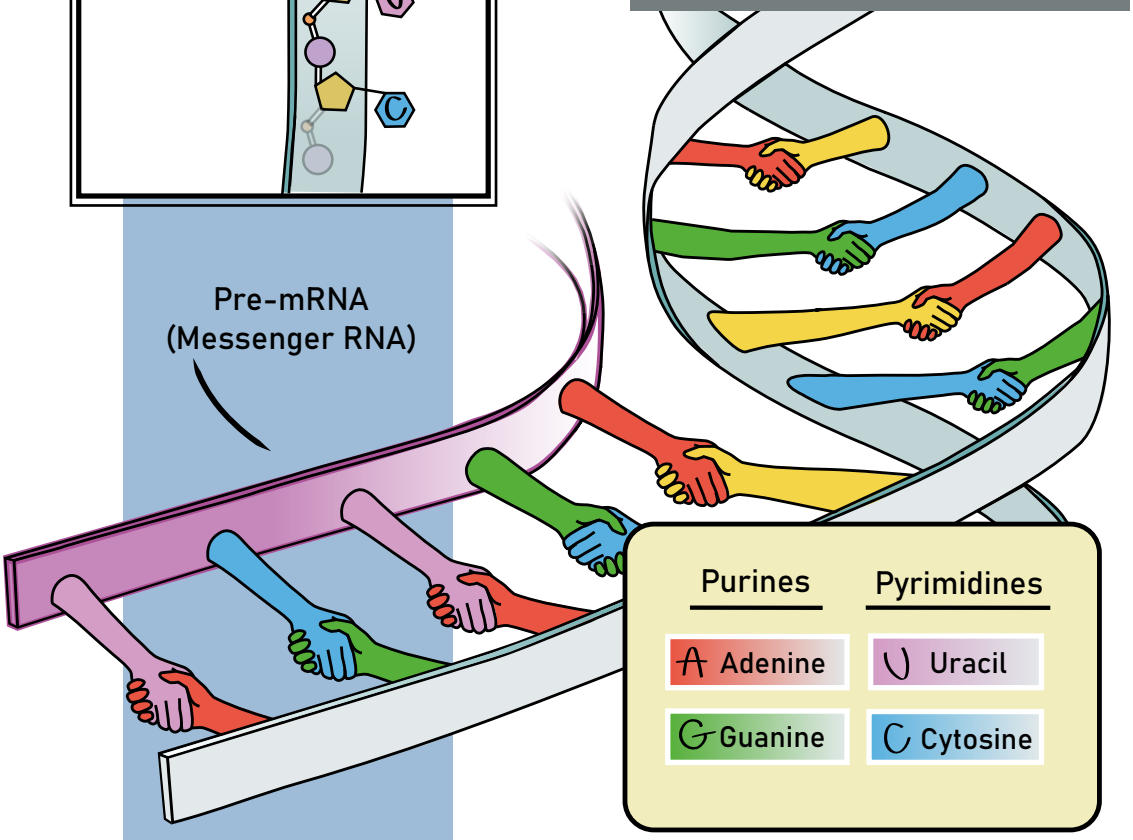


Ribonucleic acid is usually a single stranded molecule.

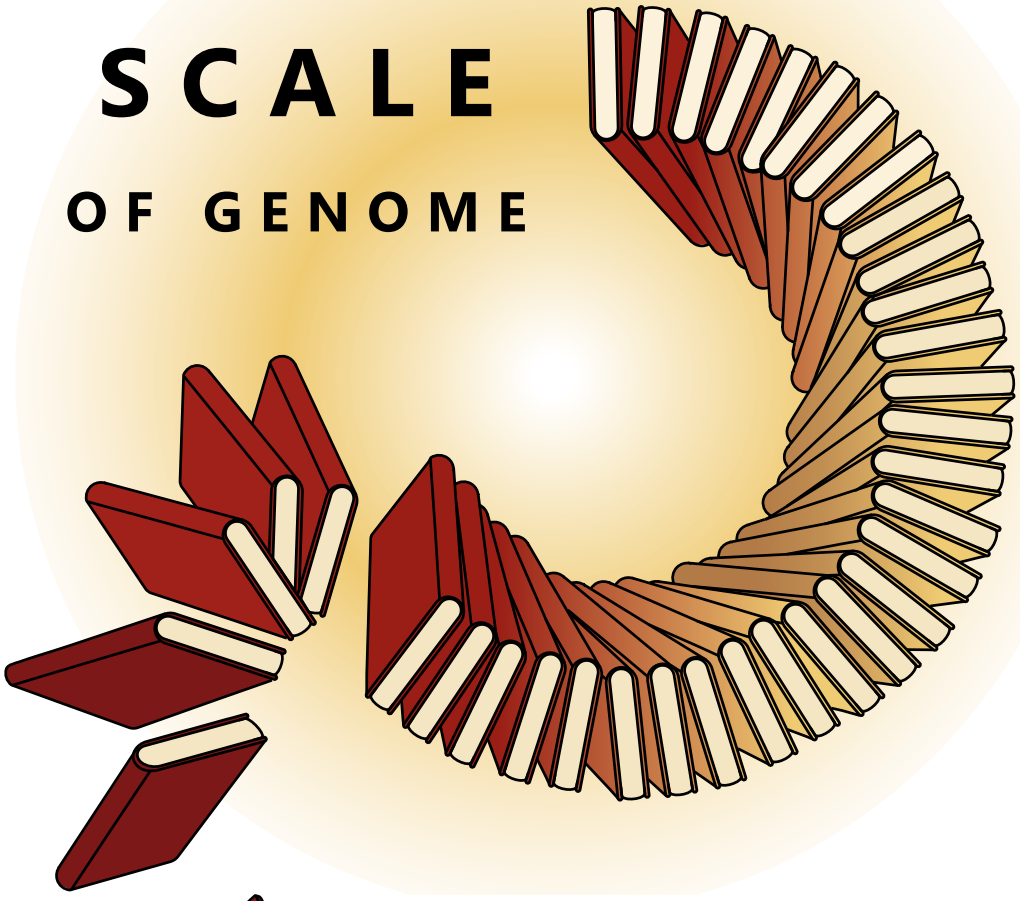
The main differences with DNA are that the sugar in the backbone is Ribose, and the base Uracil is used instead of Thymine.

It has a number of different functions in the cell.





SCALE OF GENOME

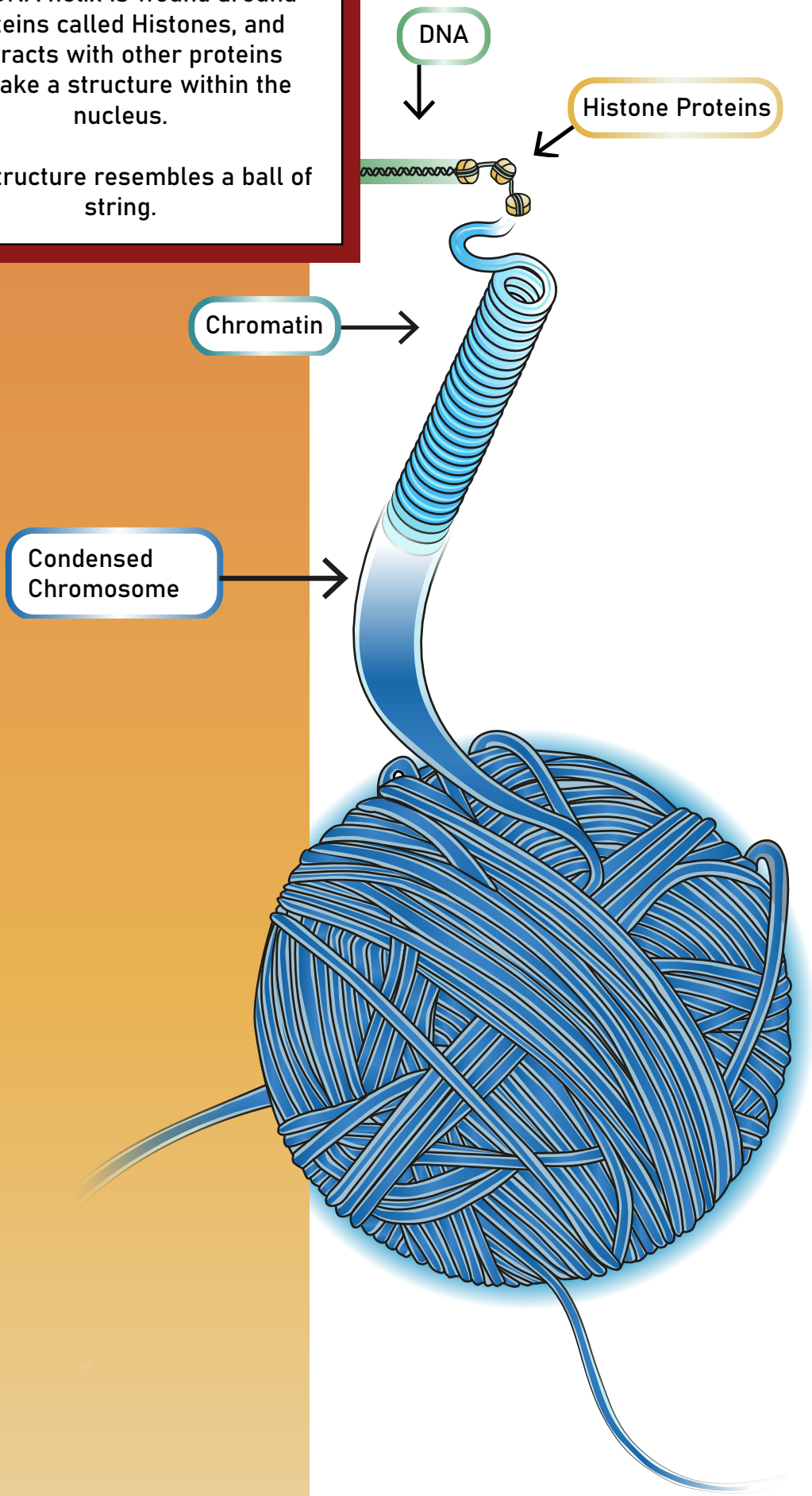


The human genome has approximately 3 billion bases, although only a small proportion of them (1-2%) encode genes.

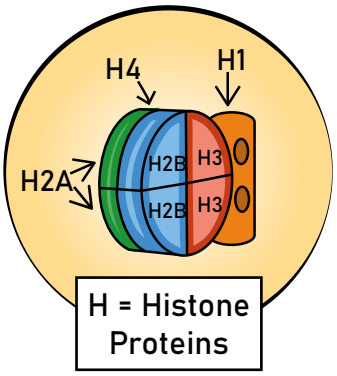
This is approximately the same length as 6,000 copies of "War and Peace" written by Leo Tolstoy.

The DNA helix is wound around proteins called Histones, and interacts with other proteins to make a structure within the nucleus.

This structure resembles a ball of string.

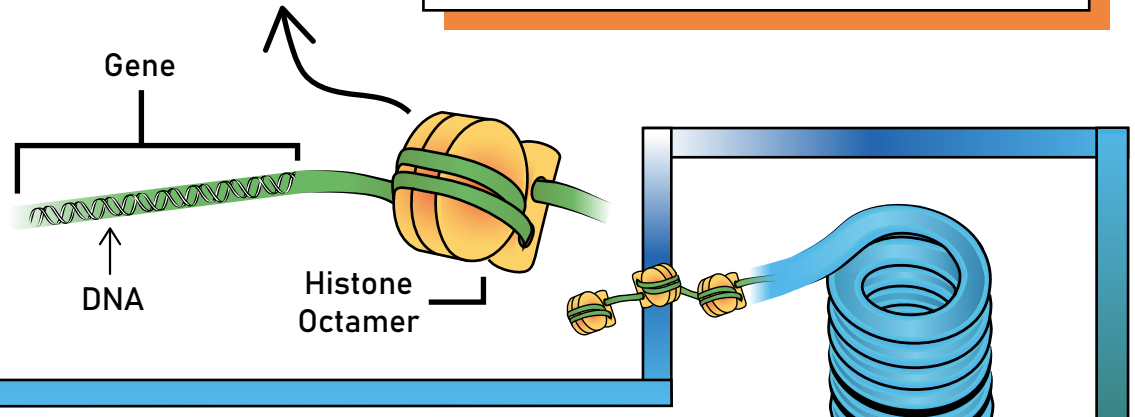


STRUCTURE OF GENOME

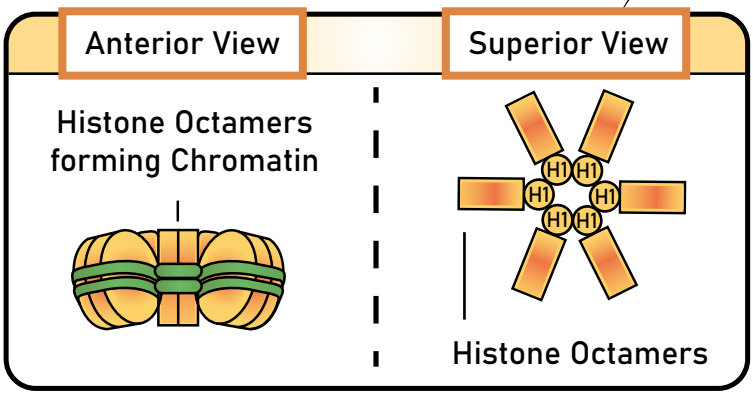


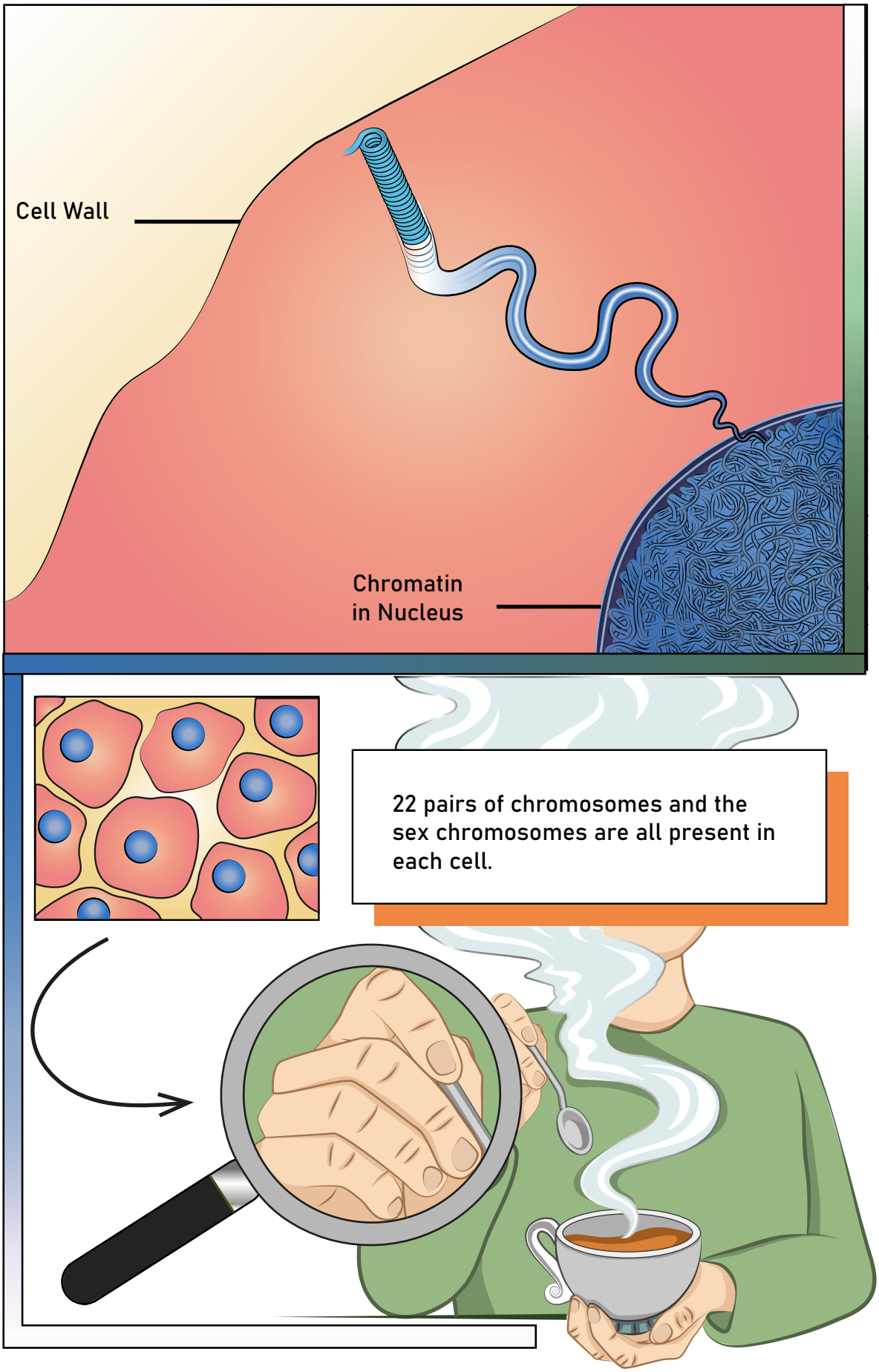
H = Histone Proteins

DNA wraps around a highly basic (positively charged) histone octamer, and is then coiled further to make the chromosome structure.



Condensation of DNA controls gene function. DNA that is tightly coiled (condensed) is less transcriptionally active.



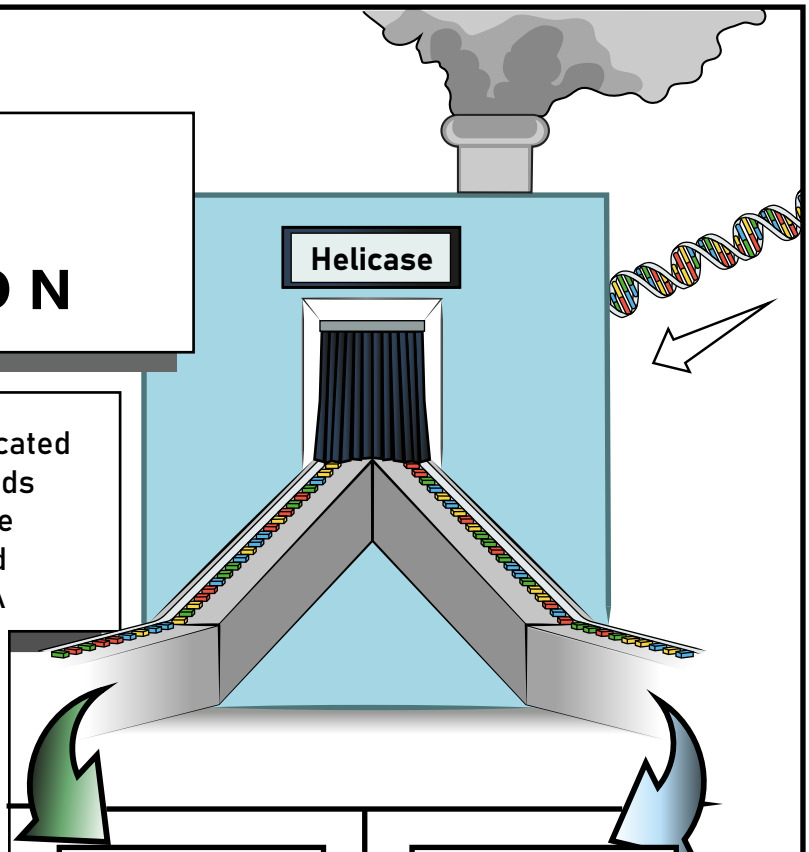

Cell Wall
Chromatin
in Nucleus
22 pairs of chromosomes and the sex chromosomes are all present in each cell.

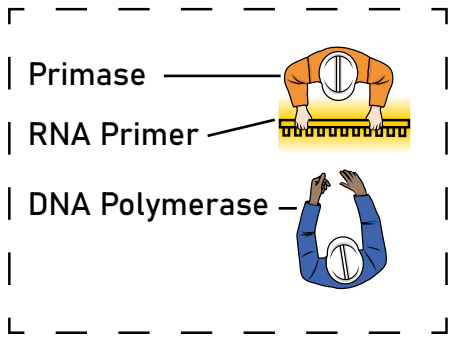
DNA

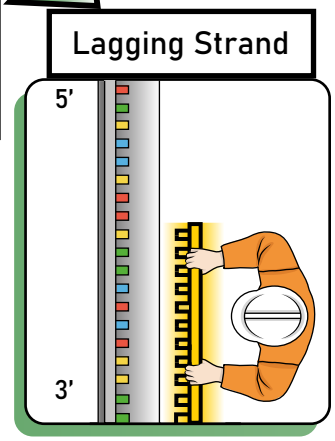
REPLICATION

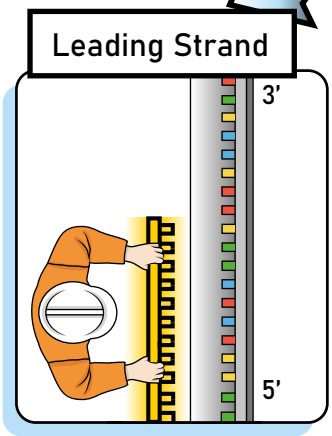
A DNA strand can only be replicated in one direction. After the strands are separated and unwound, the leading strand has bases added in the 5' to 3' direction by a DNA polymerase.

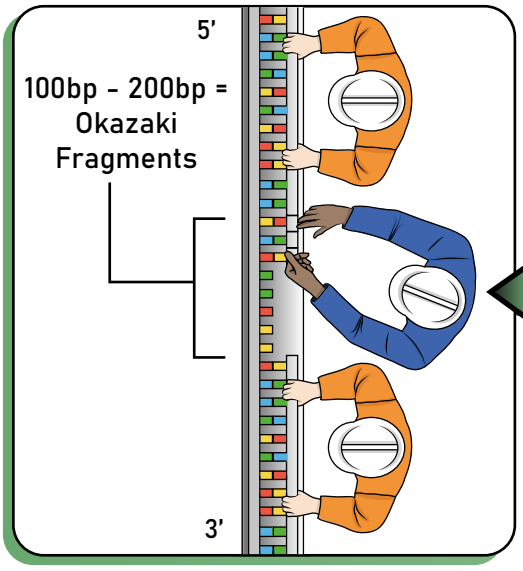
On the lagging strand, short stretches of DNA are synthesised as it unwinds (called Okazaki fragments) and these are then joined by a DNA ligase.

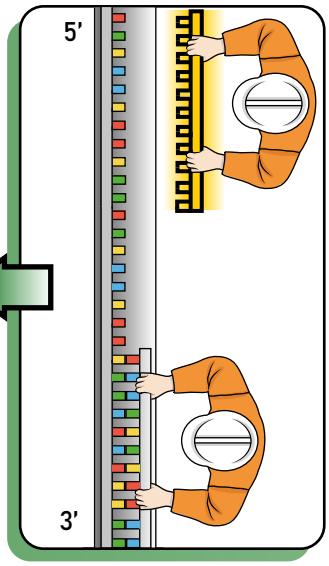


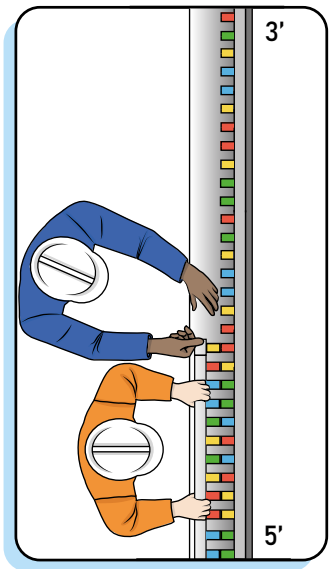


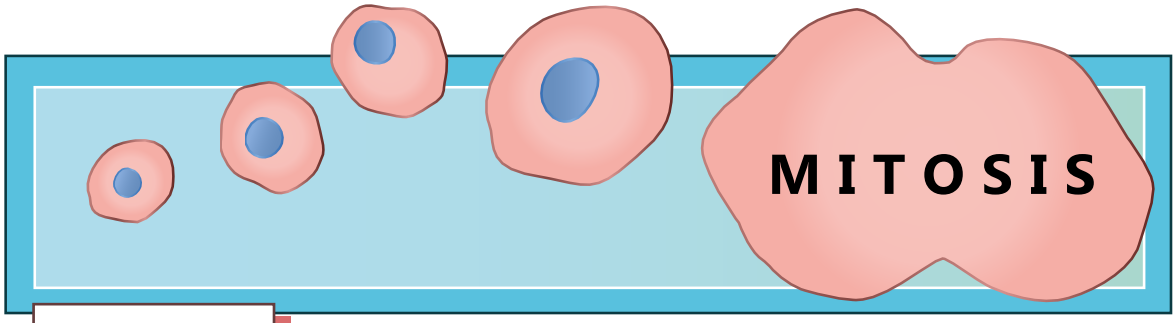










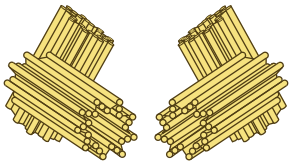


Interphase

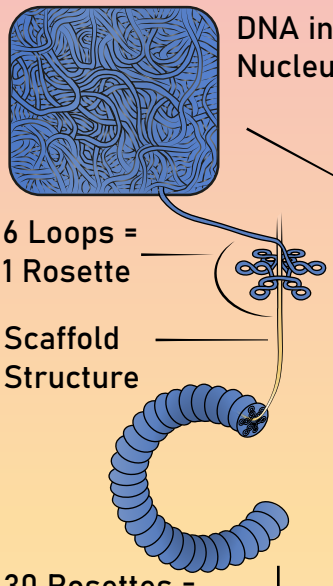
Mitosis is the process of somatic cell division. One parent cell becomes two almost genetically identical daughter cells.

In Interphase, the cell looks normal – the cell may be in G0, G1 or S phase in the cell cycle. DNA is replicated during S phase.

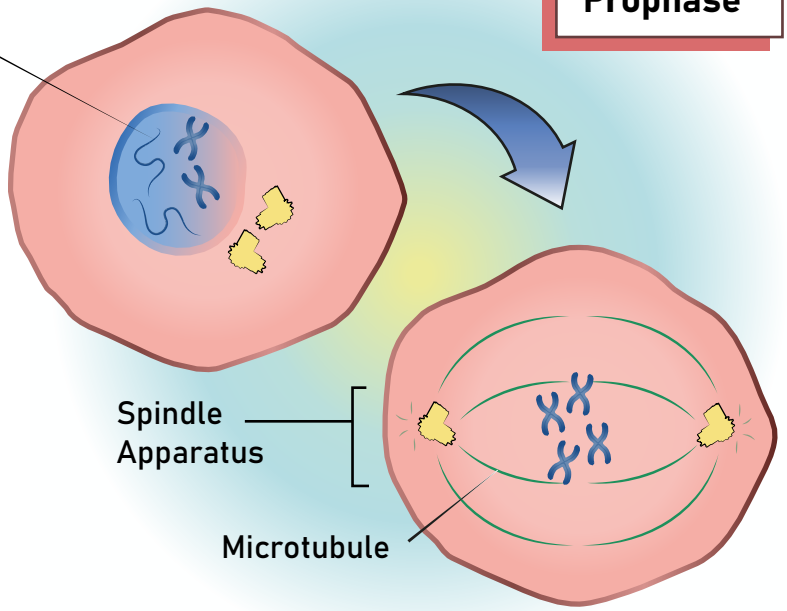
Replicated DNA



X2 Centrosomes



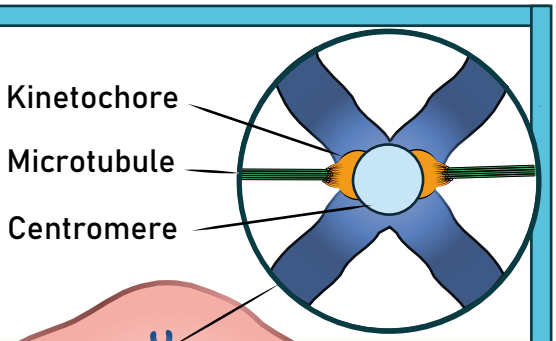
Stage 1 - Prophase

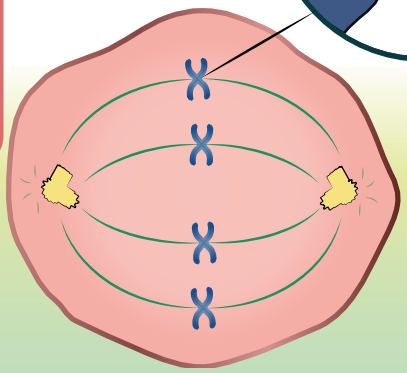


In Prophase the chromosomes condense and form part of the mitotic spindle.

Stage 2 - Metaphase

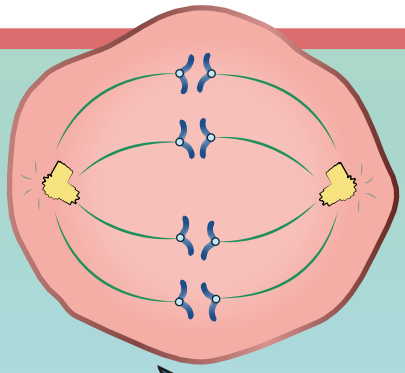
In Metaphase the chromosomes line up in the centre of the dividing cell. Microtubules are attached to each chromosome centromere.

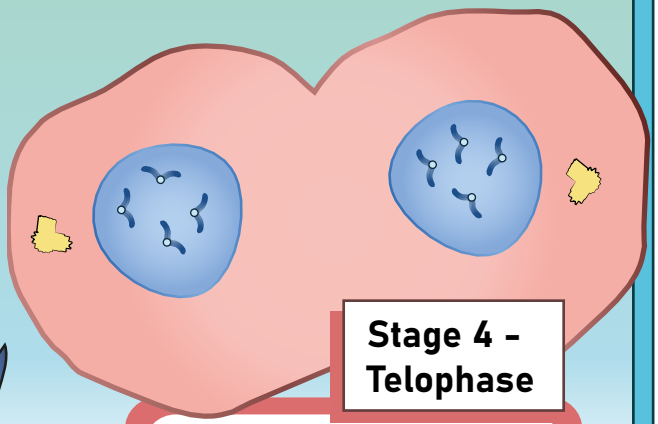




Stage 3 - Anaphase

At Anaphase, the duplicated chromosomes are separated by contraction of the microtubules.

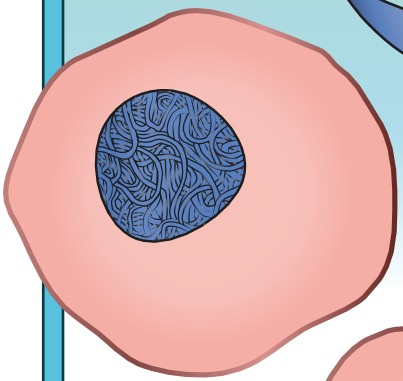


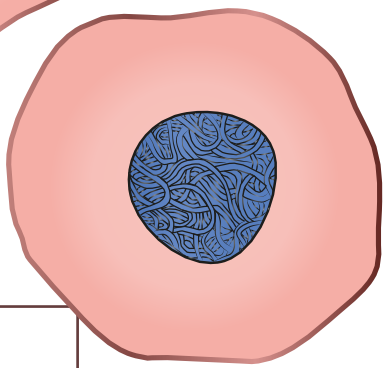


Stage 4 - Telophase

During Telophase, the chromosomes reach the poles of the cell, and the two cells separate (Cytokinesis).

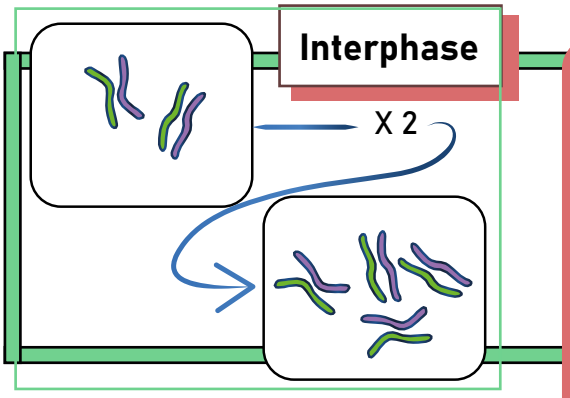
The chromosomes decondense and form part of the nucleus once more.





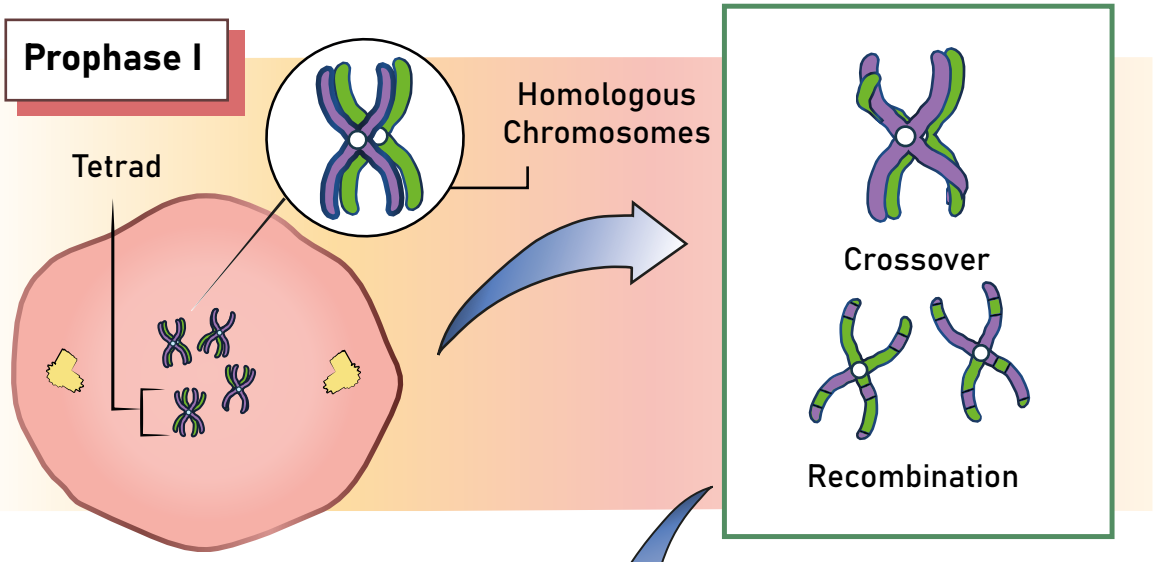
Cytokinesis (Daughter Cells)

MEIOSIS

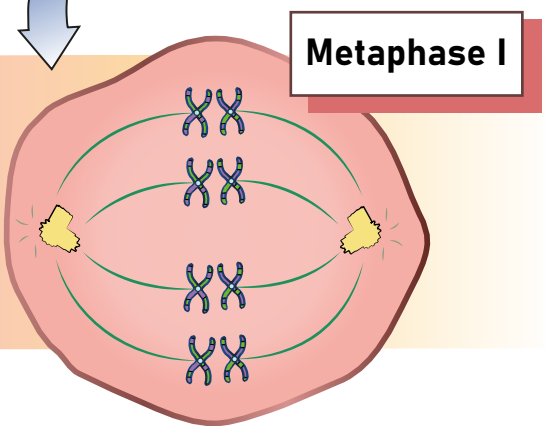


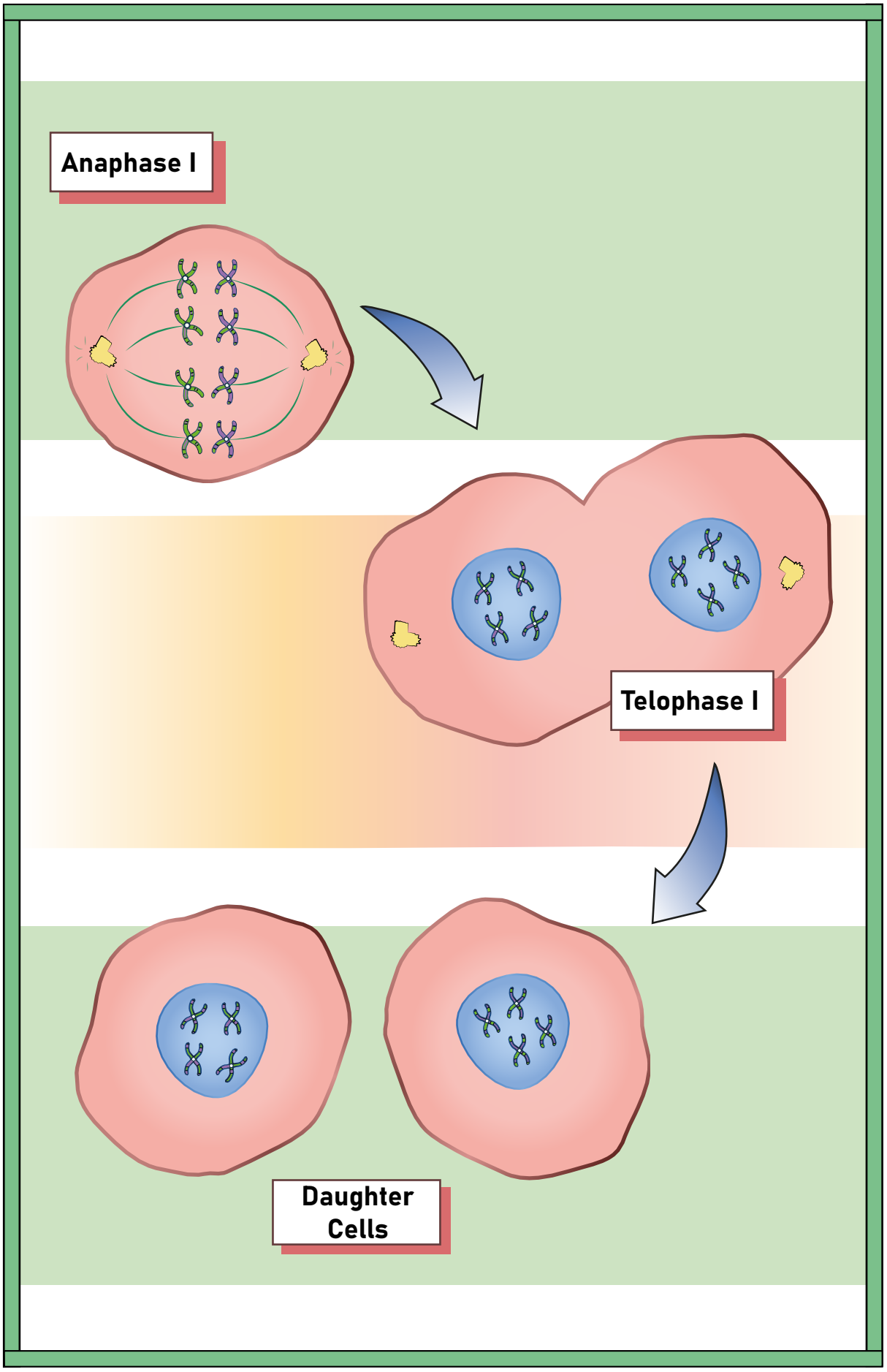
Meiosis in humans only occurs during gamete production. Key features are that the gametes are haploid and that recombination occurs between homologous chromosomes. This recombination ensures that genetic variants on the same chromosome segregate independently.

Meiosis I



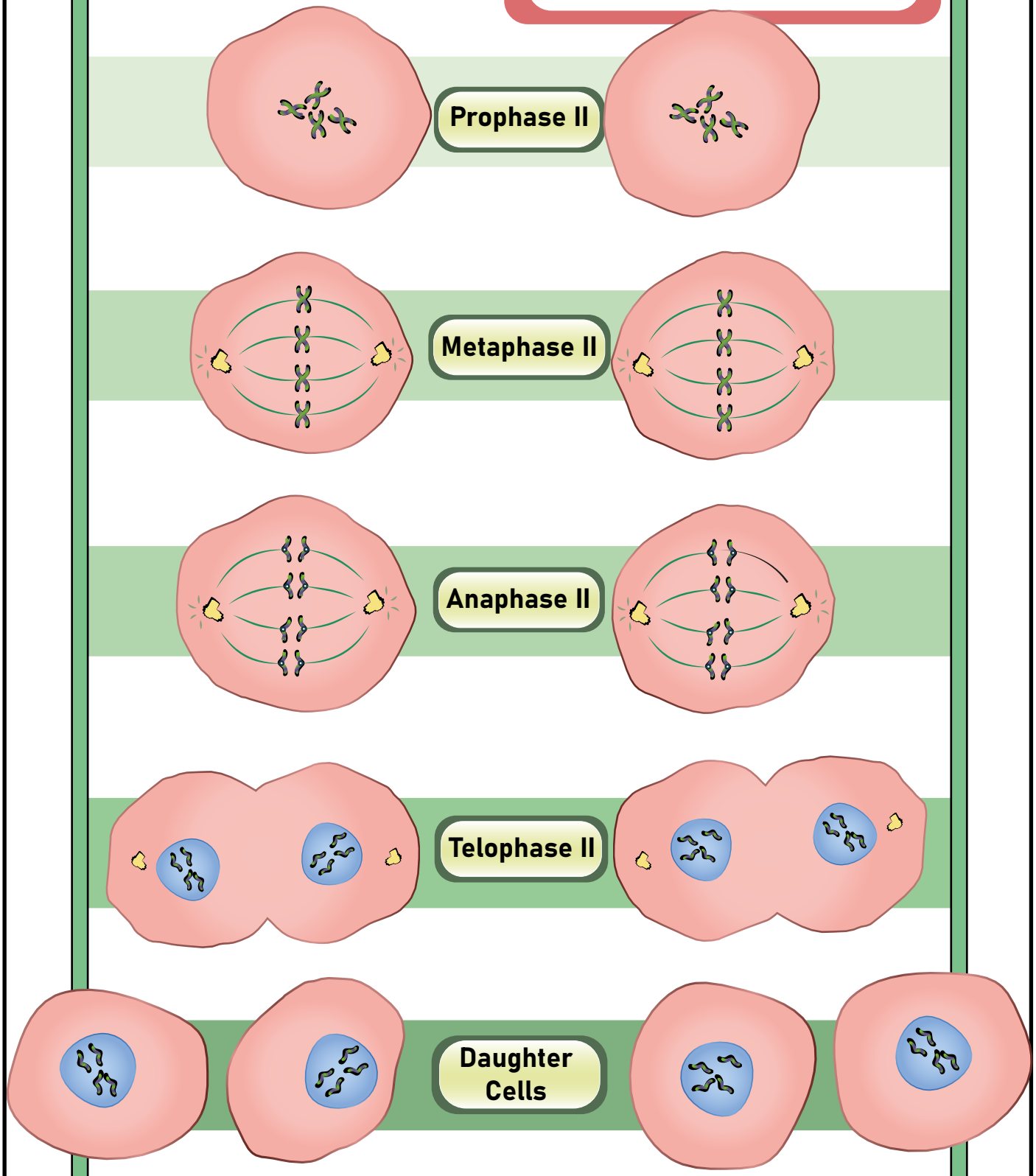
Meiosis occurs in 2 stages, Meiosis I and Meiosis II. During Prophase in Meiosis I, homologous chromosomes pair and there is crossing over between the homologous chromatids.



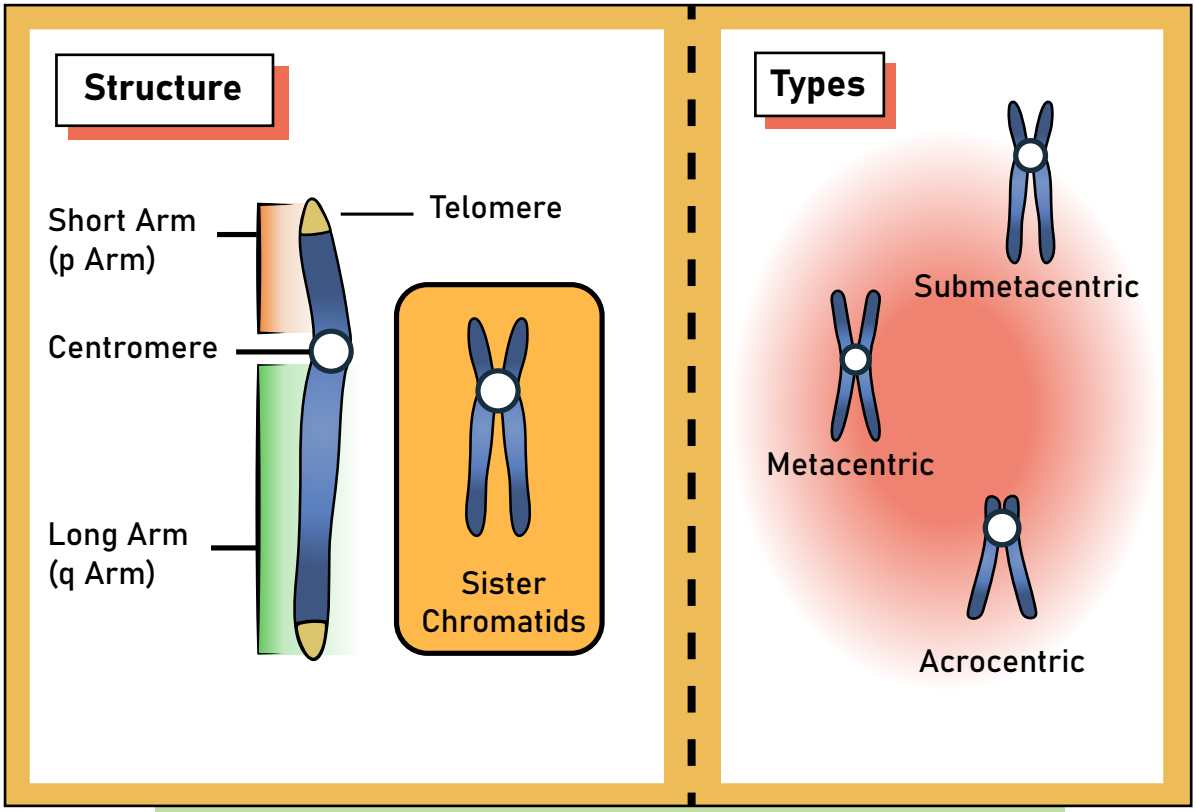

Anaphase I
Telophase I
Daughter Cells

Meiosis II

In Meiosis II, a second round of cell division leave 4 haploid daughter cells.



NORMAL CHROMOSOME STRUCTURE



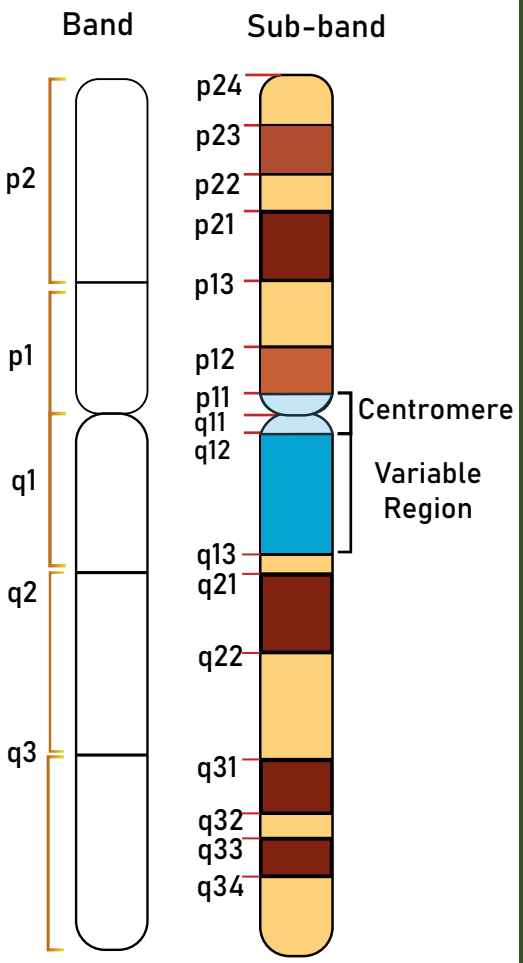
Chromosomes are recognised by their size, the position of the centromere and their banding pattern.

A metacentric chromosome has the telomere close to the middle, with a shorter “p” arm and longer “q” arm. An acrocentric chromosome has the centromere at one end, with only satellite DNA on the short “p” arm.

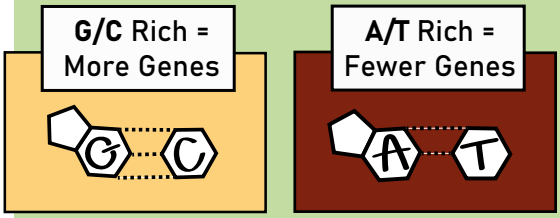
Banding

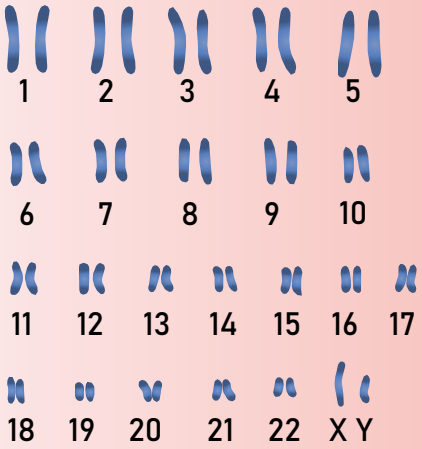
Chromosomes are visualised at metaphase in mitosis. Staining gives each chromosome a characteristic banding pattern, with dark bands showing gene poor regions and light bands showing gene rich areas.

Chromosome 9



Chromosome Staining

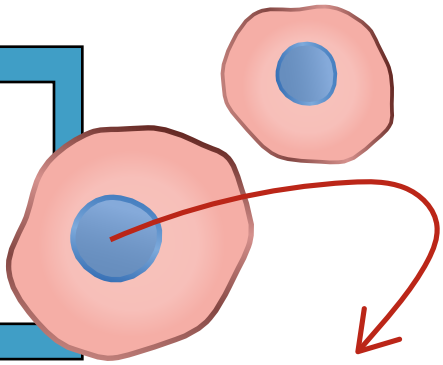




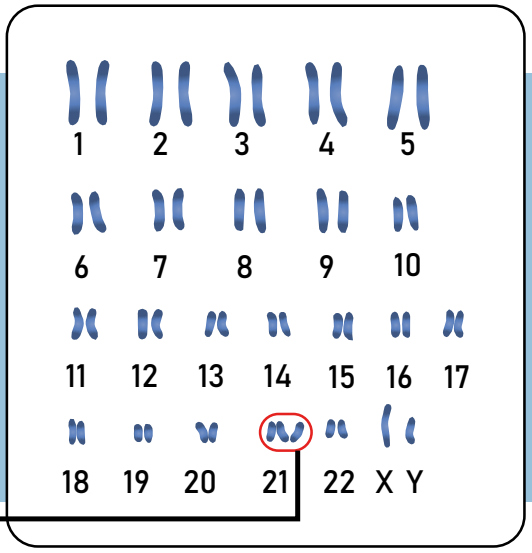
The normal chromosome complement is 22 pairs of chromosomes and 2 sex chromosomes, either 2 X chromosomes, or an X and a Y, written as 46,XX or 46,XY.

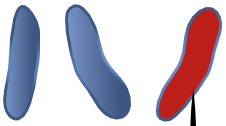
A chromosome complement is said to be balanced if there is the normal amount of each chromosome (whether the chromosomes are normal or there is a rearrangement).

UNBALANCED CHROMOSOMES



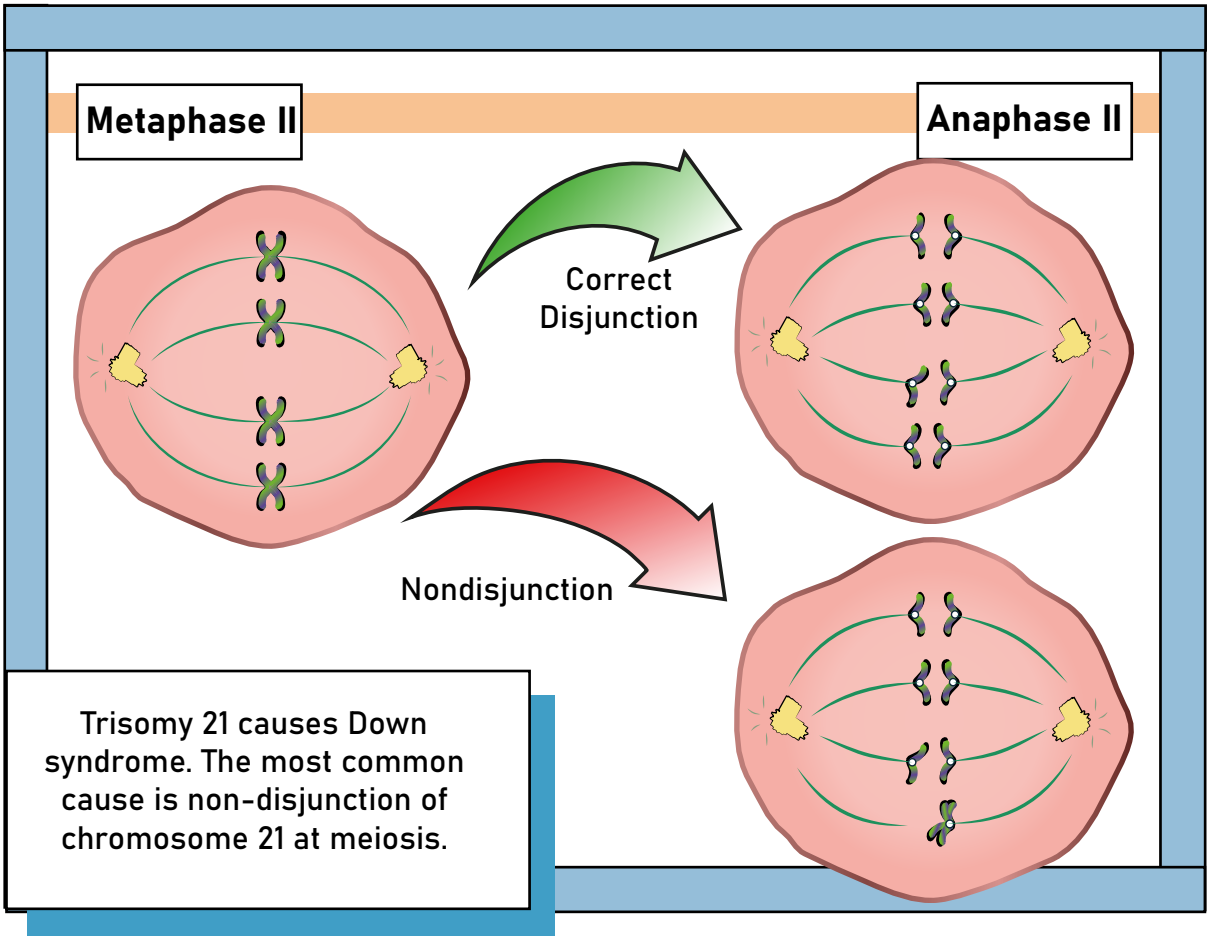
A chromosome complement is said to be unbalanced if there is extra or missing chromosomal material. In this case there is an extra chromosome 21. Written as 47 XY +21. (47 chromosomes, Male and the extra chromosome is a chromosome 21).





Karyotype 47,XY,+21 - Down Syndrome

Extra Chromosome

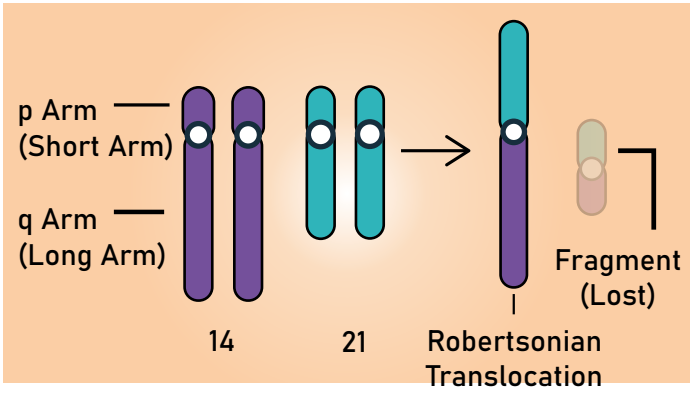


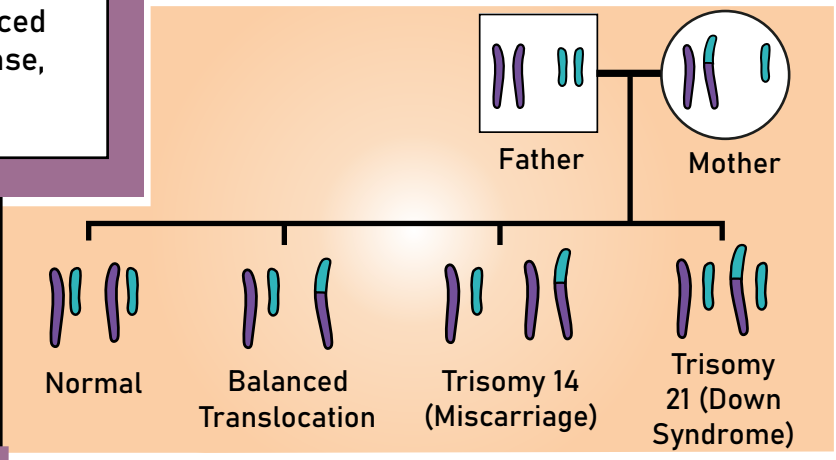
Trisomy 21 causes Down syndrome. The most common cause is non-disjunction of chromosome 21 at meiosis.

TRANSLOCATIONS

Robertsonian Translocation

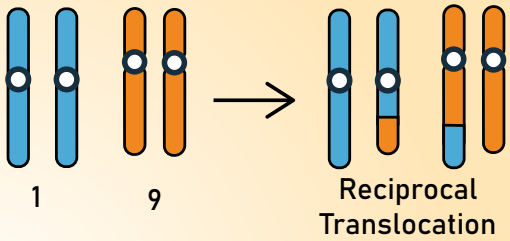
A Robertsonian Translocation is where two acrocentric chromosomes become joined end to end. The short "p" arms are lost, but do not contain significant genes in acrocentric chromosomes. If a parent has a balanced Robertsonian translocation there is an increased risk of a child inheriting unbalanced chromosomes – in this case, trisomy 21.

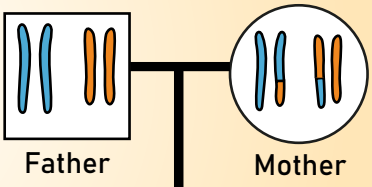


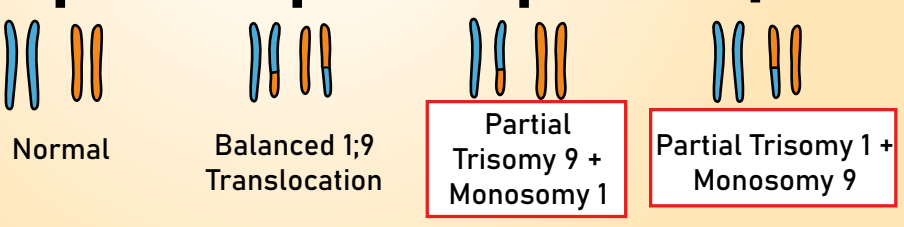


Reciprocal Translocation

In a reciprocal translocation, there has been a swap of genetic material between chromosome arms. A parent with a balanced reciprocal translocation is at high risk of having children with unbalanced chromosomes. This can cause a miscarriage (if the size of imbalance is bigger) or a child with a significant developmental disorder (smaller size of imbalance).

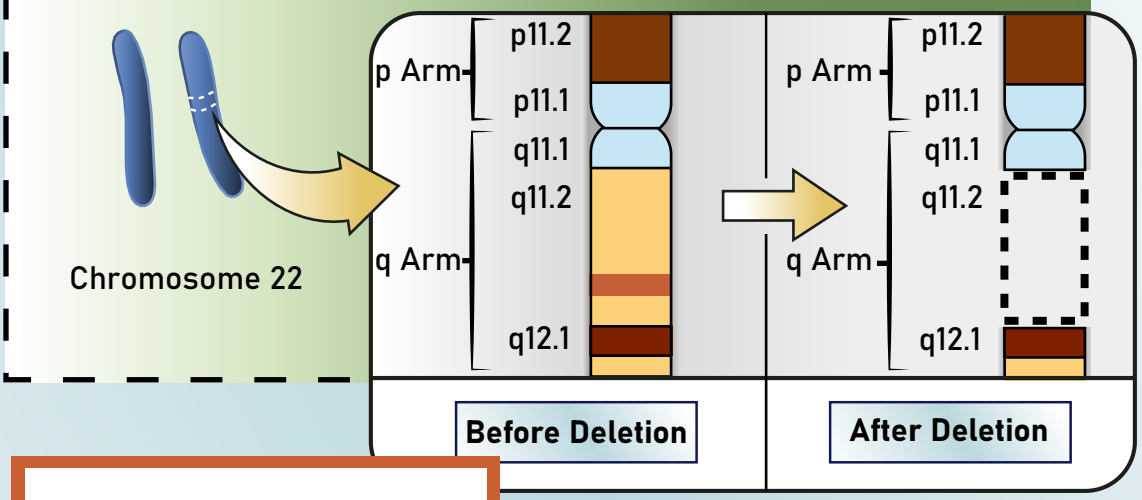


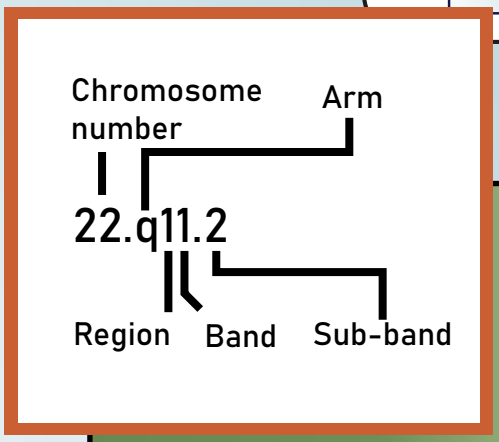




CHROMOSOME DELETION

DiGeorge Syndrome (22.q11.2 Deletion)

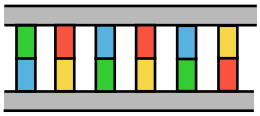




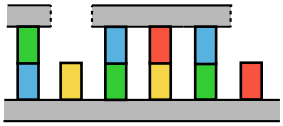
There are a number of chromosome changes that can cause disease. Deletions or insertions of genetic material can occur. With analysis by microscopy, any change smaller than 5 million base pairs is unlikely to be visible.

FISH | FLOURESCENCE IN SITU HYBRIDISATION

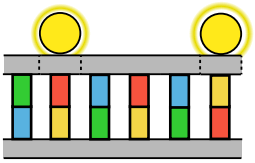
1. Probe DNA



Complementary DNA/RNA probe



Nicks are created in the DNA

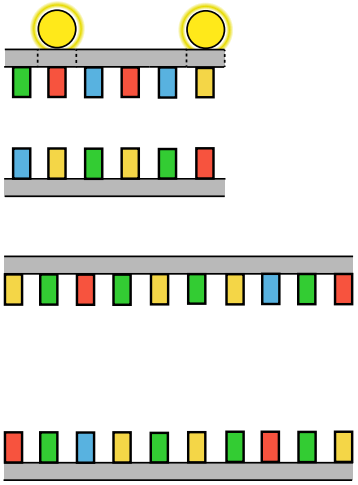


Nucleotides with a Fluorophore attached are incorporated into the strand

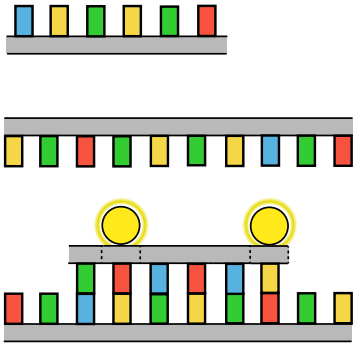
2. Denaturation

Complementary DNA

Chromosomal Target DNA

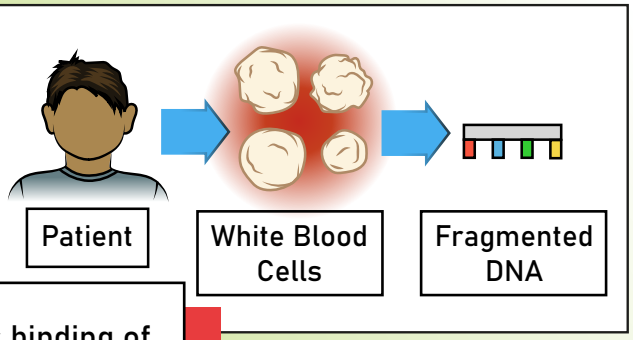


3. Hybridisation

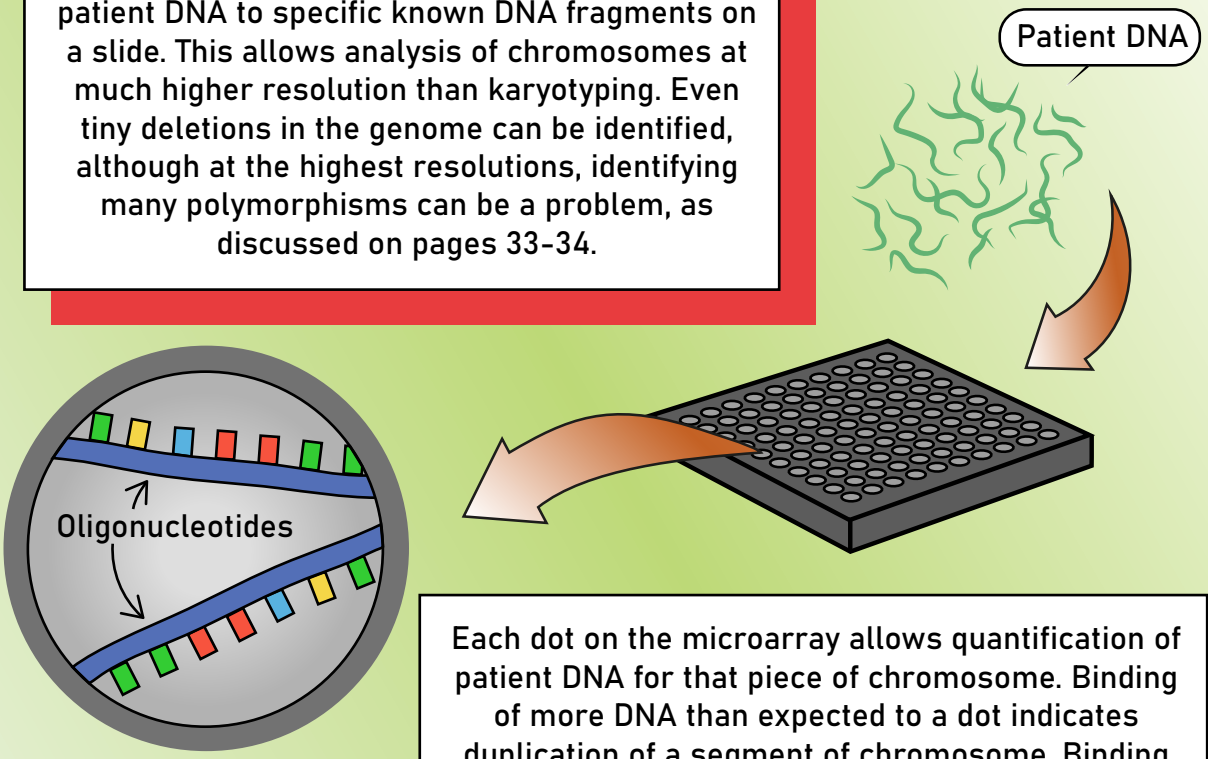


Fluorescence in-situ hybridisation is a technique that allows you to look for the presence of a specific chromosomal region. The region is highlighted by hybridisation of a region-specific probe. This technique is now mainly used to detect specific chromosomal abnormalities in cancer.

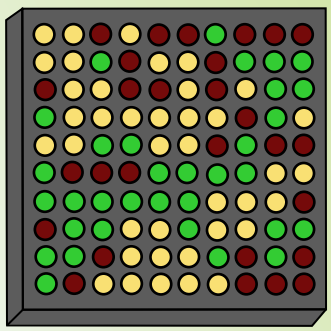
CHROMOSOME MICROARRAY



Chromosome Microarray (CMA) uses binding of patient DNA to specific known DNA fragments on a slide. This allows analysis of chromosomes at much higher resolution than karyotyping. Even tiny deletions in the genome can be identified, although at the highest resolutions, identifying many polymorphisms can be a problem, as discussed on pages 33-34.



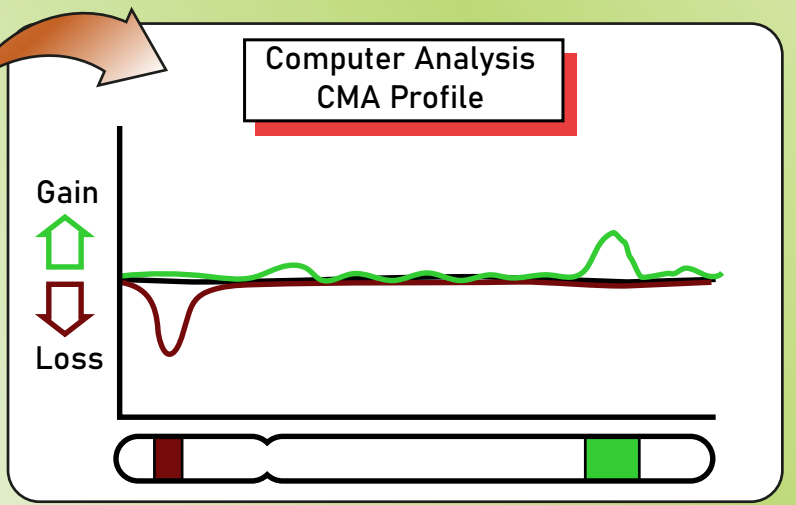
Each dot on the microarray allows quantification of patient DNA for that piece of chromosome. Binding of more DNA than expected to a dot indicates duplication of a segment of chromosome. Binding of less DNA indicates a deletion. Chromosome Microarray can usually only detect unbalanced chromosomes.

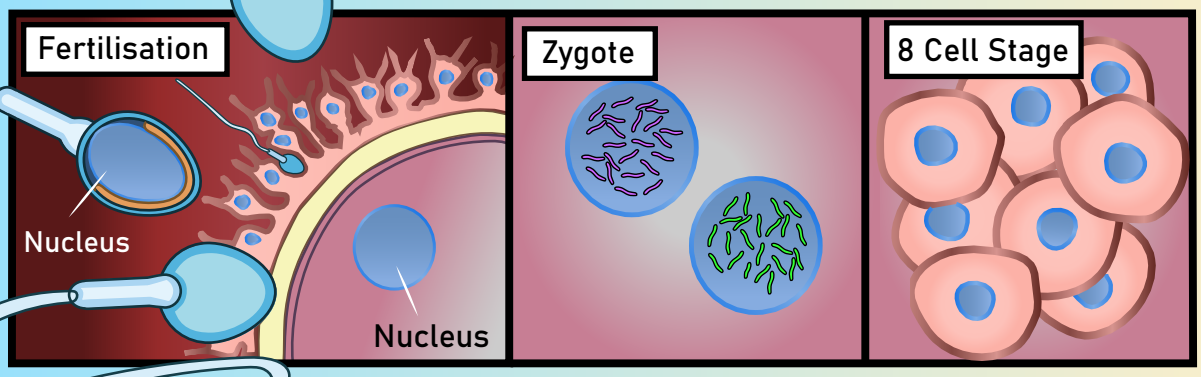


Strong Patient DNA Binding - Duplication

Normal Quantity of DNA

Weak Patient DNA Binding - Deletion



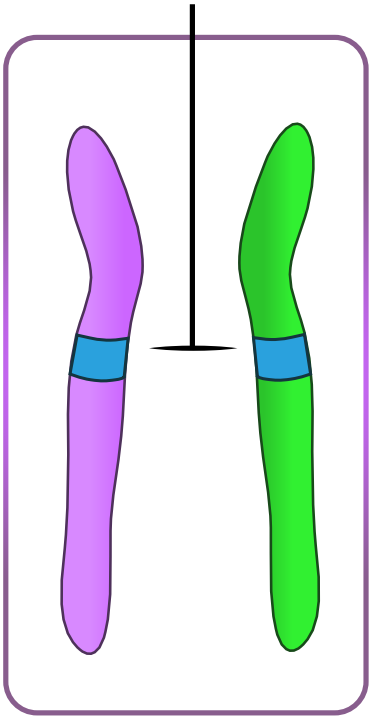


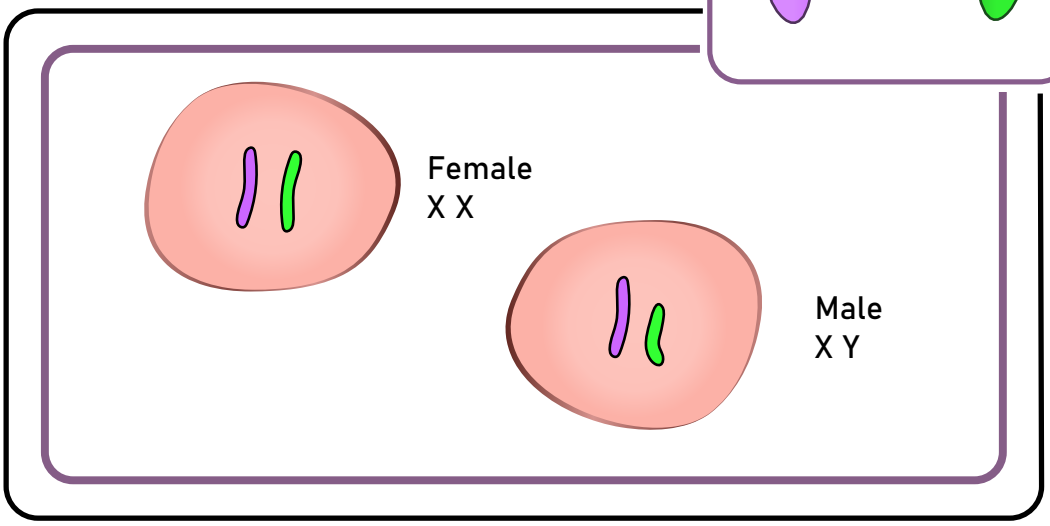
X-INACTIVATION

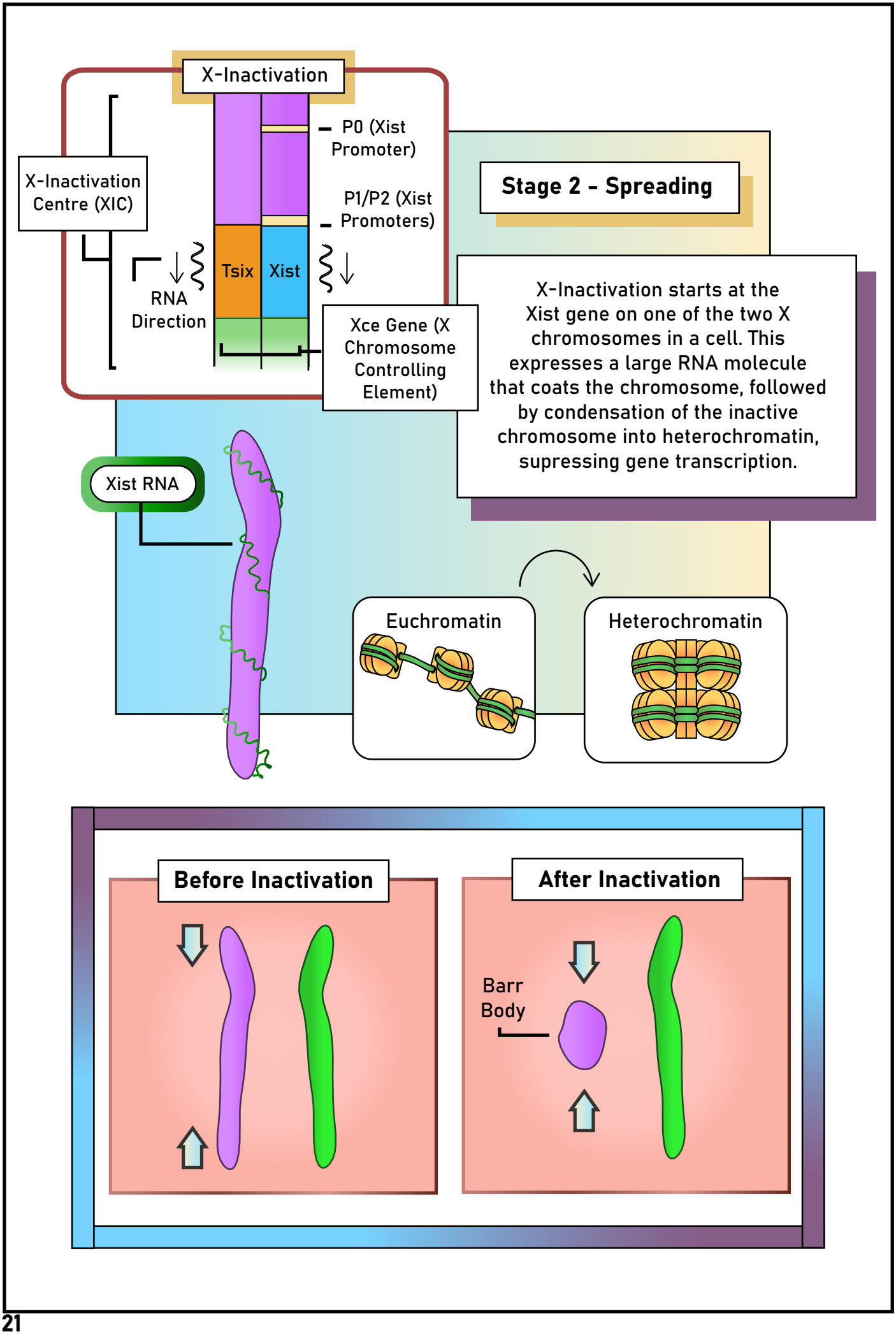
Step 1 - Regulation

Females have 2 X-chromosomes, but men only have 1. In women, one X-chromosome has to inactivate. This happens in early embryonic development, and usually one X chromosome in each nucleus is inactivated at random, the other remaining active.

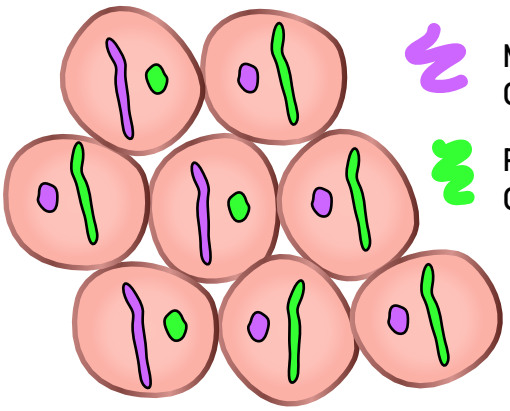
X-Inactivation Centre
(Located in Xq13 Band)






X-Inactivation
X-Inactivation Centre (XIC)
P0 (Xist Promoter)
P1/P2 (Xist Promoters)
Tsix
Xist
RNA Direction
Xce Gene (X Chromosome Controlling Element)
Stage 2 - Spreading
X-Inactivation starts at the Xist gene on one of the two X chromosomes in a cell. This expresses a large RNA molecule that coats the chromosome, followed by condensation of the inactive chromosome into heterochromatin, supressing gene transcription.
Xist RNA
Euchromatin
Heterochromatin
Before Inactivation
After Inactivation
Barr Body

Random X Chromosome Inactivation



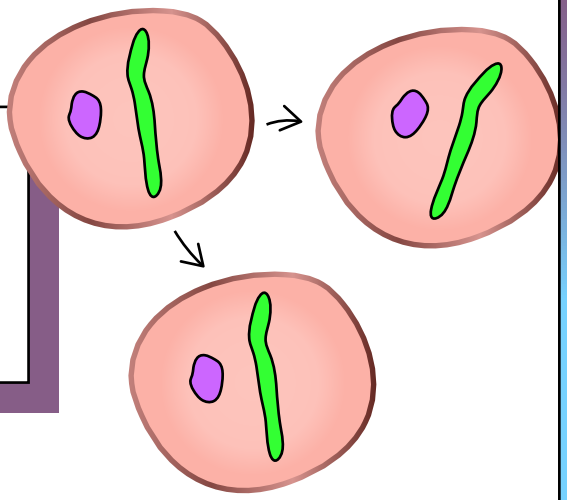
Maternal Chromosome

Paternal Chromosome

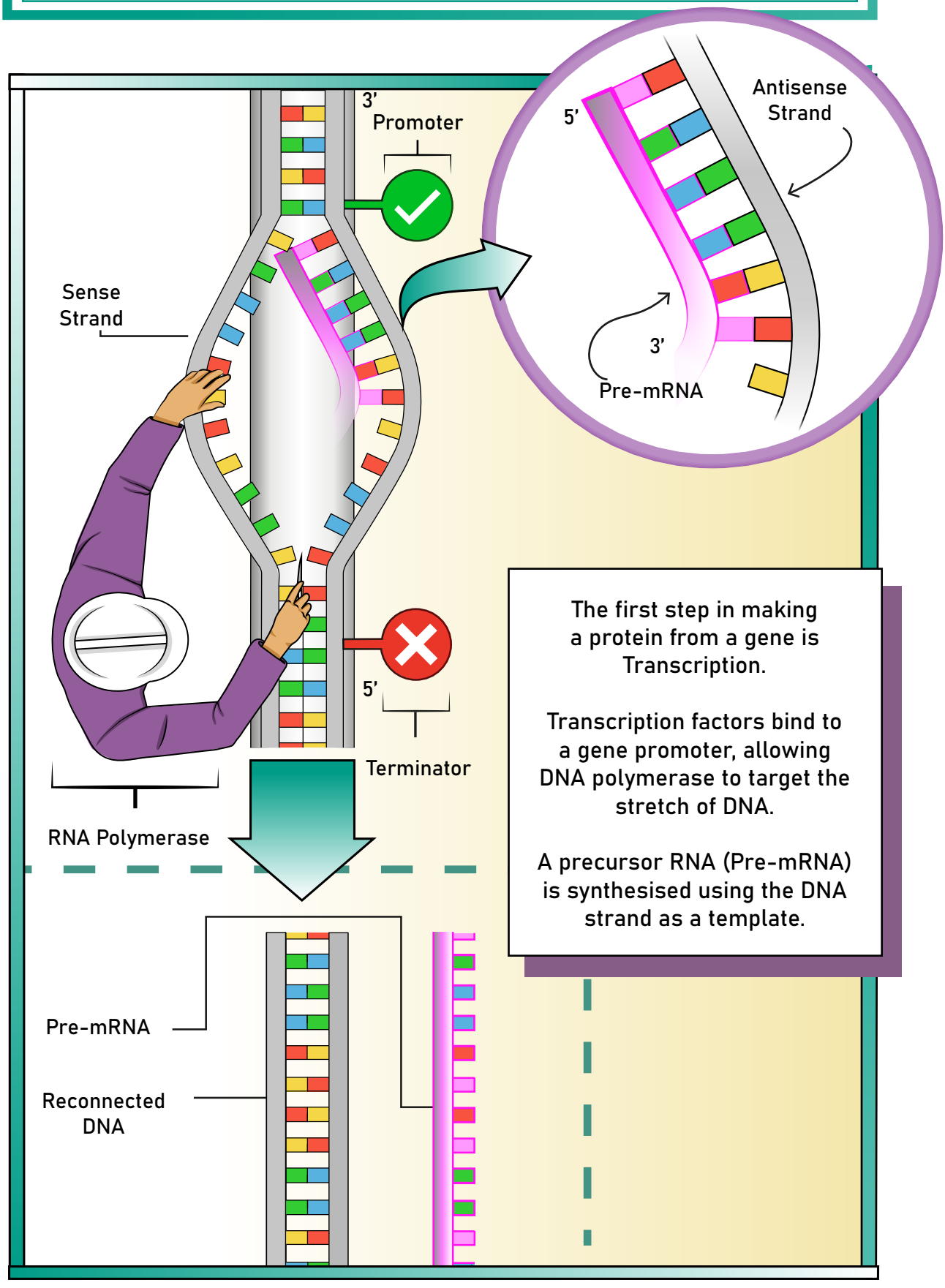
On average, in females, 50% of the nuclei will have the maternally derived X chromosome active and the other 50% have the paternally derived X chromosome active.

Stage 3 - Maintenance

The X-Inactivation pattern remains constant throughout the life of the cell, and is maintained during cell division. It is only removed in germ cell formation.



DNA TRANSCRIPTION



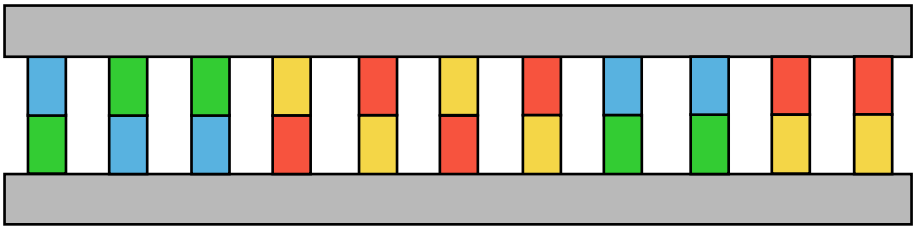
The first step in making a protein from a gene is Transcription.

Transcription factors bind to a gene promoter, allowing DNA polymerase to target the stretch of DNA.

A precursor RNA (Pre-mRNA) is synthesised using the DNA strand as a template.

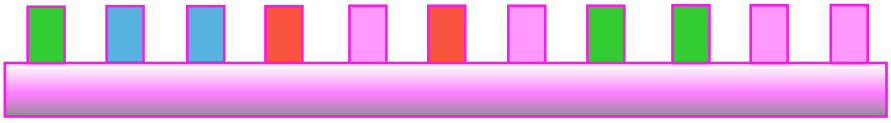
CENTRAL DOGMA

DNA



Transcription

Pre-mRNA



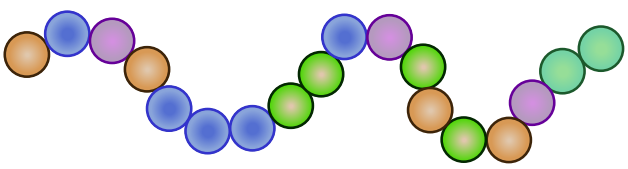
Removal of Introns

Splicing

mRNA

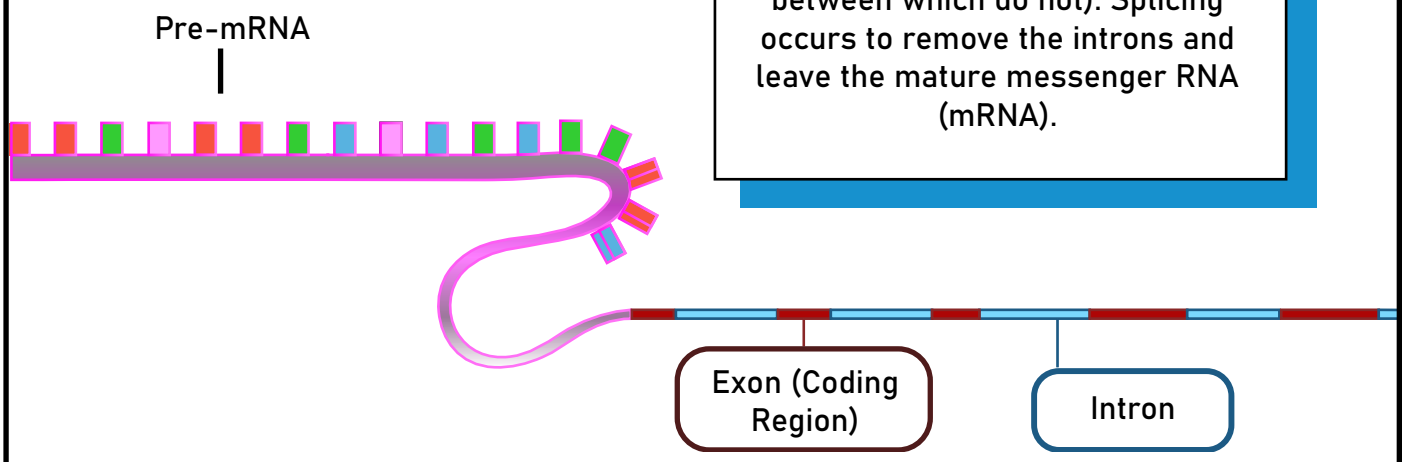
Translation

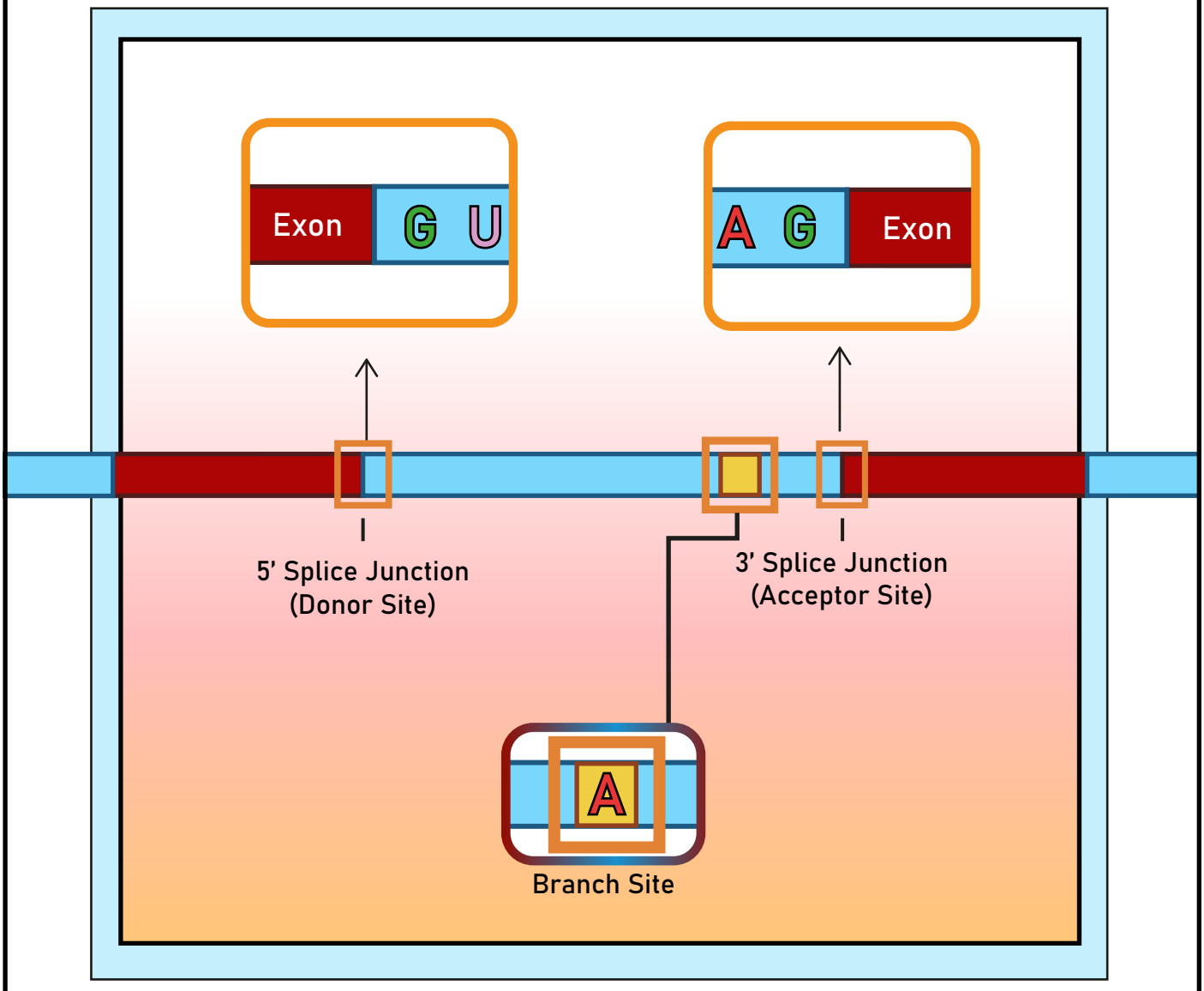
Protein



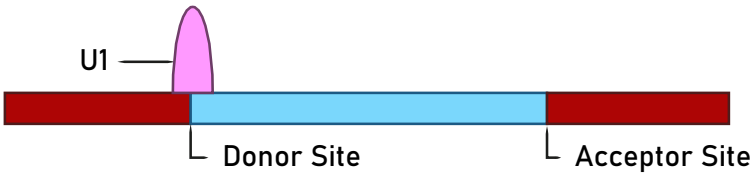
SPLICING

The precursor RNA will include sequences from the exons of the gene (which encode protein) and the introns (the sequences between which do not). Splicing occurs to remove the introns and leave the mature messenger RNA (mRNA).

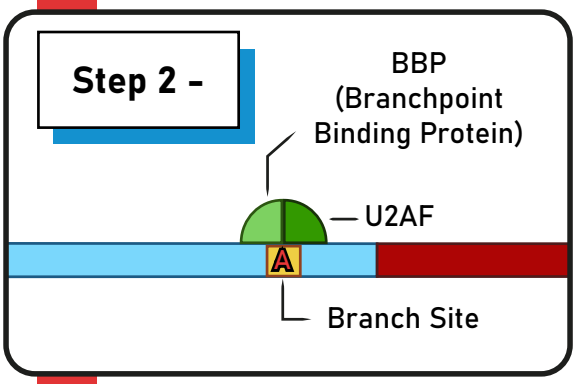




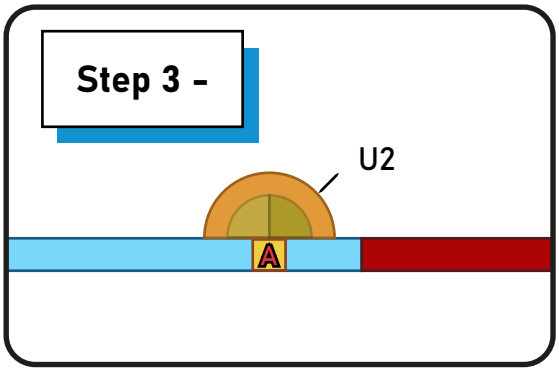
Step 1 -



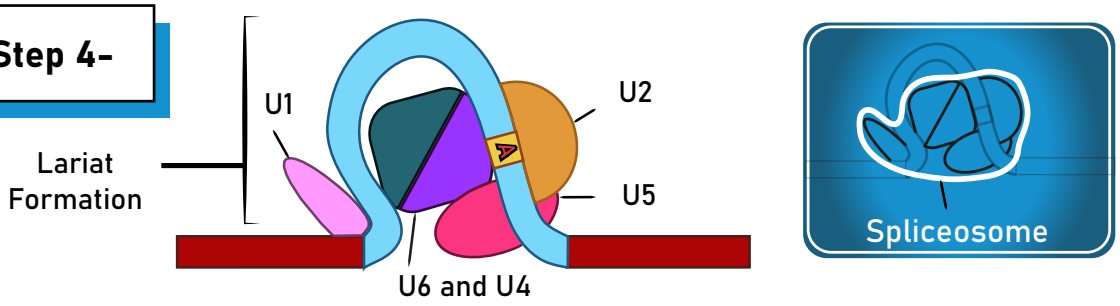
Step 2 -



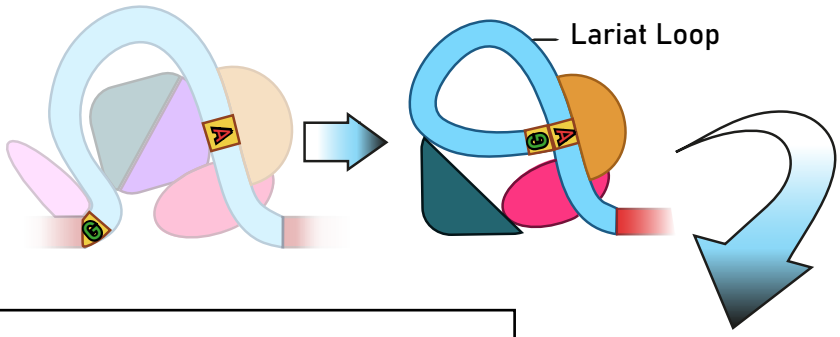
Step 3 -



Step 4-



Step 5 -



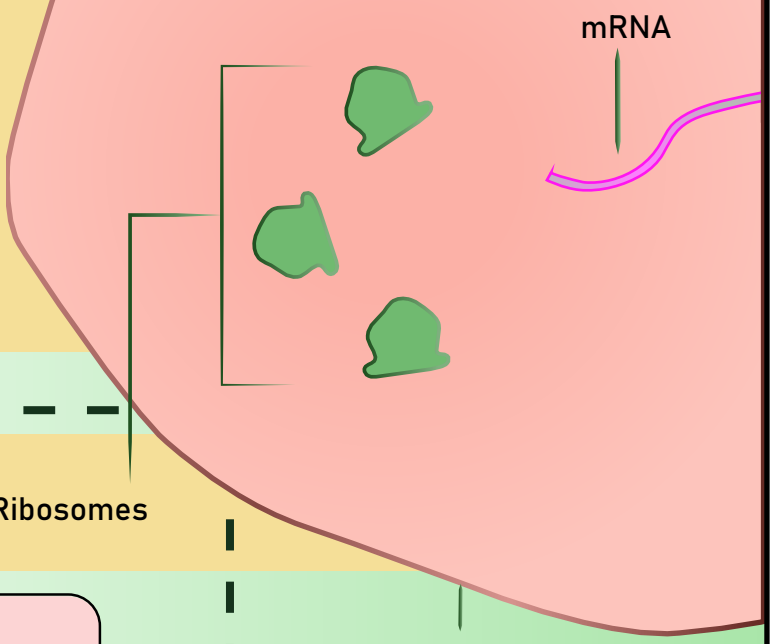
Splicing occurs in the nucleus. Small Nuclear Ribonucleoproteins (SNRPs) recognise specific RNA sequences (called Motifs), including Splice Acceptor, Splice Donor and Lariat sequences, forming a spliceosome. The spliceosome contains small nuclear RNAs (U1 – U6). The intron is removed and only exon sequence remains in the mature mRNA.

Mature mRNA

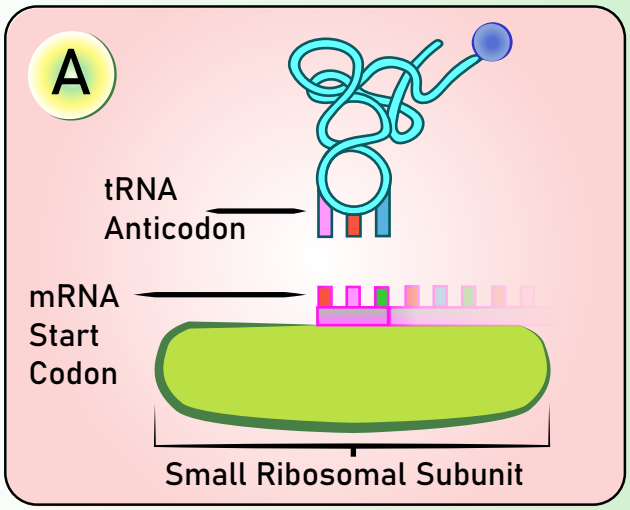
TRANSLATION

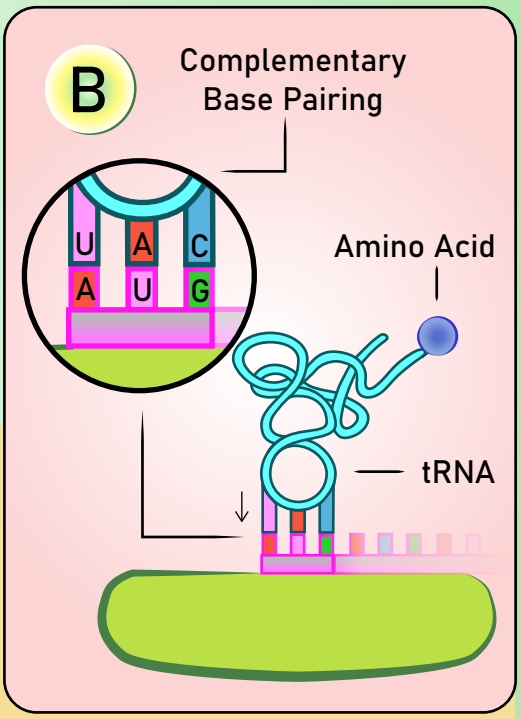
Step 1 -

Translation occurs at the ribosome. This ribosome has 2 subunits which are made of a combination of RNA and protein.



Step 2 -

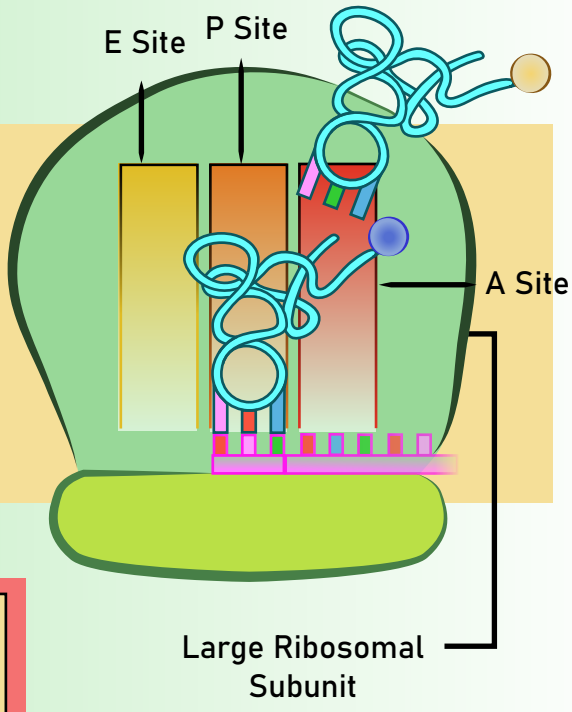


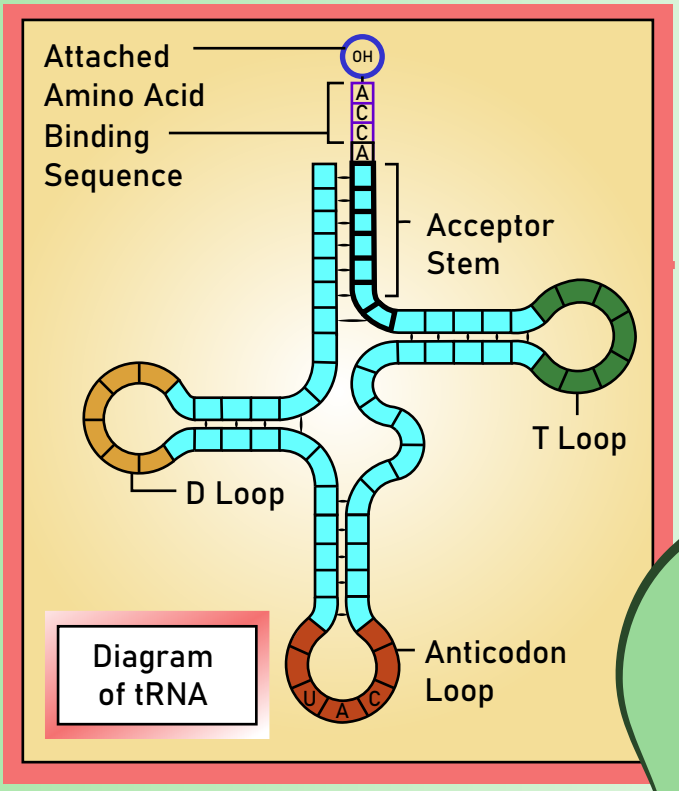


Transfer RNAs (tRNAs) carry an amino acid, and bind to the mRNA. For most 3 base sequences in the mRNA (a codon) there is a tRNA with a matching anti-codon, that attaches to a specific amino acid. The 3 base codon, therefore, specifies which amino acid should be included next in the peptide chain.

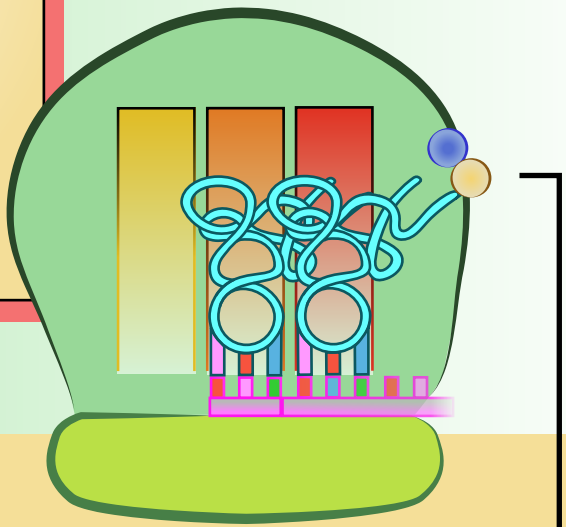
Step 3 - Translation Elongation

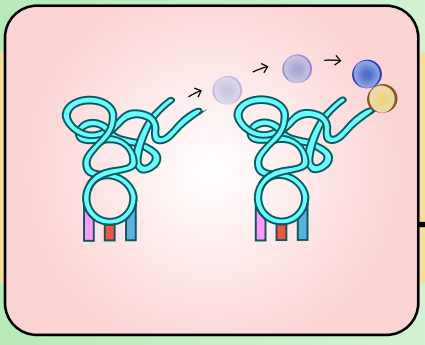
The mRNA moves along the ribosome with a new amino acid being included in the peptide chain for each 3 base codon.



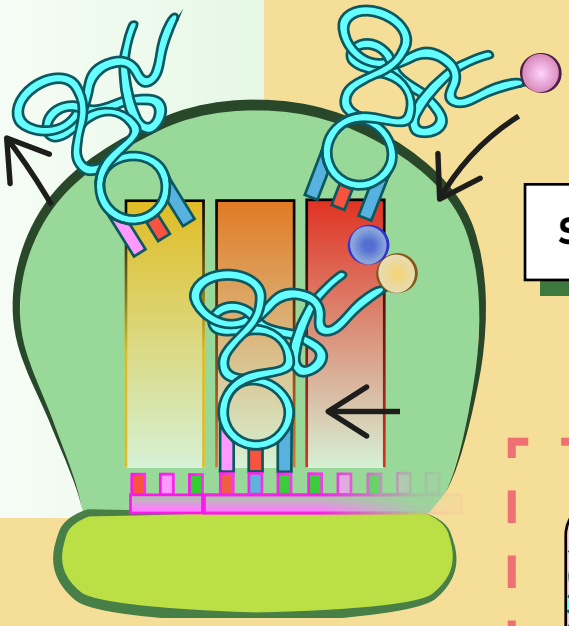


Step 4 -

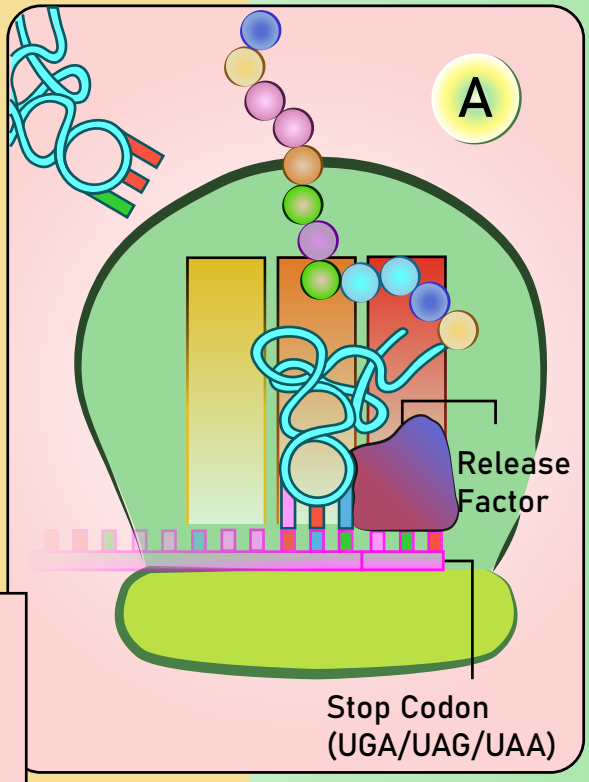




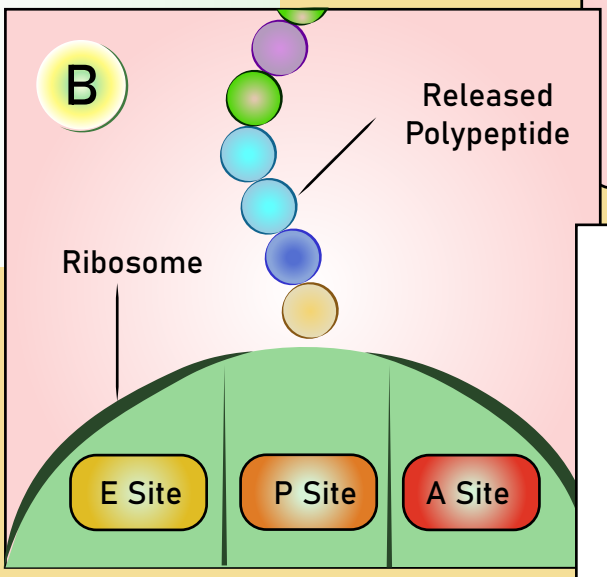
Amino Acid Bond



Step 5 -



Step 6 - Translation Termination

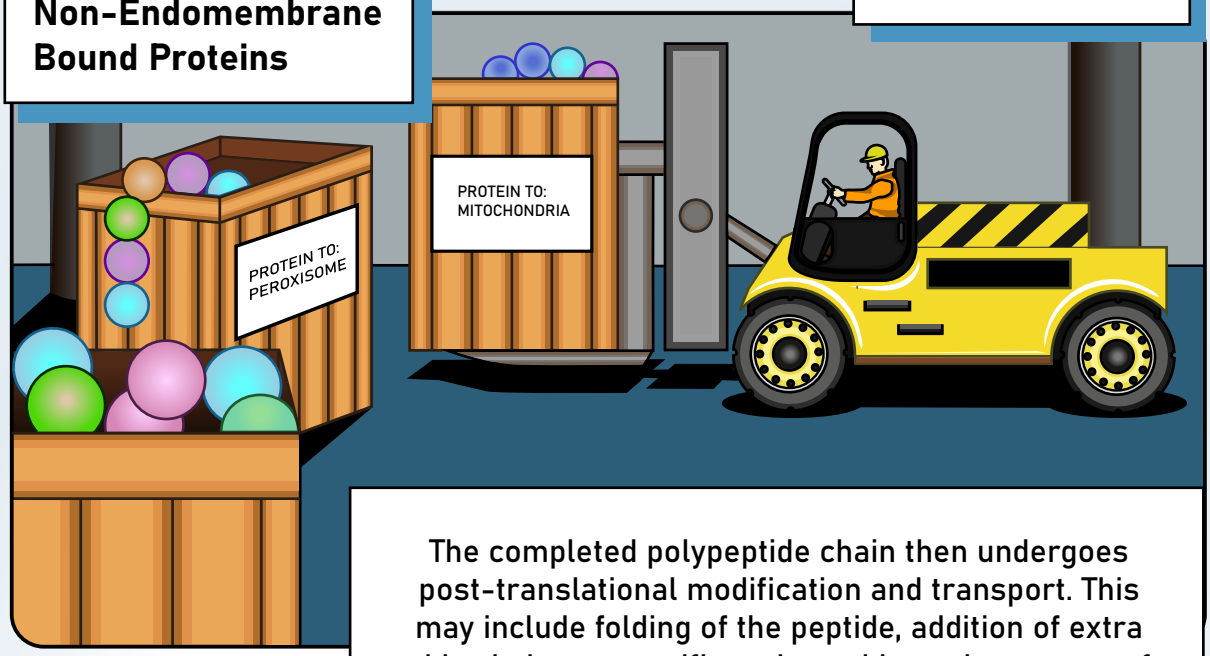


There are 3 codons that do not encode an amino acid: UGA, UAG and UAU. When one of these 'Stop' codons is reached, a release factor binds to the ribosome and causes the peptide to be released for further processing.

POST-TRANSLATIONAL MODIFICATION

Protein Targeting

Non-Endomembrane Bound Proteins

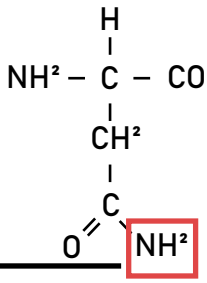


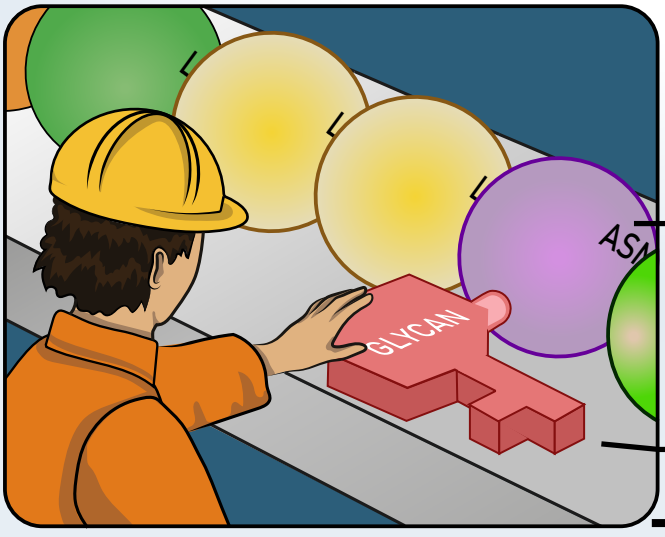
The completed polypeptide chain then undergoes post-translational modification and transport. This may include folding of the peptide, addition of extra side chains to specific amino acids, and transport of the peptide to its specific subcellular location. It may also associate with other peptides.

N-Linked Glycosylation

Glycosidic Bond at Nitrogen of Asparagine

Asparagine





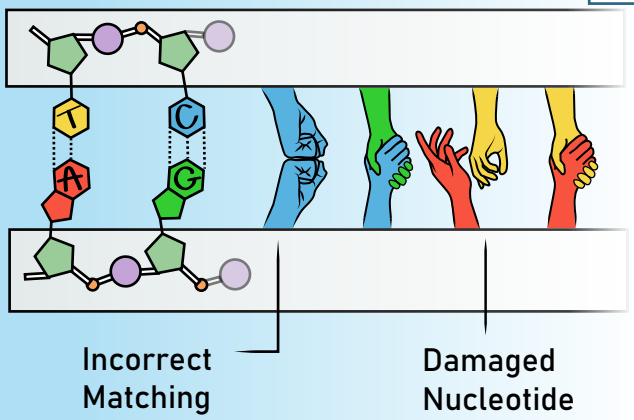
Asparagine

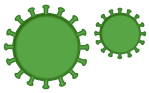
Amino Acid Chain

Glycan/ Polysaccharide

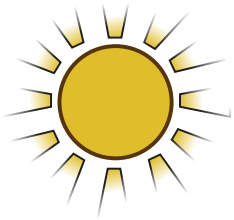
DNA REPAIR

Endogenous Damage




Viruses

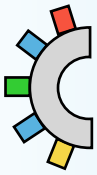

Toxins

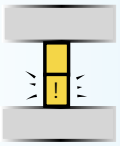

UV Exposure

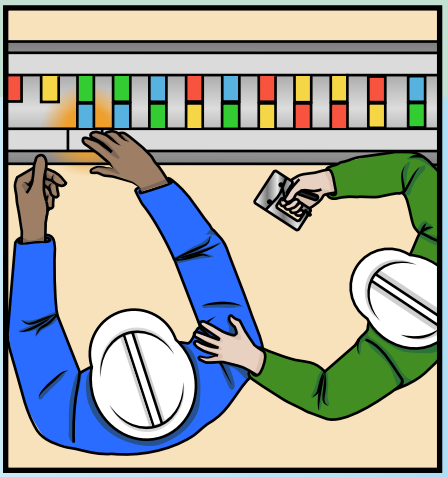
Exogenous Damage

Single Strand Repair

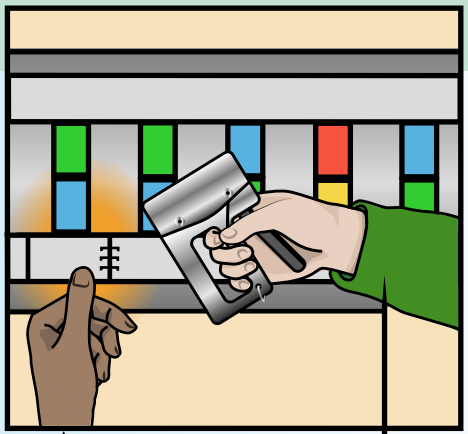

Base Excision Repair


Nucleotide Excision Repair

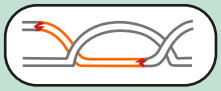

Mismatch Repair



DNA can be damaged in a number of ways, including chemical crosslinks, single and double stranded breaks and incorporation of mismatched bases. There are a number of specific repair pathways for this damage, including Base Excision Repair, Nucleotide Excision Repair and Mismatch Repair.

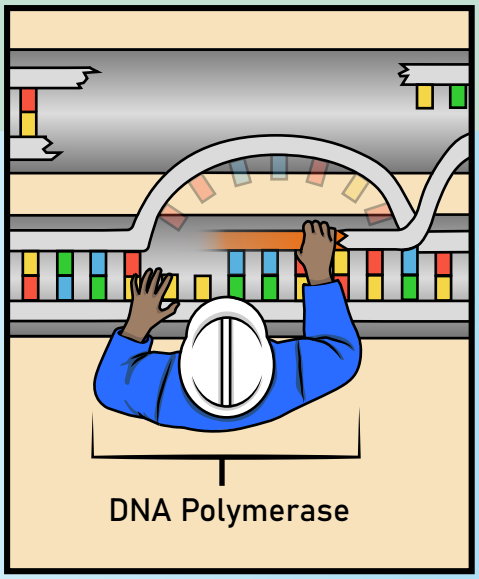


Double Strand Damage

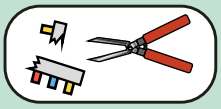


Homologous Recombination Repair (HRR)

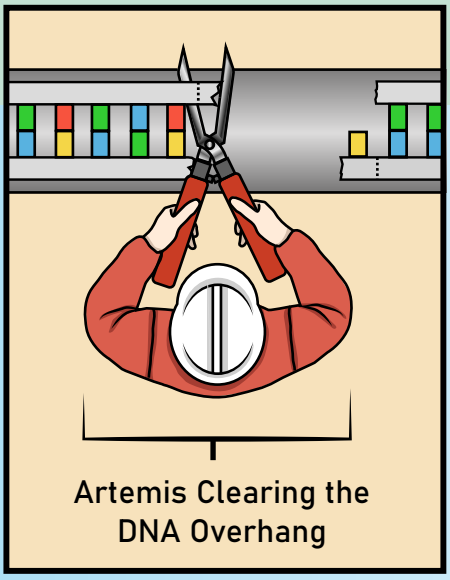
Homologous Recombination repair repairs double stranded breaks in DNA using the other allele as a template. Non Homologous End Joining (NHEJ) joins the broken strands directly, and risks the joining together of incorrect DNA strands.



DNA Polymerase



Non-Homologous End Joining (NHEJ)



Artemis Clearing the DNA Overhang

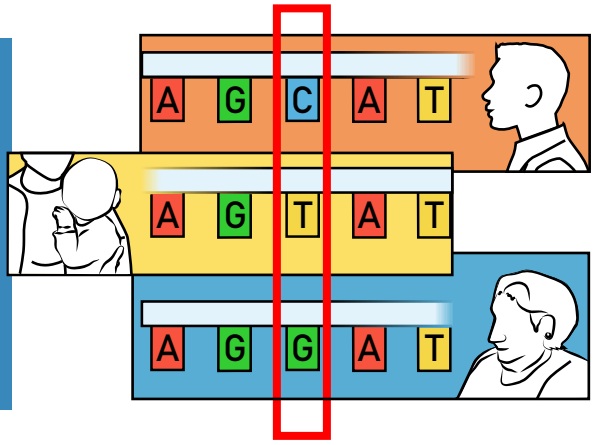
A number of human diseases can be caused by mutations in genes involved in DNA repair. These include extreme sensitivity to UV light, Xeroderma Pigmentosa caused by loss of nucleotide excision repair. Mutations in the *BRCA1* gene involved in homologous recombination repair causes a high risk of breast cancer.

POLYMORPHISMS

Polymorphisms can be of different sizes. The smallest are single changes in base sequence (Single Nucleotide Polymorphisms). Deletions and duplications of DNA, from single bases to large genomic segments over a million bases in size (described as Copy Number Variant – CNVs or Structural Variation – SV) can also be polymorphisms.

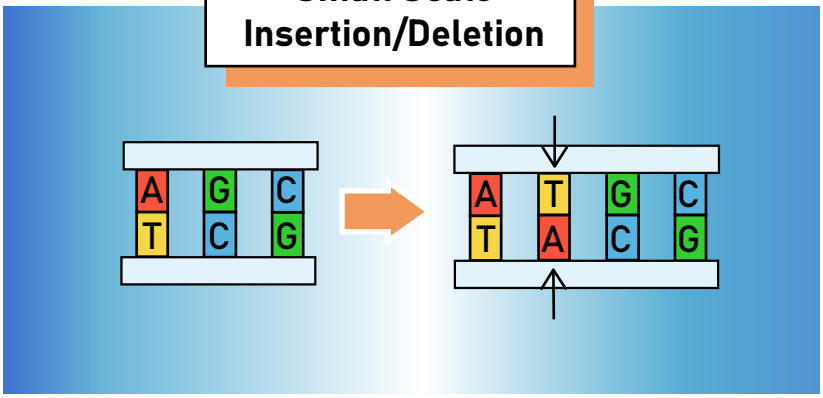
Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs) are scattered throughout the genome. They can be found in coding and non-coding genome sequence. Any person will have over 3,000,000 SNPs, variations from the reference human genome sequence.



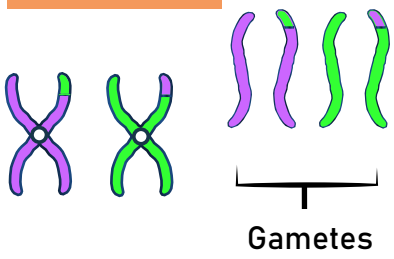
Some SNPs lying in exons can affect peptide sequence, although many do not. Other SNPs are found in regulatory sequences near genes, and may affect gene regulation. Most SNPs have no effect.

Small Scale Insertion/Deletion

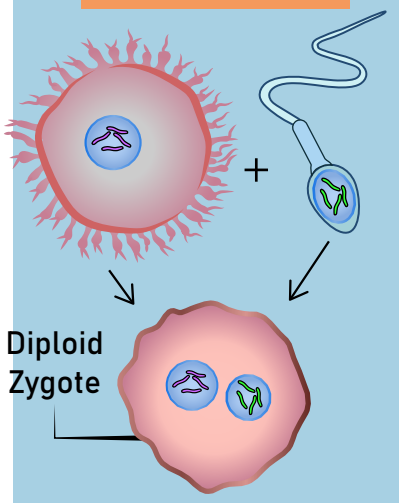


The idea of a “normal” genome sequence does not make sense, as every human will have a different genome sequence. A reference sequence, defined by the Genome Reference Consortium (GRC) is used to allow effective description of the genetic variation found in any one individual.

Crossing Over

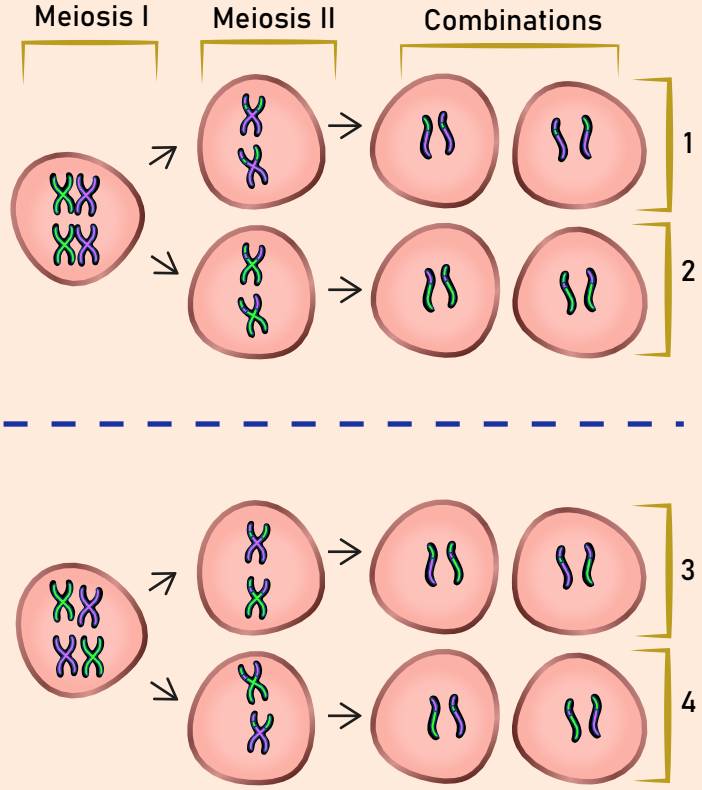


Fertilisation



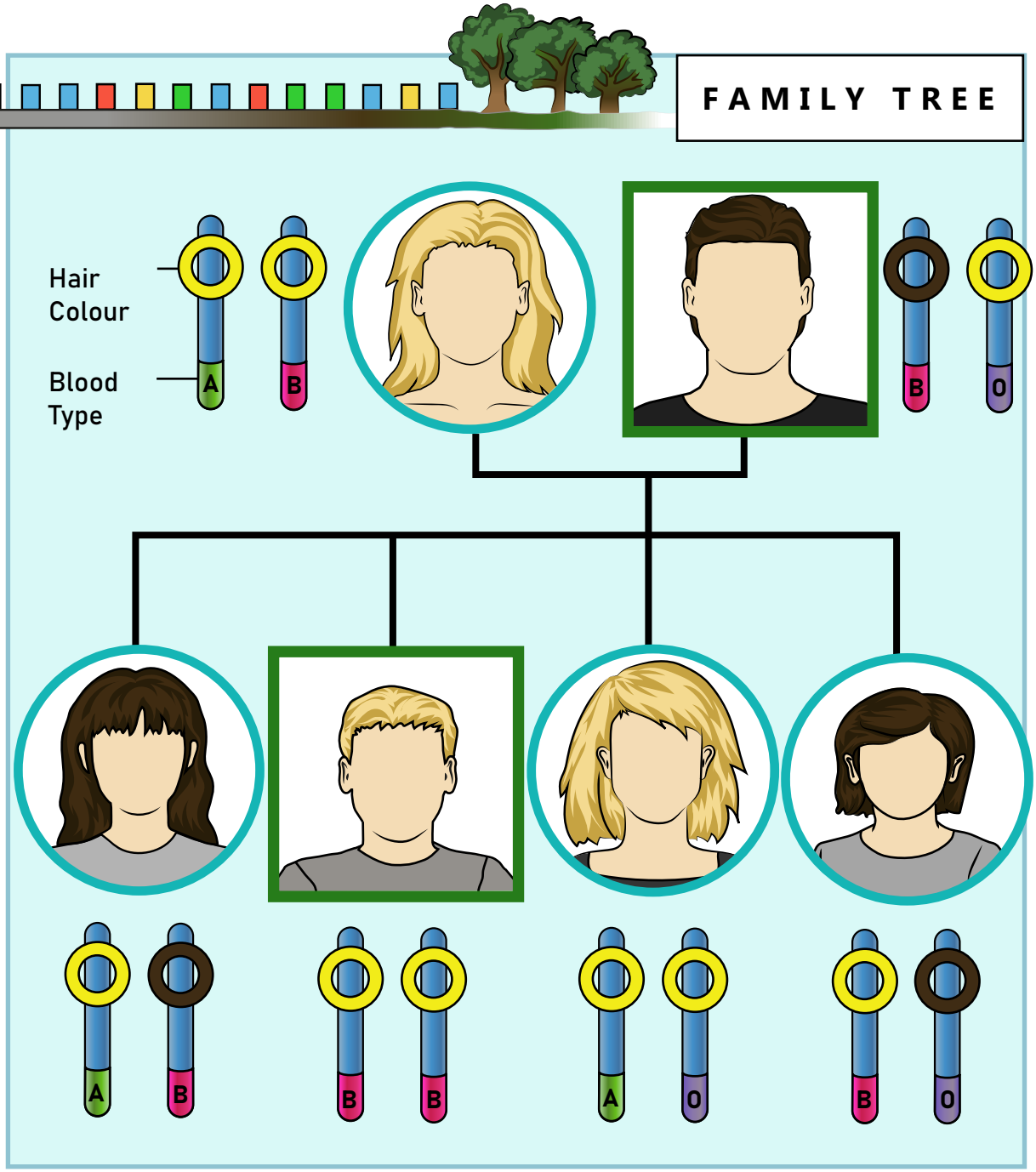
Every copy of the human genome is different. Polymorphisms usually have no effect, but a proportion of them are responsible for the differences between individuals.

Independent Assortment



At meiosis, these polymorphisms segregate independently into the gamete. This is Mendel’s Law of Independent Segregation. Recombination in Meiosis I ensures this.

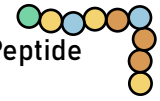
FAMILY TREE



When we draw a family tree, men are traditionally drawn as squares and women as circles. In this family tree, a man and a woman have 4 children together. Under each symbol there are genes shown on the same chromosome that control hair colour and blood group. Recombination ensures that these characteristics segregate independently.

MUTATION TYPES AND NOMENCLATURE

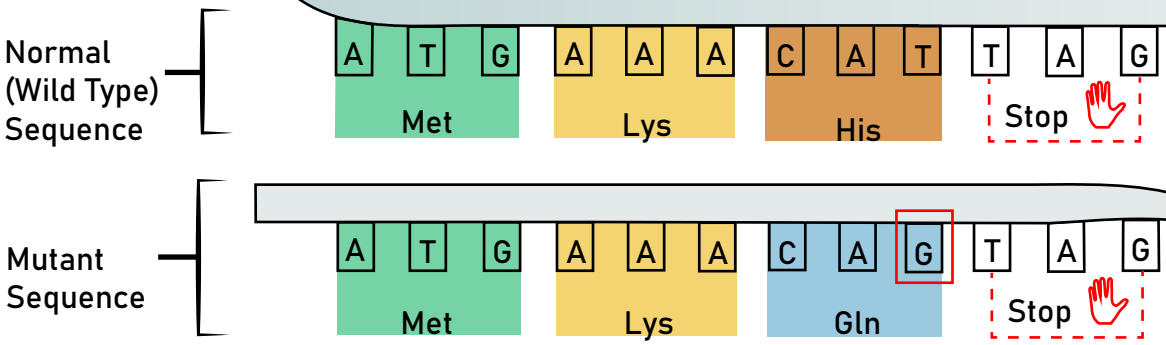
c. = Effect on mRNA/ cDNA Sequence

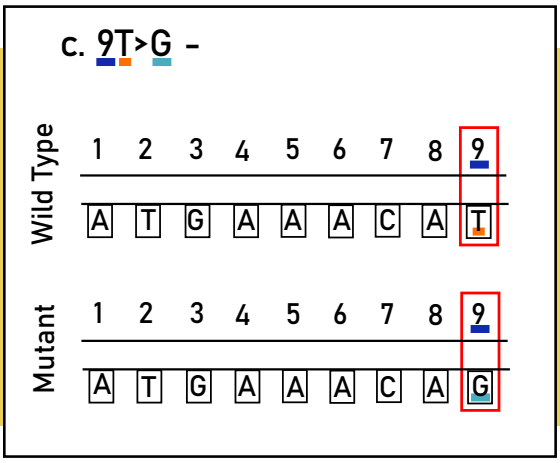


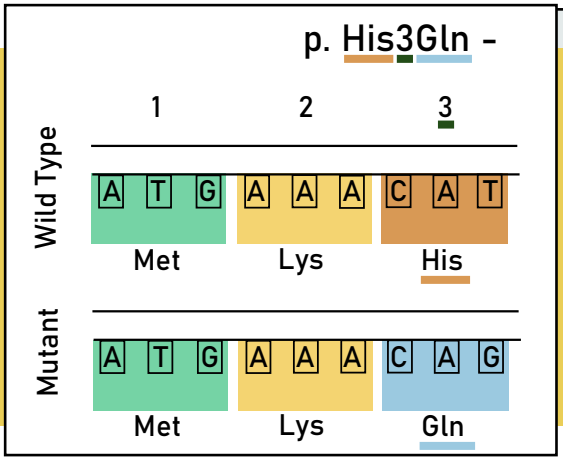
p. = Effect on Peptide Sequence

There is an international standard for describing a mutation, established by the Human Genome Variation Society (HGVS). HGVS nomenclature for a mutation can be expressed as the change in mRNA sequence, given as "c.", referenced to the first base of the coding DNA sequence that would make the first base of the mRNA. The resulting change in peptide sequence is referenced to the first amino acid, and given as "p."

Point Mutation I







In this sequence the mutation is a Thymine to a Guanine in the ninth base of the coding DNA sequence: c.9T>G. This causes the third amino acid in the peptide chain to be a Glutamine (Gln) rather than a Histidine (His): p.His3Gln. This is a typical Missense mutation.

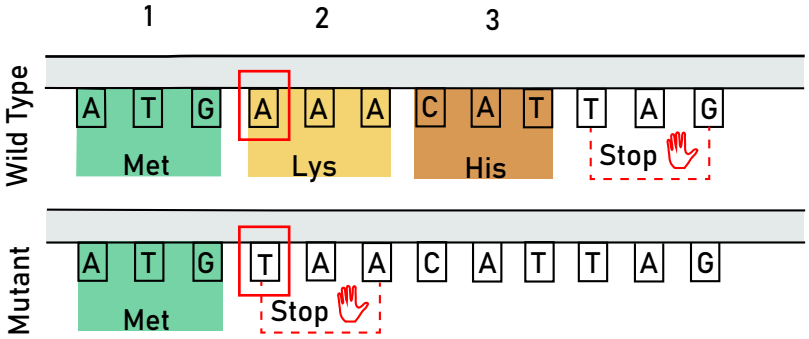
Point Mutation II

c.4A>T

p.Lys2Ter

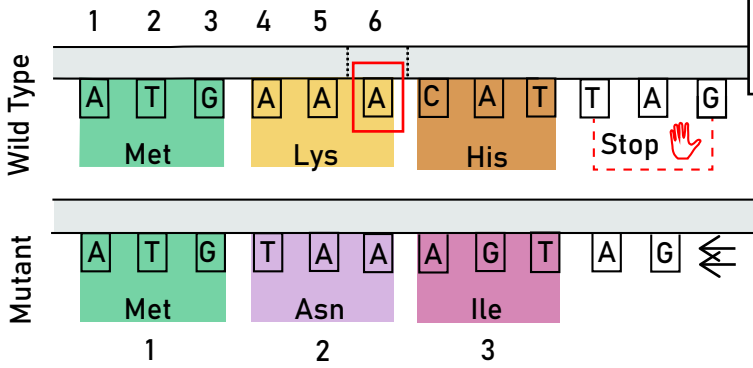
(p.Lys2*)

Terminate



An Adenine to Thymine at position 4 of the coding DNA sequence, c.4A>T causes a change from a Lysine amino acid to a stop codon, which can be written as: p.Lys2Ter or p.Lys2*.

Single Base Deletion and Frameshift



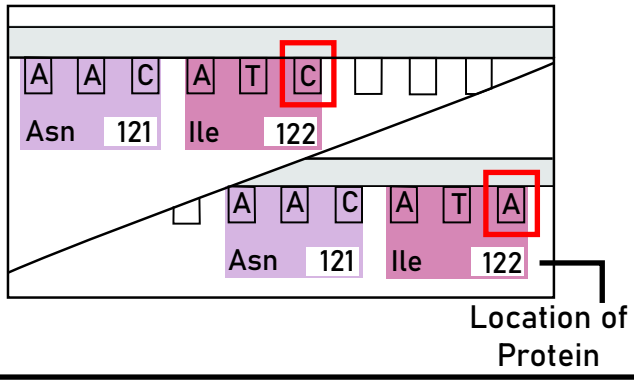
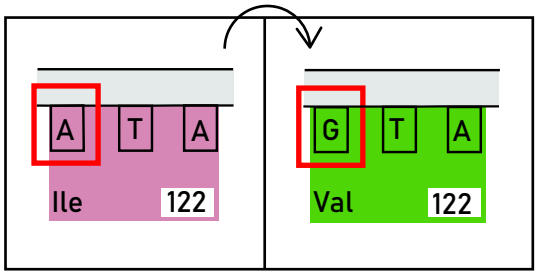
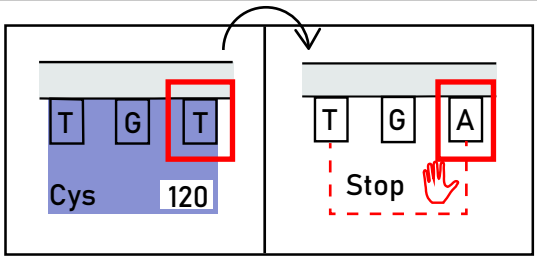
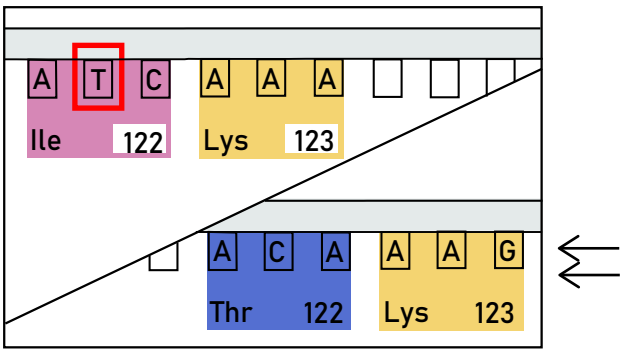
Deletion

c.6delA

p.Lys2Asnfs

Frame Shift

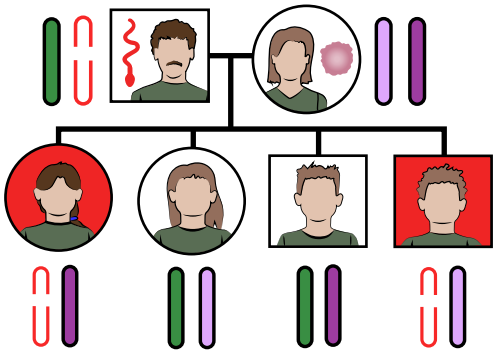
Deletion of a single base at position 6, written as c.6delA, causes a change of amino acid and then a downstream frameshift at Translation, p.Lys2Asnfs.

Example	Diagram	p. Effect
c.366C>A		p.Ile122Ile No Effect
c.164A>G		p.Ile122Val Missense
c.360T>A		p.Cys120Ter Premature Stop/ Nonsense
c.165del		p.Ile122Thrfs Deletion causes Ile to Thr and Frame Shift

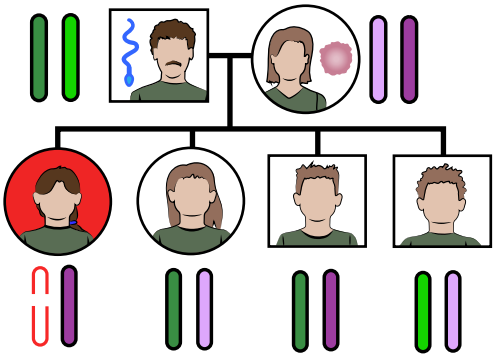
WHERE DO MUTATIONS COME FROM?

Disease causing mutations can segregate in families, inherited in the germ line. Mutations can also arise during gametogenesis – these 'De-Novo" mutations are a frequent cause of developmental disorders in children. De-novo point mutations are more frequently paternal, with a higher risk with advanced paternal age.

Germline Mutation

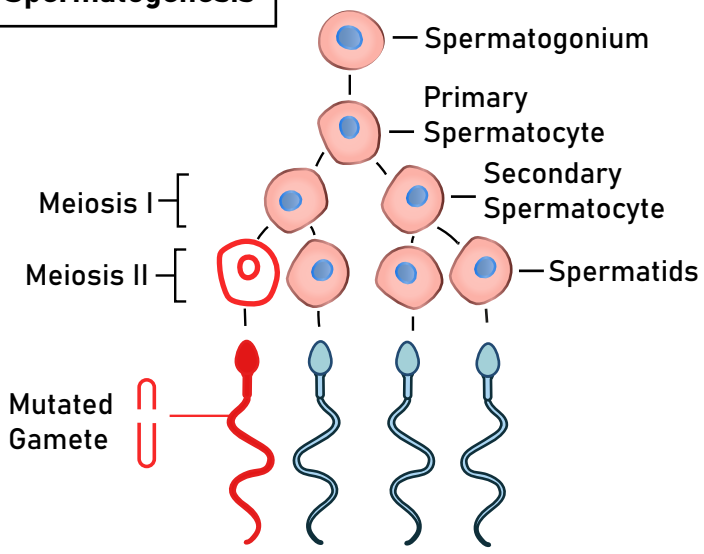


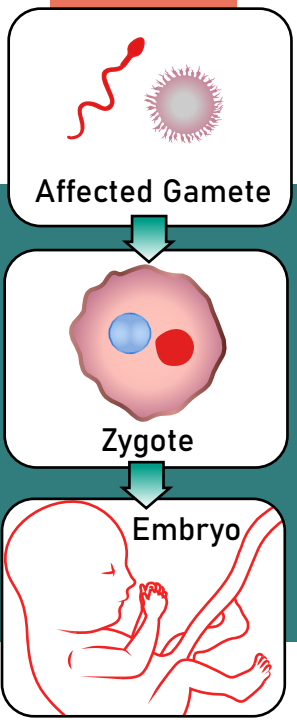
De Novo Mutation

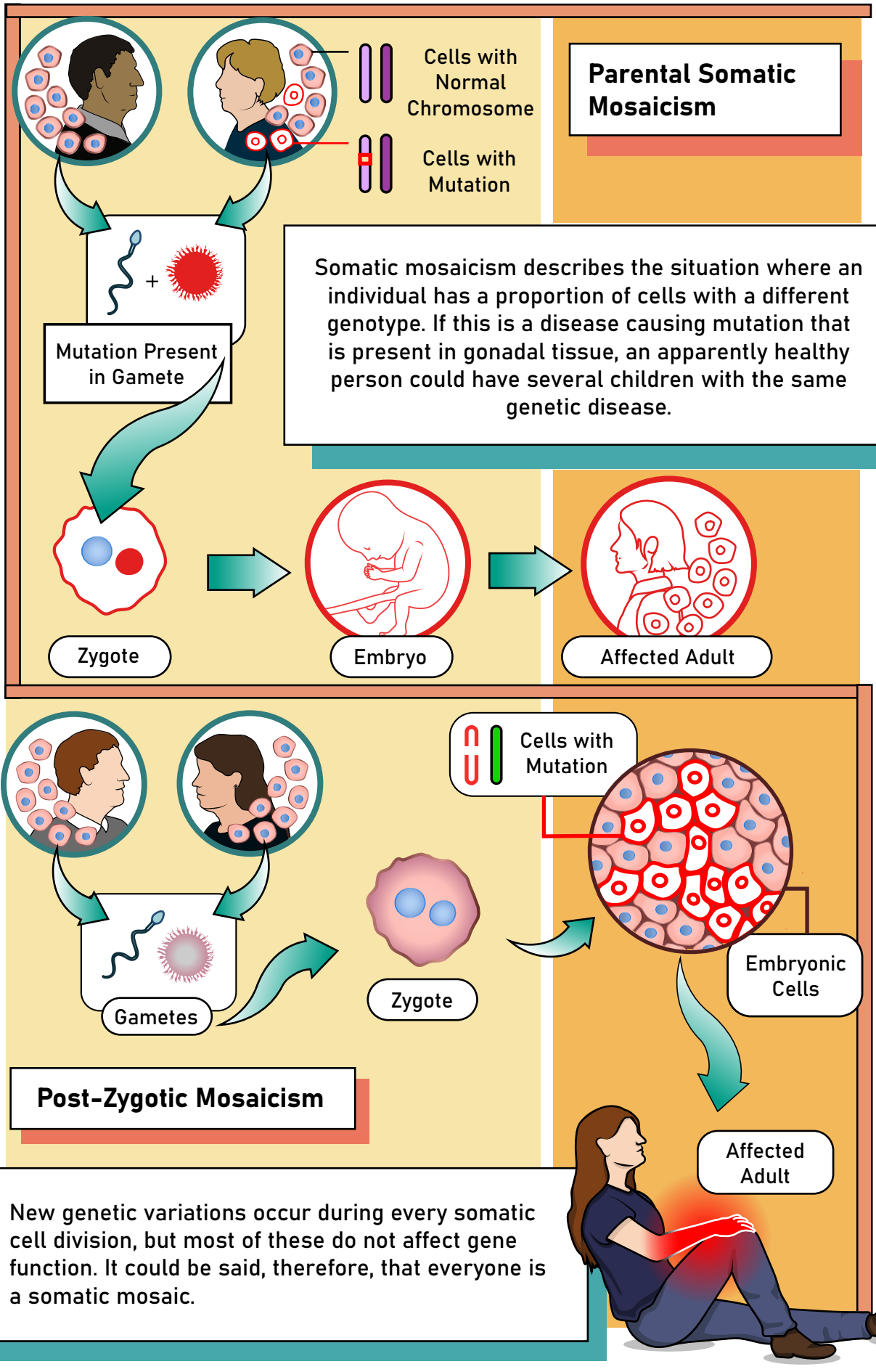


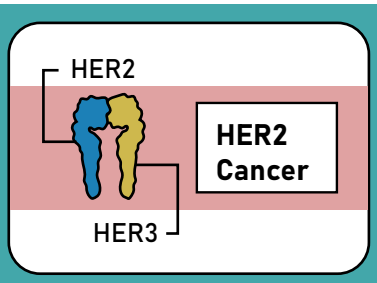
Mutation During Gametogenesis

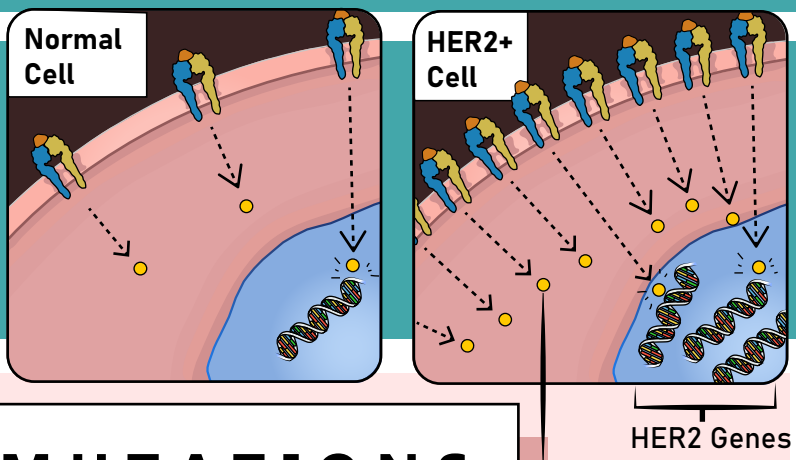
Spermatogenesis






Cells with Normal Chromosome
Cells with Mutation
Parental Somatic Mosaicism
Somatic mosaicism describes the situation where an individual has a proportion of cells with a different genotype. If this is a disease causing mutation that is present in gonadal tissue, an apparently healthy person could have several children with the same genetic disease.
Mutation Present in Gamete
Zygote
Embryo
Affected Adult
Cells with Mutation
Embryonic Cells
Gametes
Zygote
Post-Zygotic Mosaicism
Affected Adult
New genetic variations occur during every somatic cell division, but most of these do not affect gene function. It could be said, therefore, that everyone is a somatic mosaic.

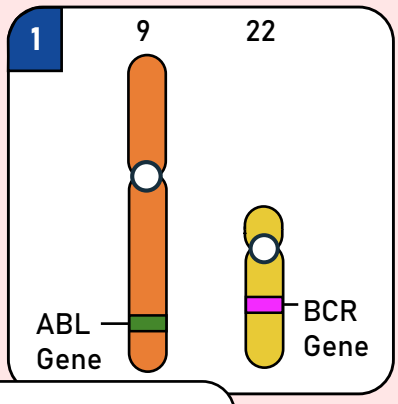




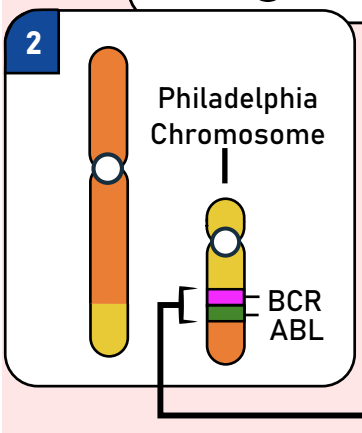
SOMATIC MUTATIONS AND CANCER

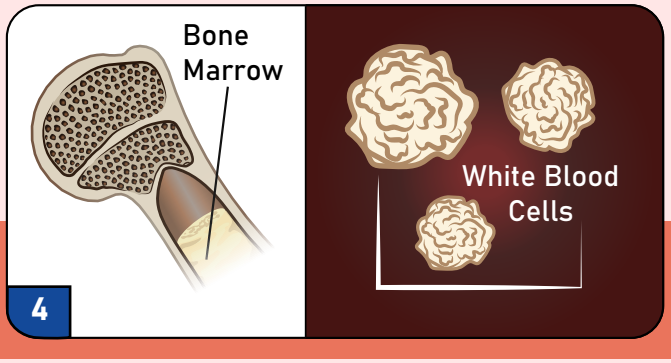
The formation of cancer is largely due to somatic mutations accumulating in cells. These mutations allow the cells to acquire characteristics such as uncontrolled proliferation or the ability to metastasize.

Philadelphia Chromosome



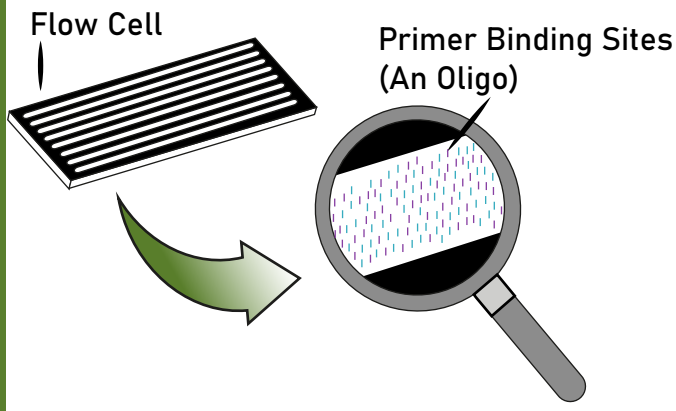
One example of a somatic cancer causing mutation is the Philadelphia chromosome. This is a translocation between chromosome 9 and chromosome 22 that activates the ABL oncogene, a major event in the causation of chronic myeloid leukaemia (CML). The drug Imatinib specifically targets the ABL oncogene and is a highly specific and effective treatment.





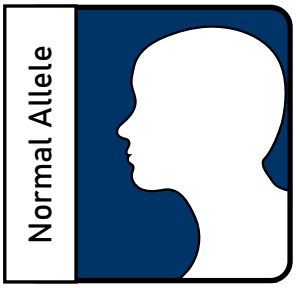
NEXT GENERATION SEQUENCING

Next Generation Sequencing (NGS) describes a number of technologies that can sequence large amounts of DNA. These technologies make it possible to sequence the entire genome of an individual for an acceptable cost, within a short period of time. The majority of current technologies break genomic DNA into fragments, and sequence a very large number of these fragments.



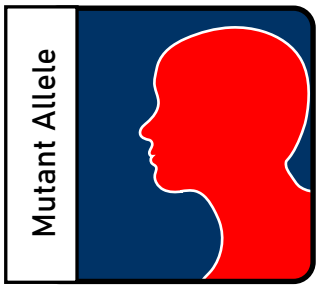
Reference Sequence

A G C C G A G A T G A C T A C A T C A A G C T



A T G A C
A T G A C
A T G A C
A T G A C

A T G A C



A T C A C
A T G A C
A T C A C
A T G A C

A T C A C

Data Analysis

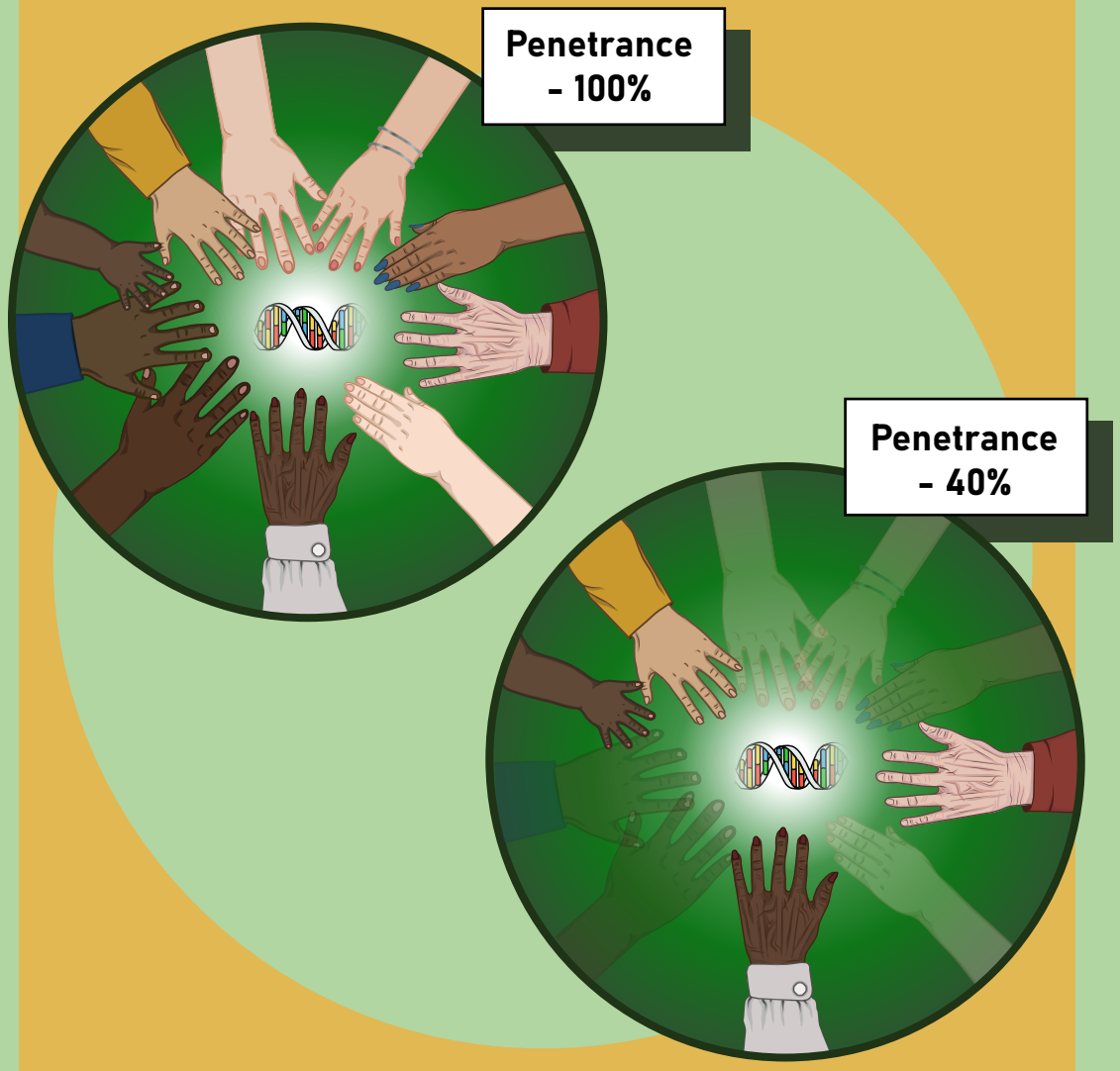
The sequences of the fragments are aligned against a reference genome sequence. If there is a variant in one copy of the genome (remembering that humans are diploid), half the sequences will show the genetic variation compared to the reference sequence.

On average, approximately 3,000,000 variants are detected when you sequence the entire genome in a person, these are polymorphisms. Usually we are looking for a single pathogenic variant (mutation).

To identify this pathogenic variant, the list of variants is filtered to remove those that are unlikely to be disease causing - for example, for a rare disease the variant will not be a polymorphism, it will have a critical effect on the gene, and the gene affected will be one that is known to cause the phenotype.

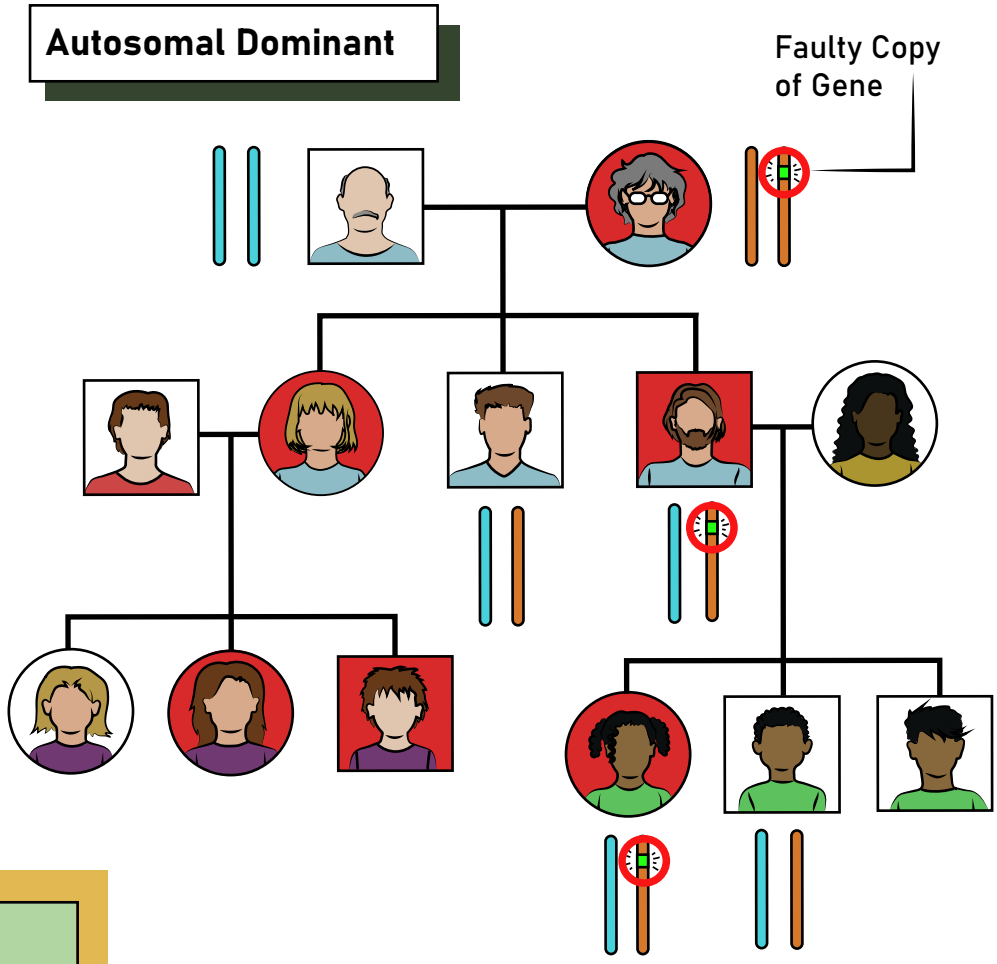
If a single variant meets these criteria, it may be the variant causing disease in the individual.

PENETRANCE AND MENDELIAN INHERITANCE

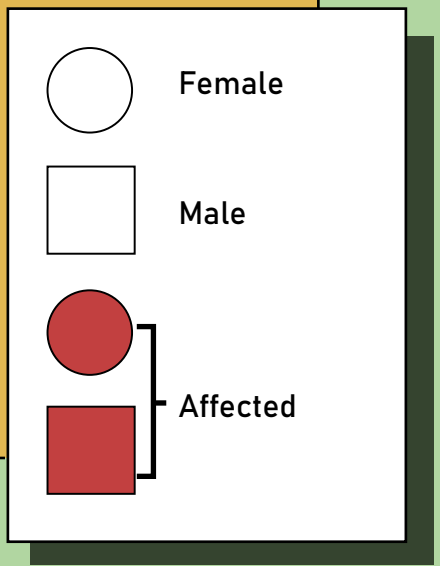


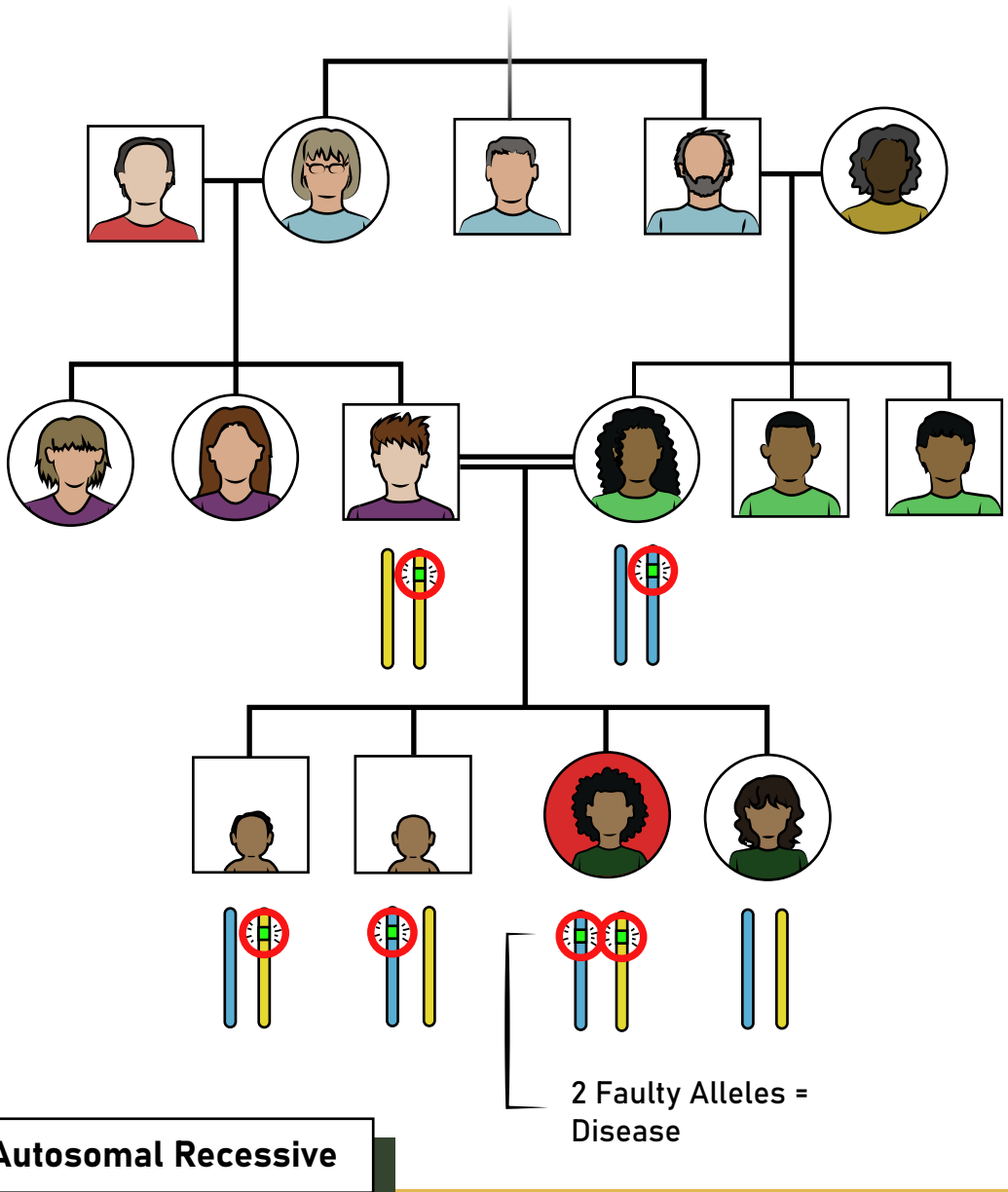
"Penetrance" of a mutation is the likelihood that it will cause a disease phenotype in an individual. If penetrance of a mutation is 100%, everyone who has the variant will have the disease. if penetrance is 40%, only 40% of people with the mutation will be affected.

Autosomal Dominant



For autosomal dominant inheritance, a single faulty allele (copy of the gene, described as a pathogenic variant) is sufficient to cause disease. An affected person has a 50% chance of passing the pathogenic variant to the child. The disease is usually seen in each generation, with males and females equally affected.

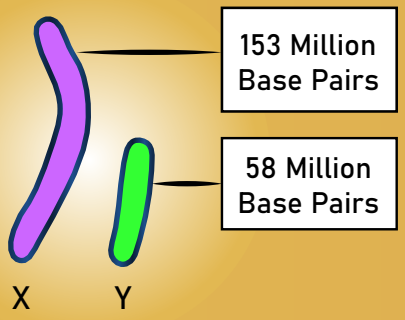


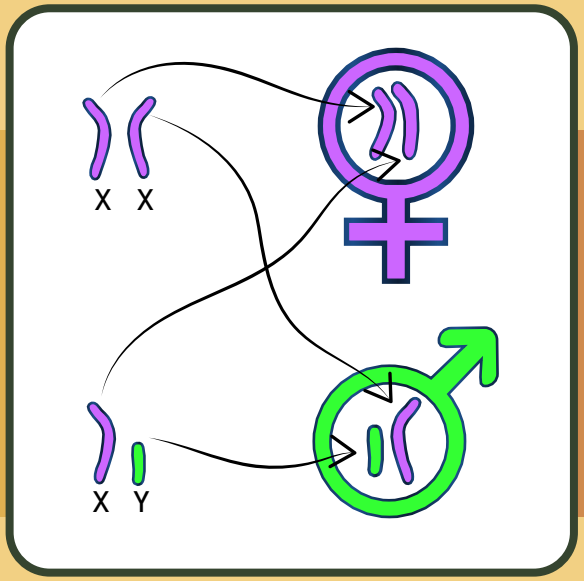


Autosomal Recessive

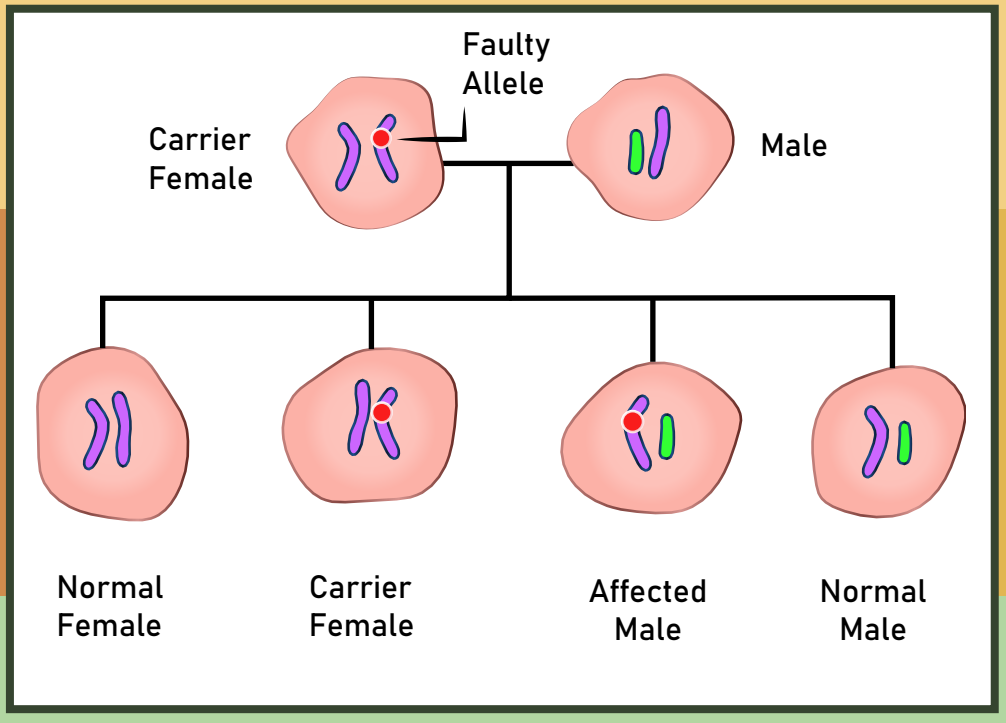
For autosomal recessive inheritance, an affected person has 2 faulty alleles (pathogenic variants affecting both copies of the gene). Parents usually both carry one faulty allele and one working allele. This happens more commonly in consanguineous parents. The risk of a child being affected is 1 in 4, and half of children will be carriers of one pathogenic variant.

X-Linked Inheritance

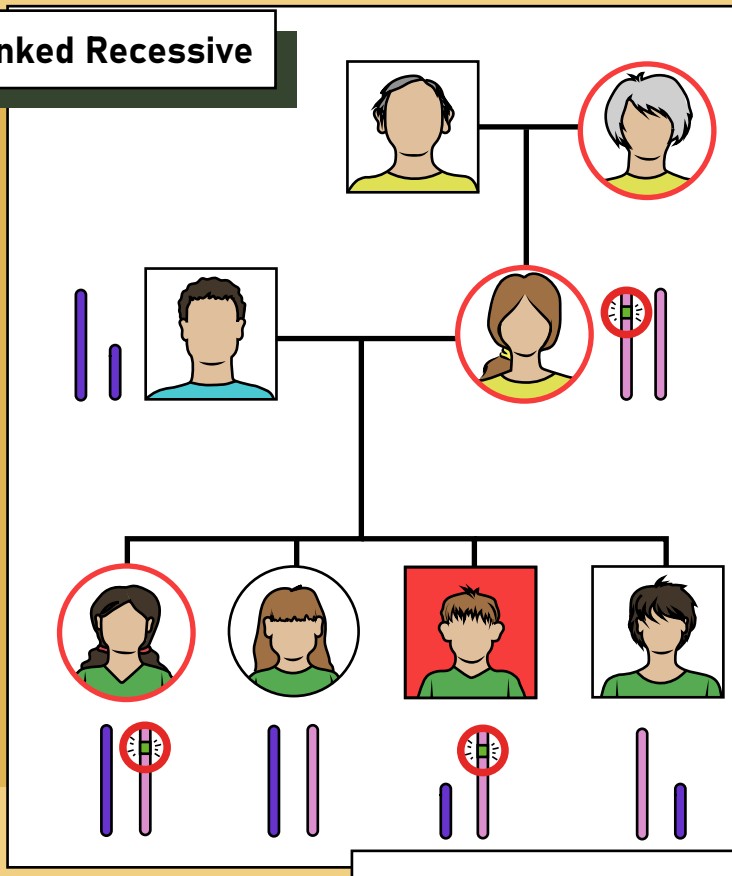




X-linked inheritance occurs where the pathogenic variant is found on the X chromosome. X-linked recessive inheritance describes the situation where a female with one pathogenic allele and one normal allele does not show major clinical features of the disease, but a male with a single faulty allele will be fully affected.

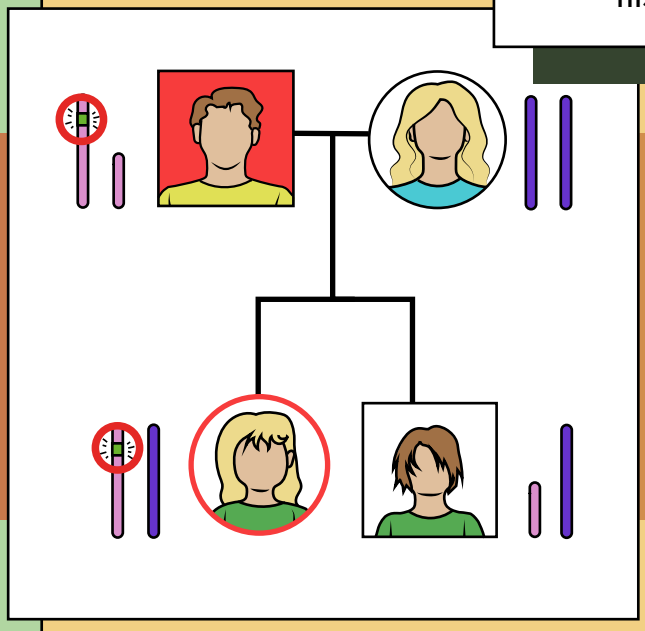


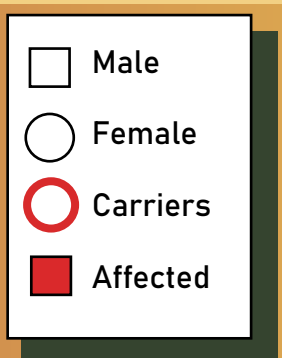
X-Linked Recessive



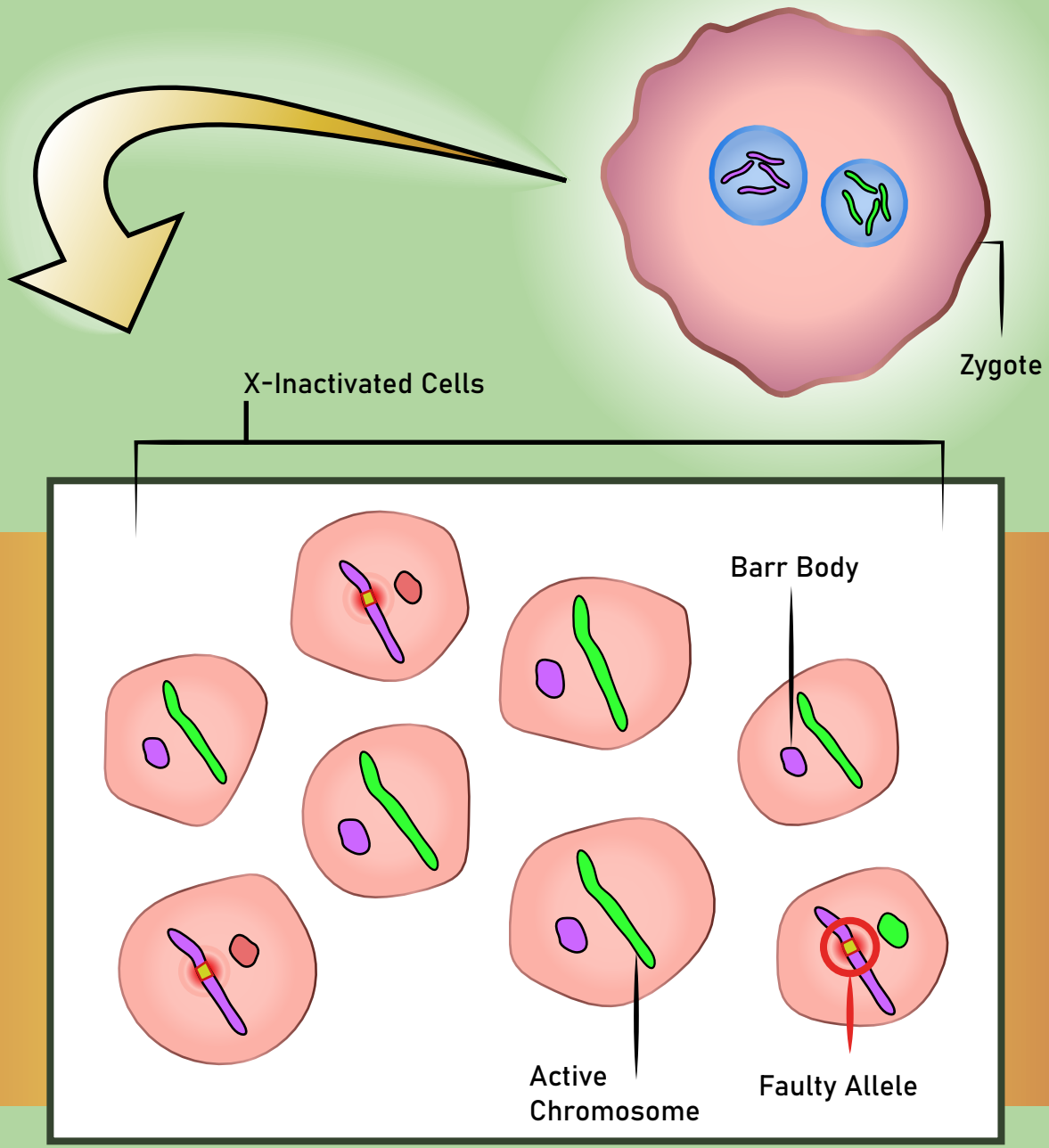
In X-linked inheritance, a carrier female can have an unaffected son (1/4), unaffected daughter (1/4), a carrier daughter (1/4) or an affected son (1/4).

If an affected male has children, all his daughters will be carriers and all his sons will be unaffected.





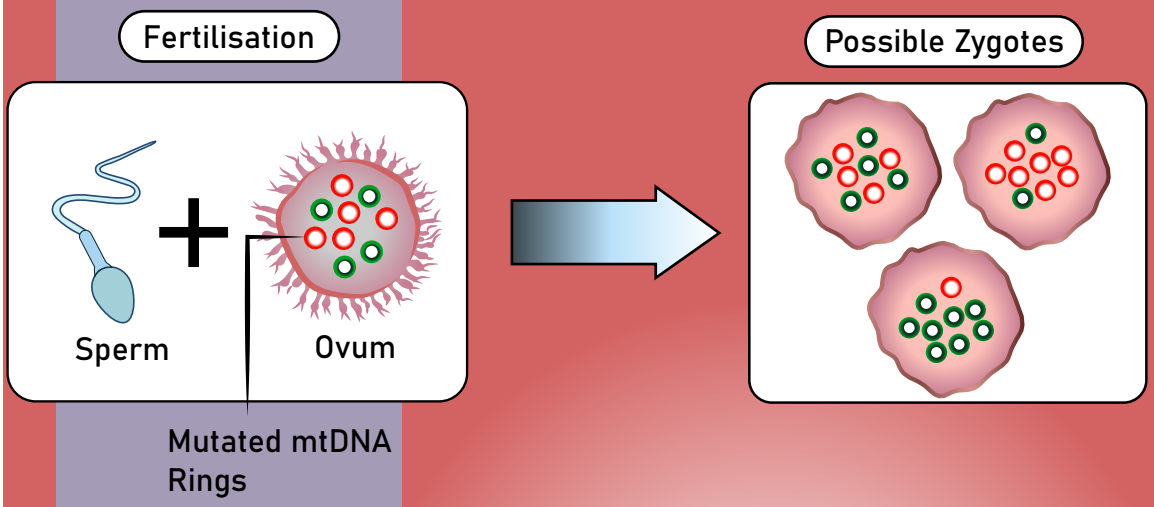
Where a female has a pathogenic variant on one X chromosome, X-inactivation will mean that on average, half her cells will have the functioning/normal allele active, and half will have only the pathogenic allele active. This explains why women who carry an X-linked pathogenic variant can show mild or sub-clinical features of an X-linked disorder.

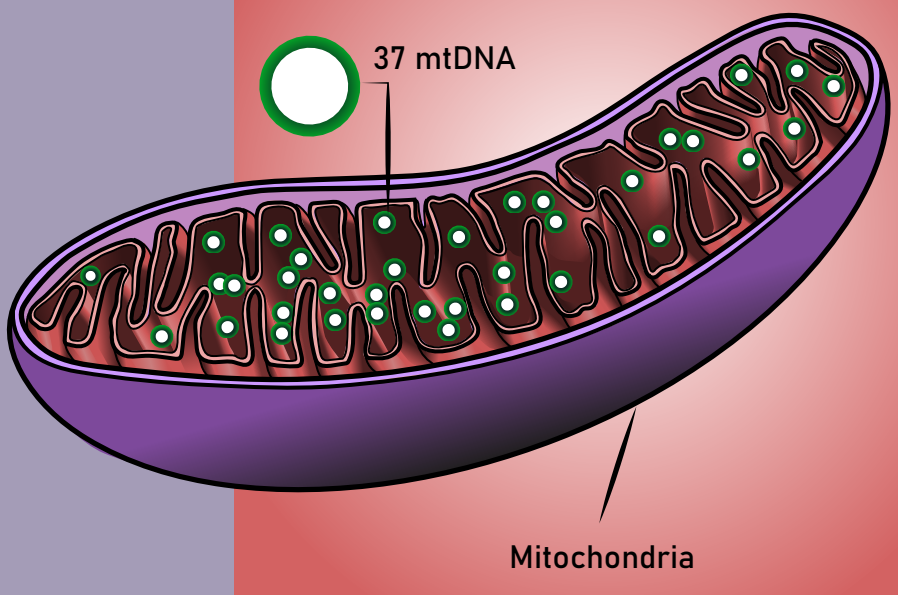


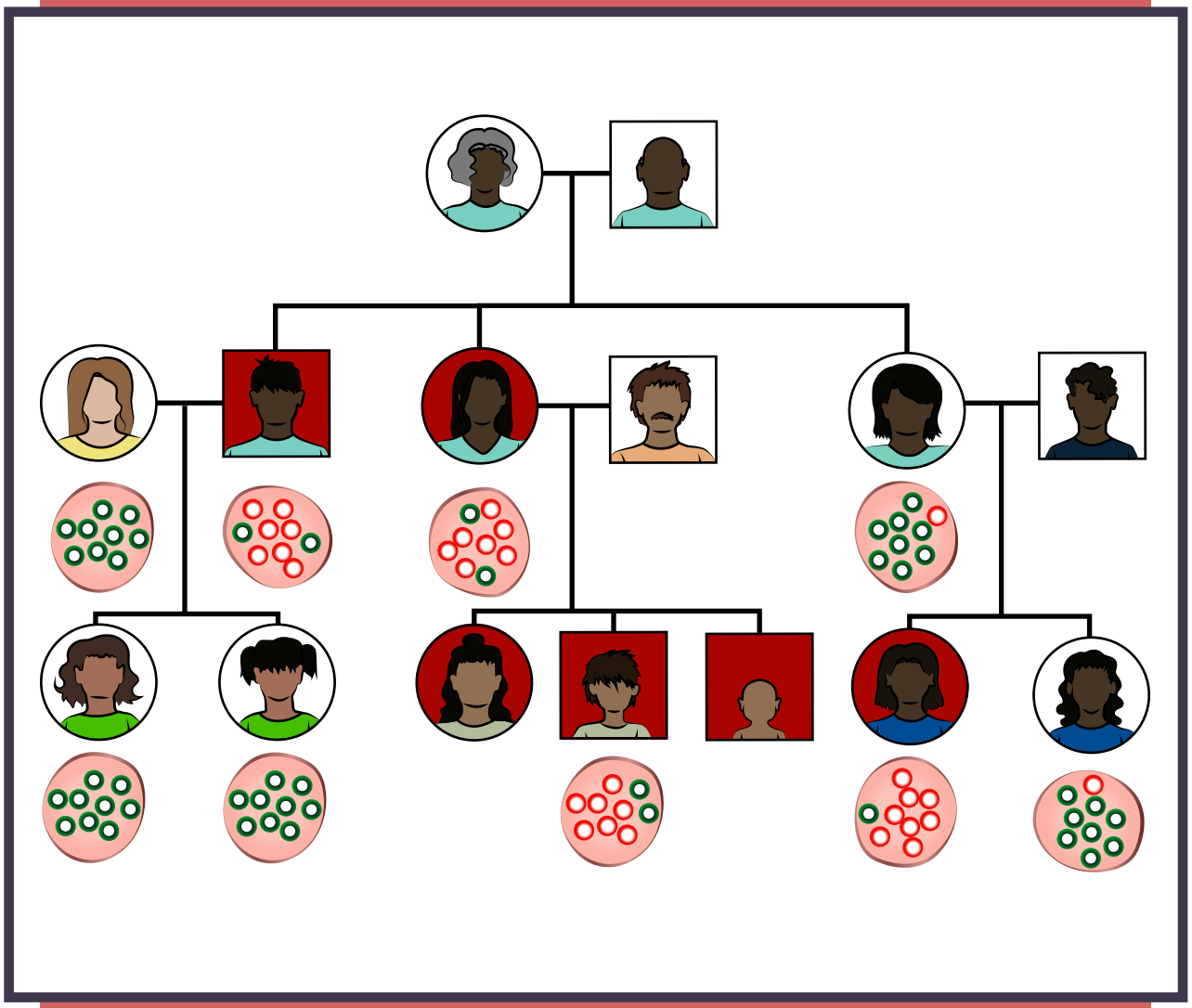
NON MENDELIAN-
MITOCHONDRIAL INHERITANCE

The mitochondria in the cell have their own genome of a single loop of 16,569 base pairs. With multiple mitochondria in each cell, there are multiple copies of the mitochondrial genome in each cell. In many cases, a mutation is only present in a proportion of mitochondria, and the proportion will vary between cells within an individual. Mitochondrial DNA is only transmitted maternally - in the ovum.

Heteroplasmy







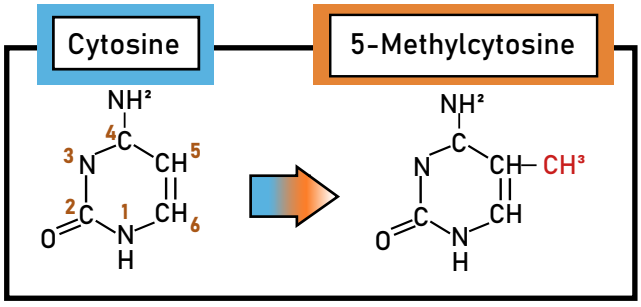
As mitochondrial DNA is only transmitted maternally in the ovum, mitochondrial shows a pattern where usually, males and females are equally affected, but only an affected mother passes the condition to her children.

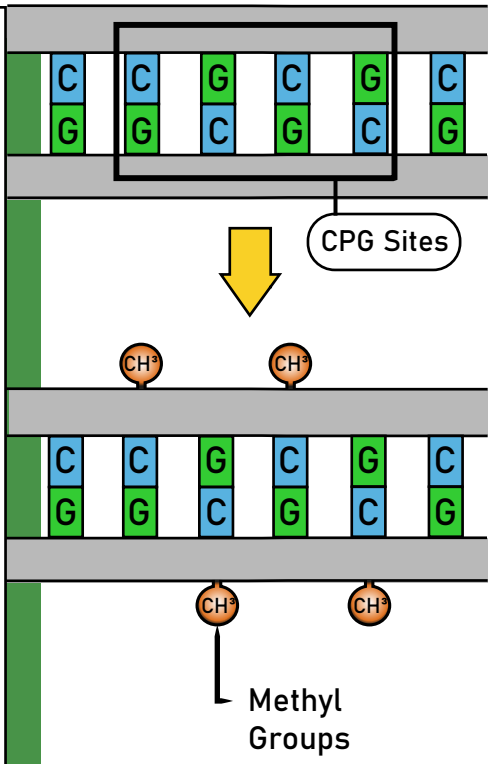
DNA METHYLATION

De Novo Methylation

A key mechanism for control of gene expression is DNA methylation. This is an epigenetic modification of DNA, one that does not change the base sequence.

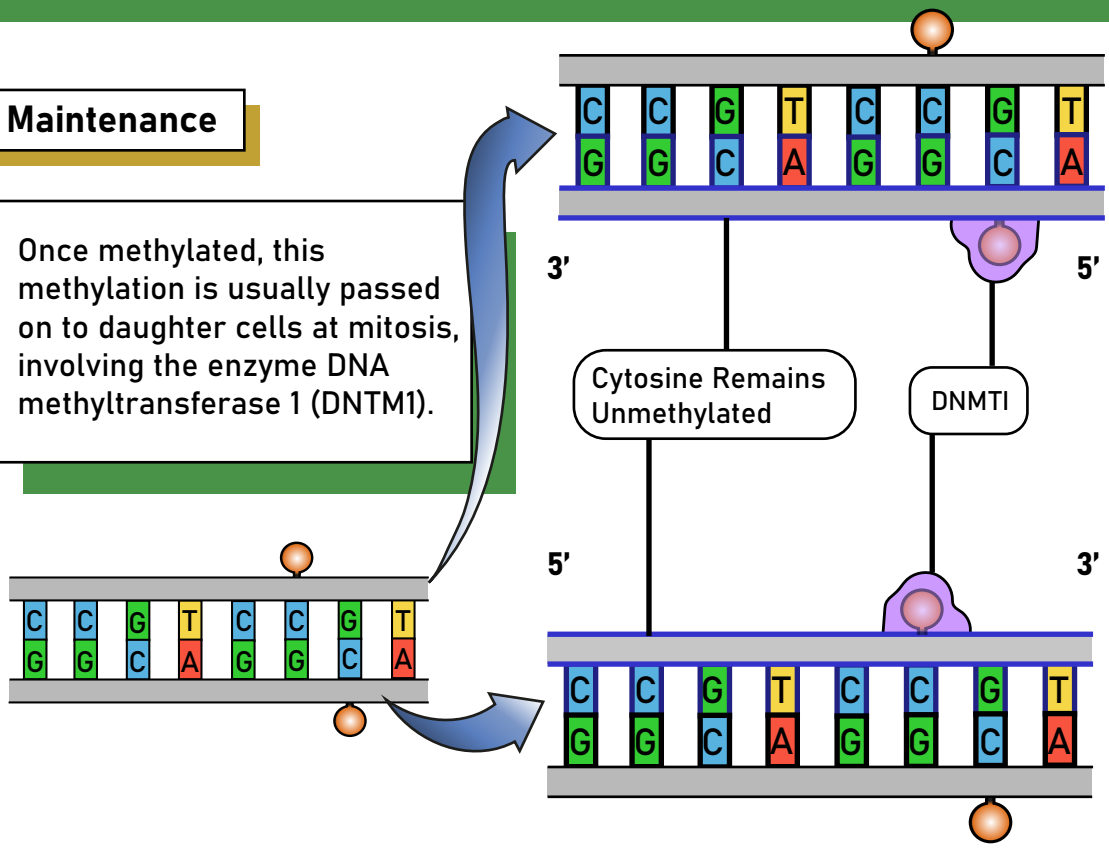
Where a Cytosine is adjacent to Guanine on a DNA strand, it is commonly methylated, becoming 5 methylcytosine.



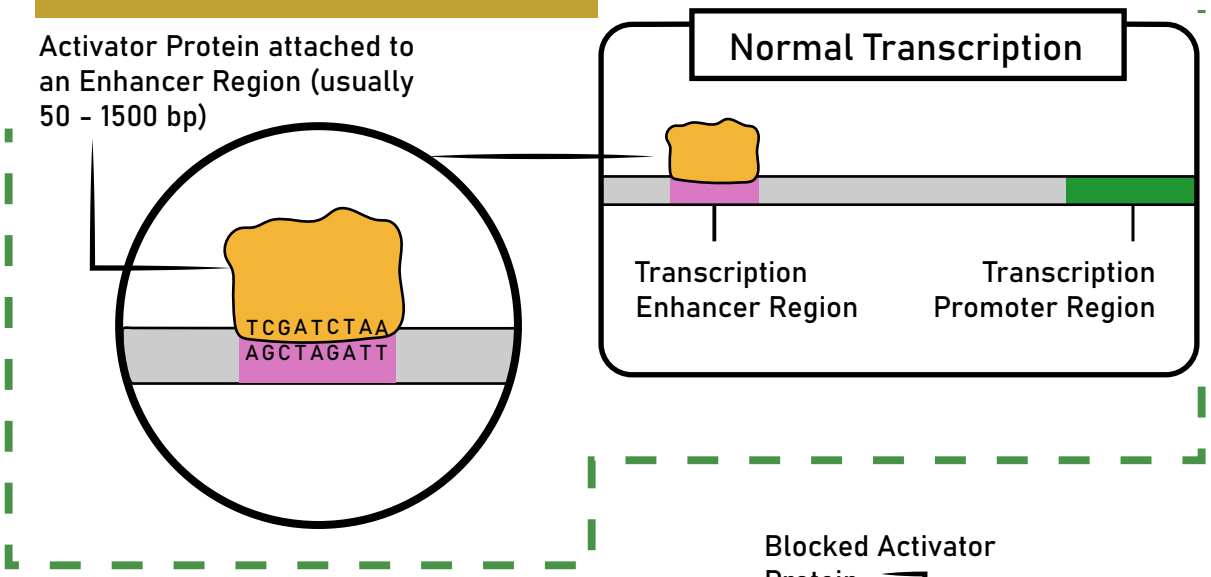


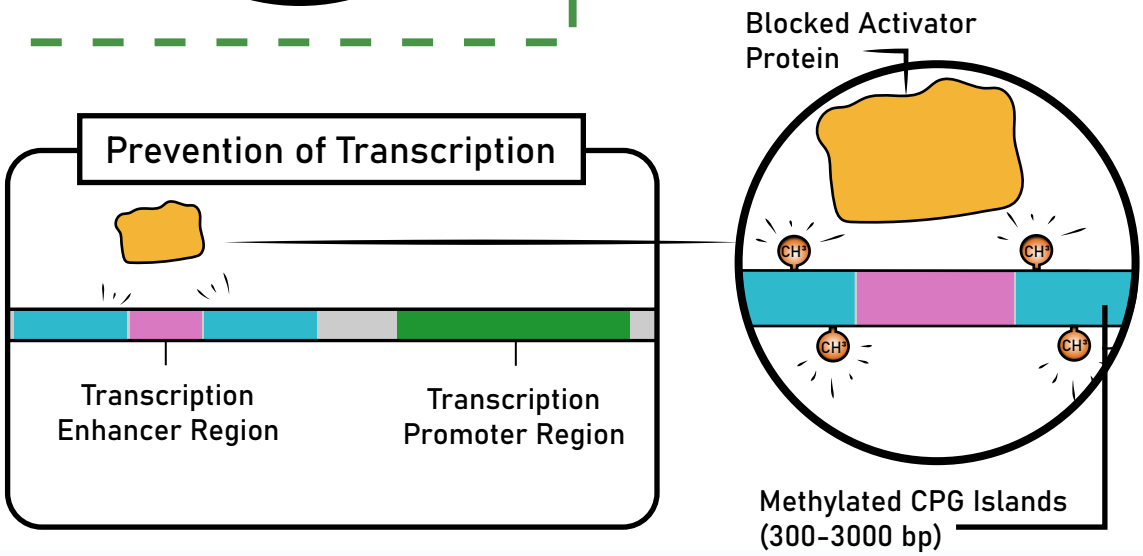
Maintenance

Once methylated, this methylation is usually passed on to daughter cells at mitosis, involving the enzyme DNA methyltransferase 1 (DNTM1).

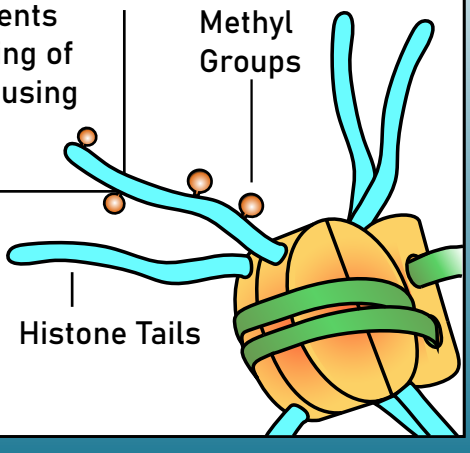


Regulation of Gene Expression

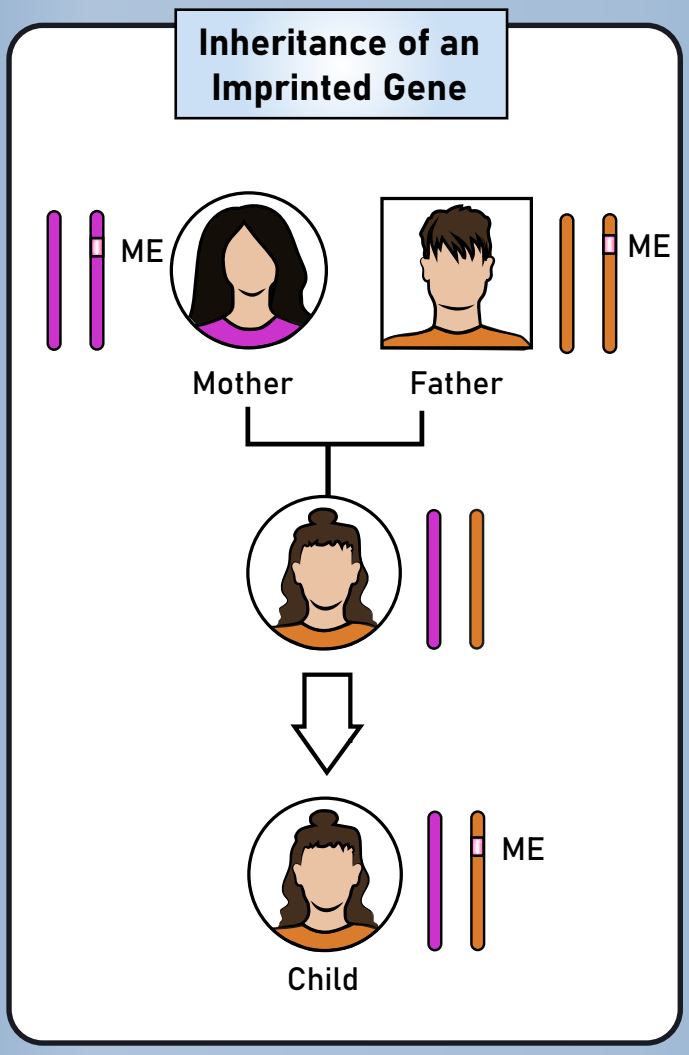




Methylation of a gene promoter prevents gene transcription by preventing binding of transcription factors, and indirectly causing deacetylation of histones.



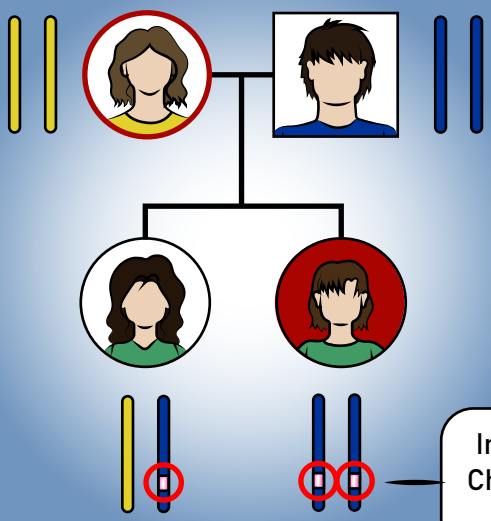
GENOMIC IMPRINTING



Some genes are only active in the allele inherited from one parent. This is called "Imprinting". A paternally imprinted gene is one that is only expressed in the allele inherited from the mother. A maternally imprinted gene is one that is only expressed in the paternally inherited allele. Imprinted genes are found in specific chromosomal regions, for example on chromosome 15q.

Inheriting Angelman Syndrome

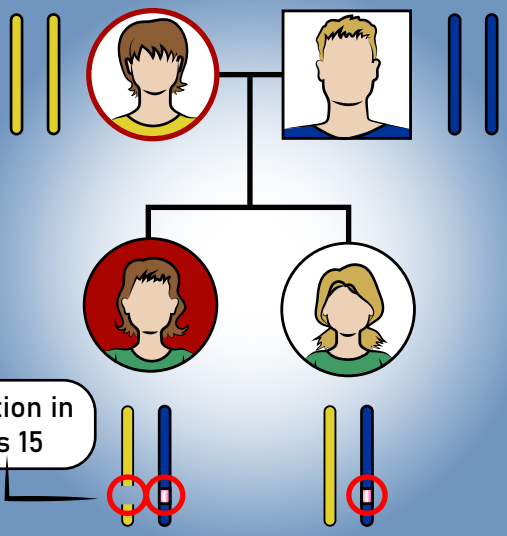
Imprinted Area



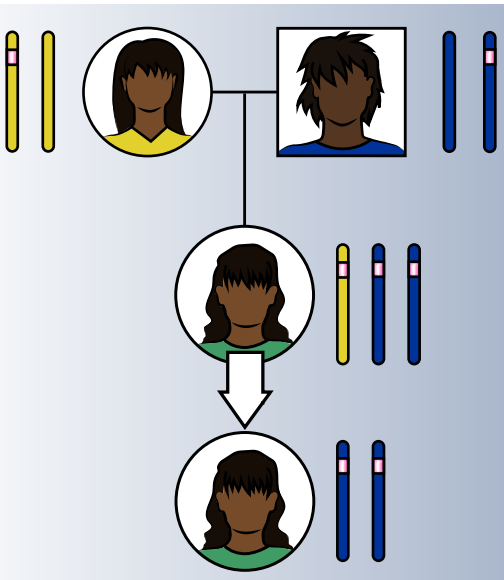
The UBE3A gene on chromosome 15 is paternally inherited, and is only expressed from the maternal allele. A child with no functioning UBE3A is affected by a condition called Angelman syndrome.

Inheriting both Chromosome 15 from Father

Other children are affected with Angelman syndrome because they have a mutation such as a deletion of their mother's copy of the gene on chromosome 15.



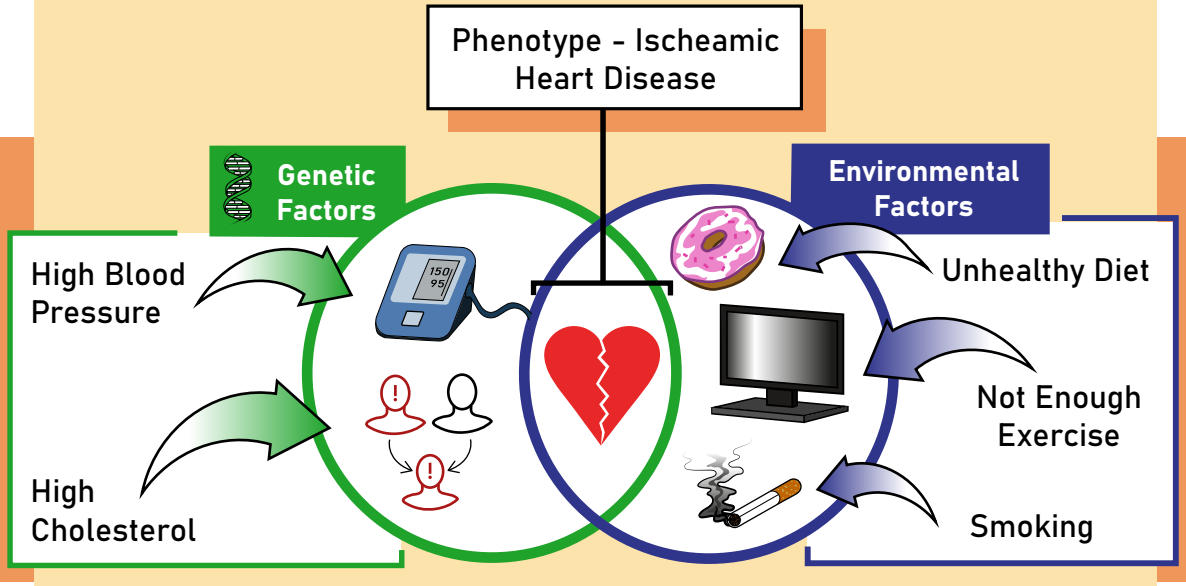
New Deletion in Mother's 15

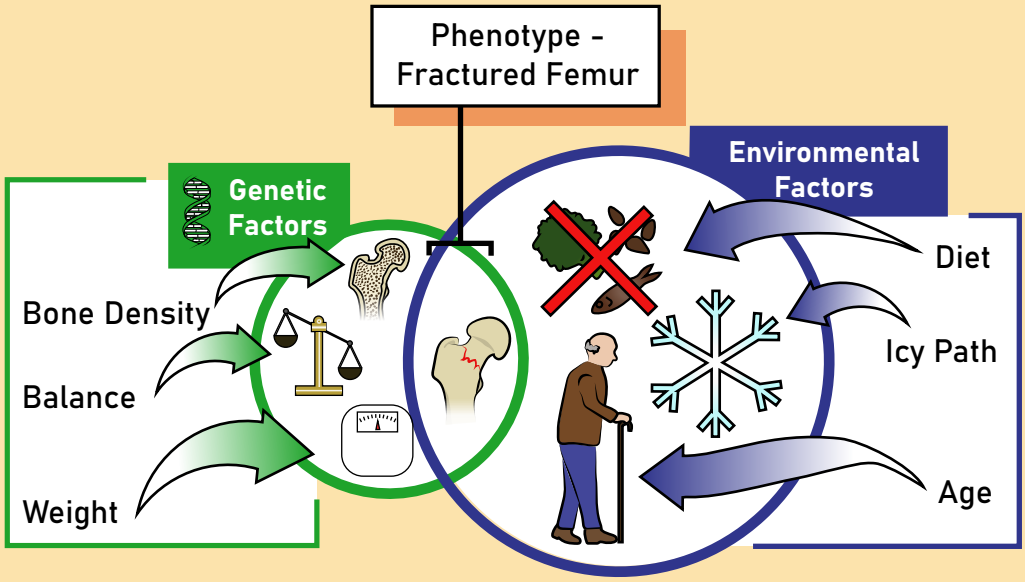


Parental Uniparental Disomy

A child may be affected because they inherit both copies of chromosome 15 from their father, called "uniparental disomy".

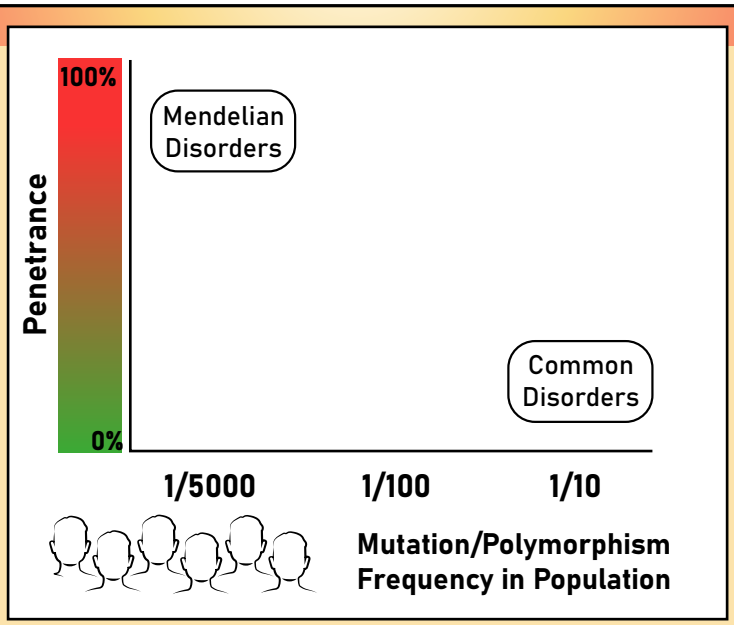
MULTIFACTORIAL INHERITANCE





Almost all common disease have a mixture of environmental and genetic causes. In some conditions, environmental factors are more important, in others there is a strong genetic component.

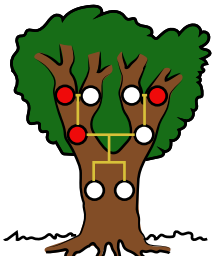
COMMON DISEASE—COMMON VARIANT



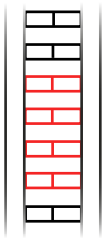
Genetic variants causing diseases that are inherited in a mendelian fashion have a high penetrance, but are rare in the population. Genetic variants that are responsible for the predisposition to common ("multifactorial") diseases are more common in the population but have a low penetrance (or size of effect).

Genetic testing is currently widely used for mendelian disorders, as it predicts the development of disease. In common diseases, genetic testing is less useful and risk is estimated from features such as family history and environmental exposure.

Mendelian Disorders = Genetic Testing

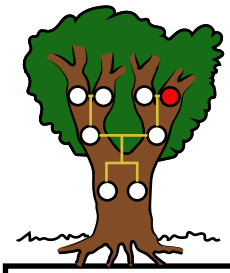


Worrying Family History



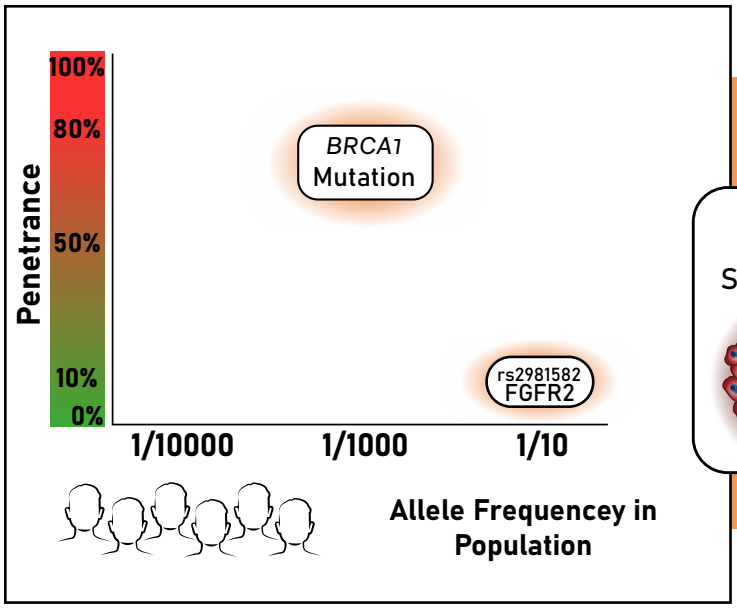
Younger Age, More Cases, Unusual Presentation

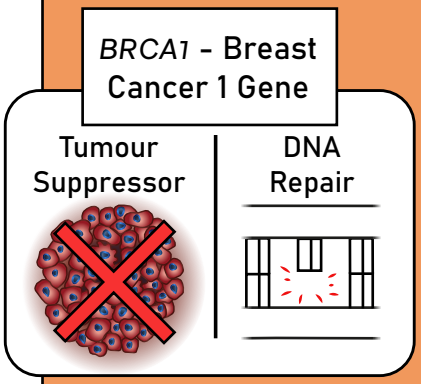
Common Disorders = Estimate Risk



Check Family History

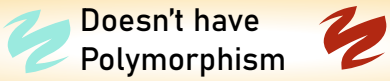
Other (i.e Environmental Factors)

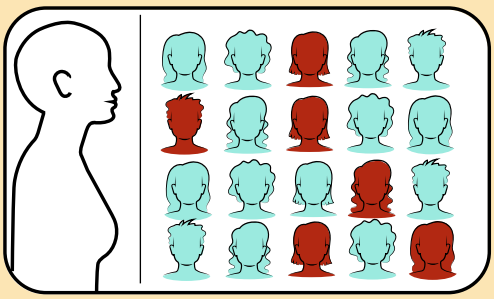




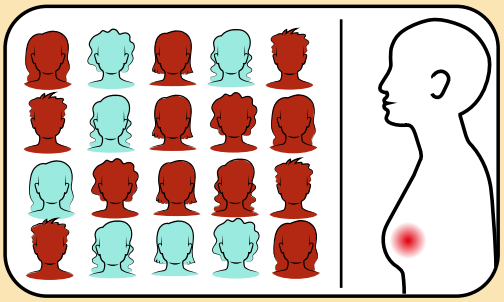
Some conditions may have a high penetrance genetic cause in some cases, but more commonly have a multifactorial cause. For example, a small proportion of women affected with breast cancer have a *BRCA1* mutation which causes a 30 times increase in risk. For most women, there are multiple low penetrance genetic and environmental factors. An example of this is a low penetrance polymorphism in the *FGFR2* gene that increases breast cancer risk by a factor of about 1.2X times population risk.

SNPs - Association Studies





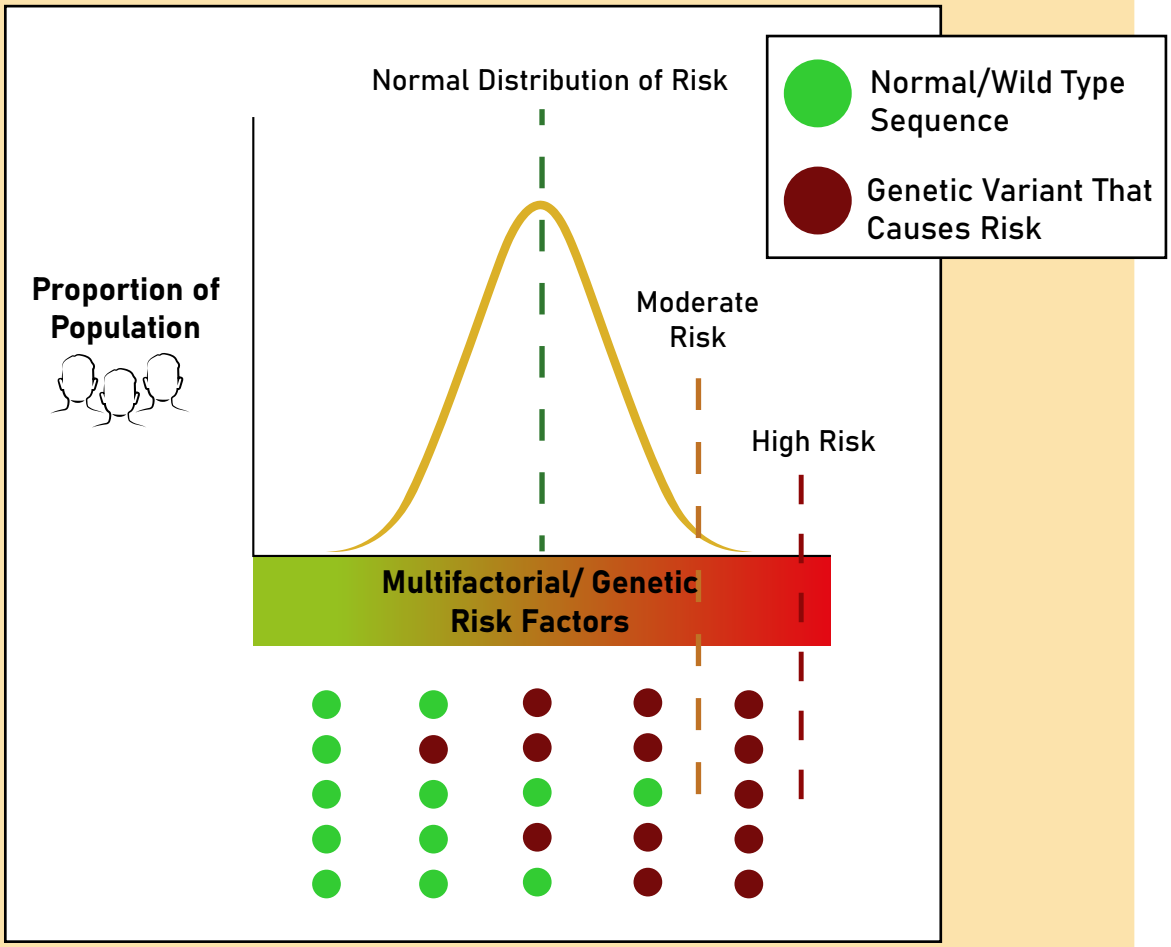
Not Affected with Breast Cancer



Affected with Breast Cancer

Low penetrance polymorphisms are identified by comparing populations of affected and unaffected individuals. If a polymorphism is more frequent in affected individuals, then it is associated with that disease. Most studies look at polymorphisms across the whole genome, and are, therefore, described as Genomewide association studies (GWAS).

COMMON DISEASE - MULTIFACTORIAL RISK

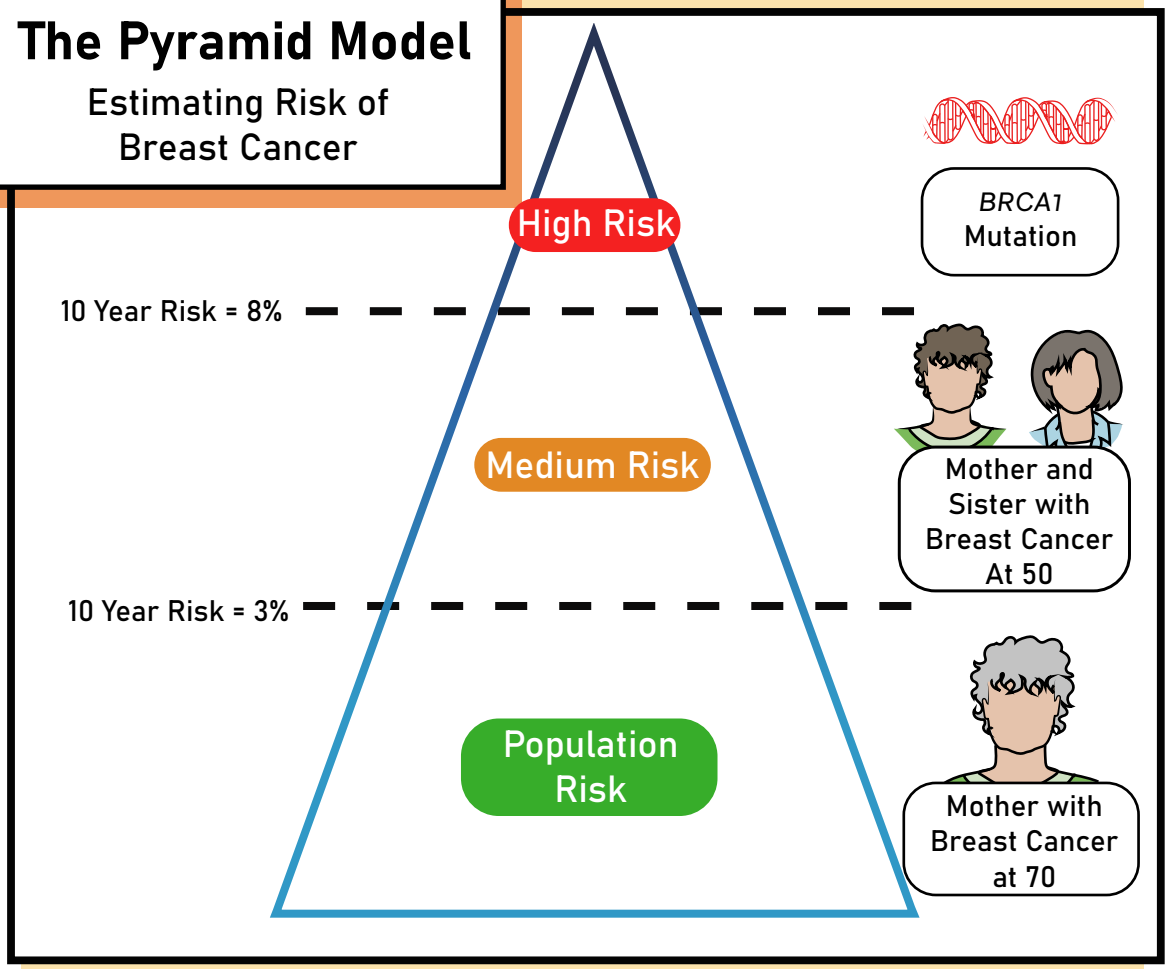


It is usually assumed that risk for a common disease follows a normal distribution. The risk that applies to an individual is a function of the number of risk factors that they have. These could be genetic or environmental factors.

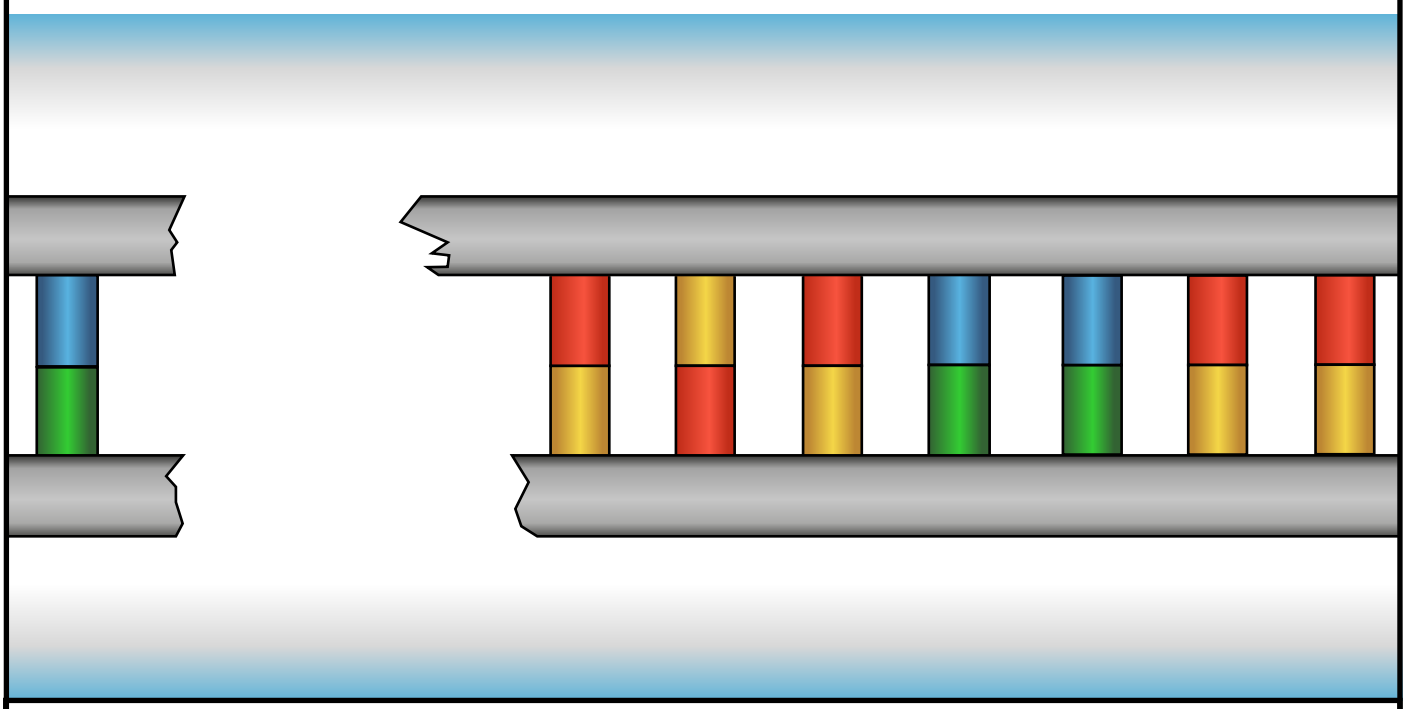
For some conditions such as breast cancer, there are national or international guidelines for the level of risk that would justify an additional intervention – for example increased screening, or risk reducing treatment.

The Pyramid Model

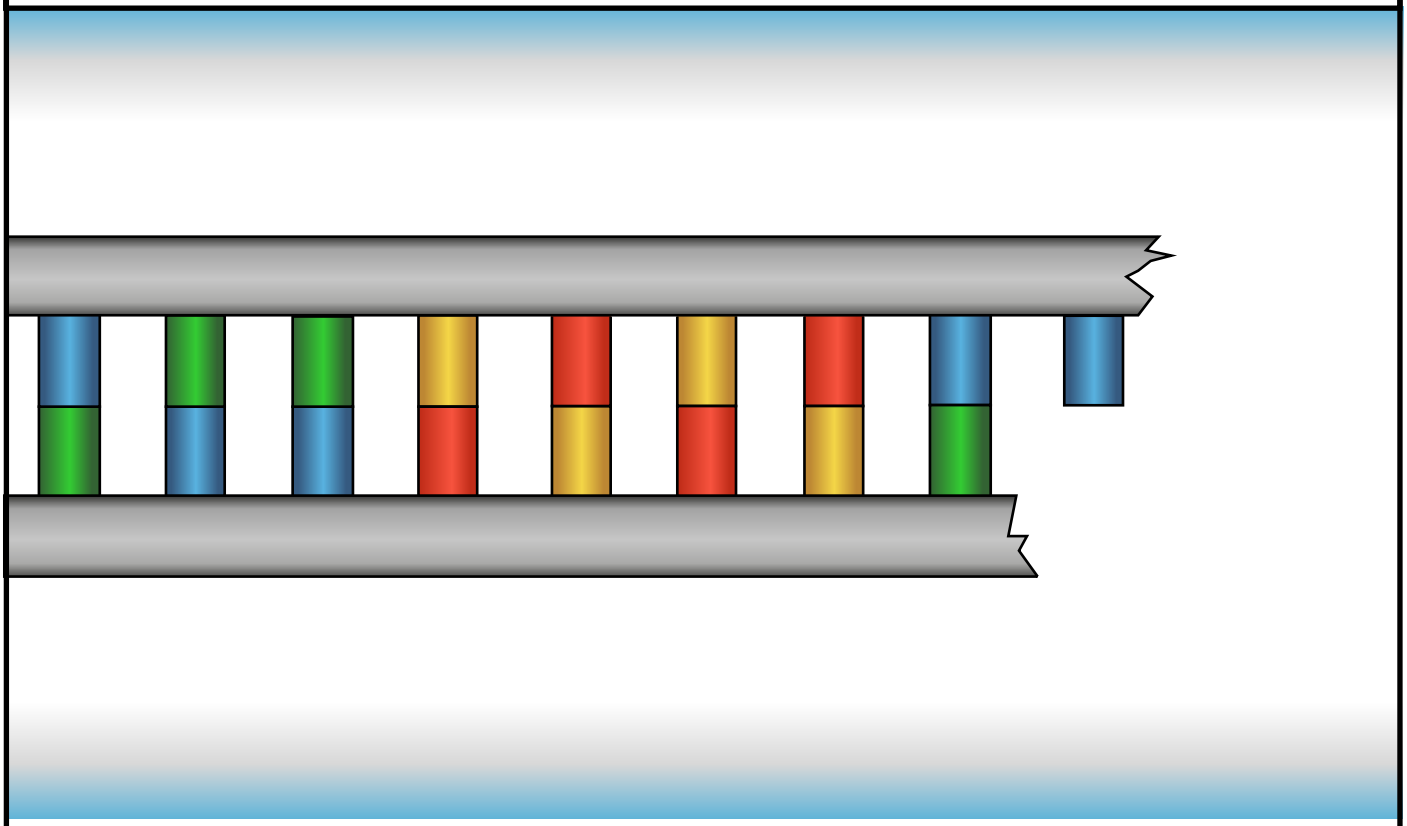
Estimating Risk of Breast Cancer



In clinical practice for some conditions in genetics, it is usual to define risk thresholds. These can be based on family history and other factors such as genetic testing. For example, in breast cancer risk, having a single relative affected at an older age does not justify a change in treatment. Having two relatives affected with breast cancer would increase risk to a "Moderate risk" level, justifying increased screening for cancer. Having a *BRCA1* mutation would increase risk to a level where early cancer screening by MRI scan and prophylactic mastectomy would be justified.



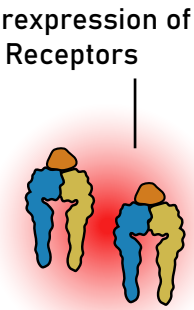
CANCER



HALLMARKS OF CANCER

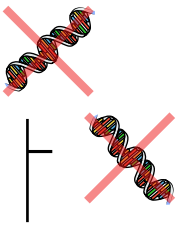
1. Sustained Proliferative Signalling

Tumours produce their own growth factors, and/ or overexpress receptors (see HER2 cancer on page 41).



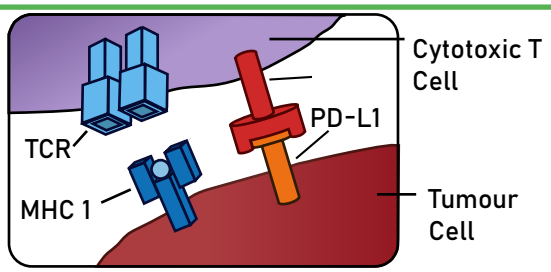
2. Evading Growth Suppressors

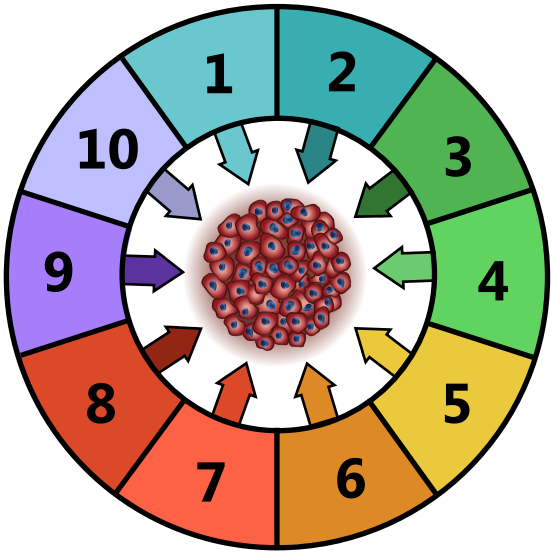
Tumour suppressor genes that are involved in the normal function of the cell cycle are inactivated or prevented by mutant proteins.



3. Avoiding Immune Destruction

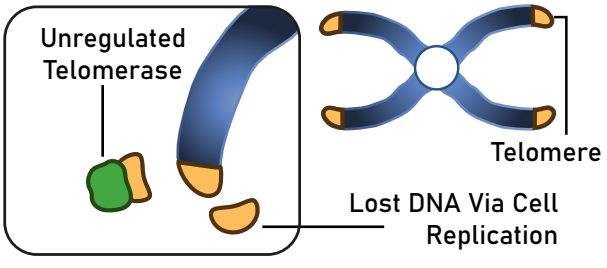
Tumour cells evade detection and ellimination during their development. Some tumour cells adapt to the immune system and its anti-tumour activity, for example, by expressing proteins such as PD-L1 to supress the binding of the T-cell receptor to MHC 1.



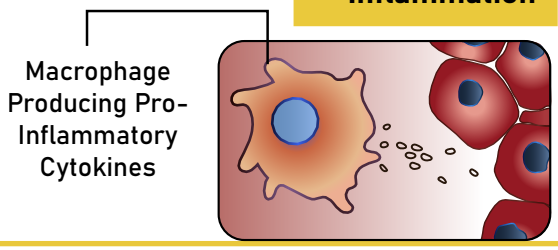


4. Enabling Replicative Mortality

Telomeres (excess DNA) shorten after multiple replications and eventually enter senescence. Tumours use the enzyme telomerase to continue adding DNA so that the cell can keep dividing.



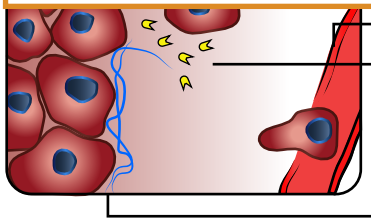
5. Tumour Promoting Inflammation



Overproduction of prostaglandin E2 (PGE2), for example, triggers an inflammatory response to the tumour. Cytokines released from tumour-associated macrophages can encourage the tumour to develop.

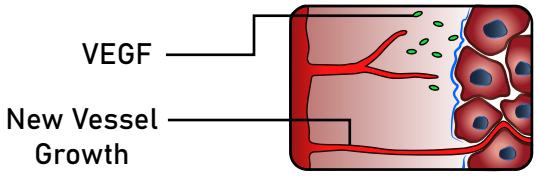
6. Activating Invasion and Metastasis

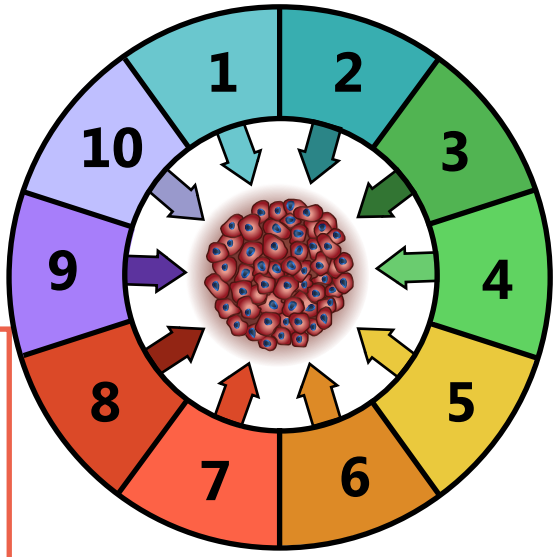
This is led by the secondary tumour cells. Matrix metalloproteinases (MMP's) are utilised by the tumour cells to break through the extracellular membrane, allowing cells to detach and enter a blood vessel, and into other environments.



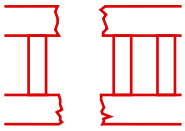
7. Inducing Angiogenesis

Tumour cells encourage the growth of new blood vessels. They can do this by releasing vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF).





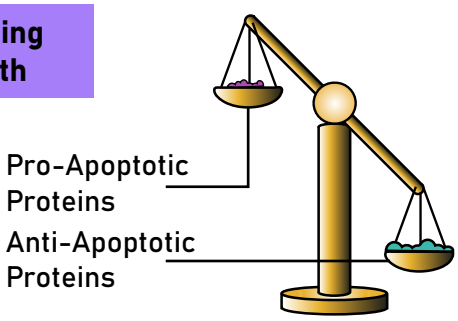
8. Genomic Instability



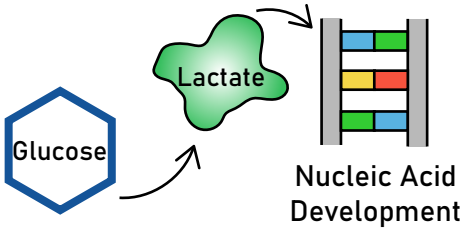
DNA repair prevents somatic mutations in dividing cells. Most cancer cells lose aspects of DNA repair and acquire genomic instability. This allows the cells to gain "driver mutations" in genes, accelerating the progression to malignancy.

Apoptosis is controlled by a balance of proteins that regulate both cell death and proliferation. When the balance is disturbed by tumour cells with overproduction of anti-apoptotic proteins, such as Bcl-2 (B-cell lymphoma 2) cell destruction is inhibited.

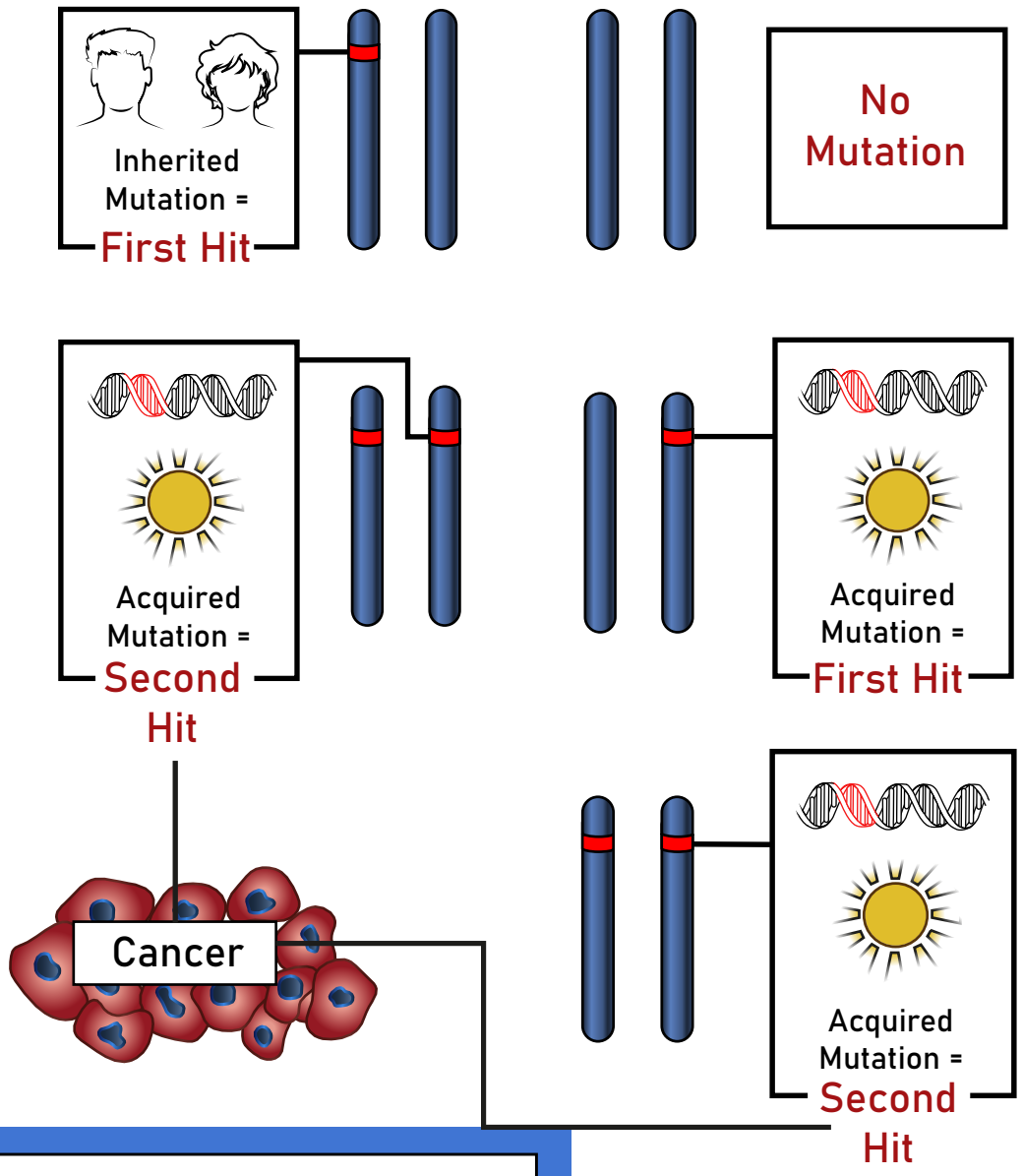
9. Resisting Cell Death



10. Deregulating Cellular Energetics



Aerobic glycolysis converts glucose to lactate in much higher percentages in tumour cells, which encourages the production of amino acid precursors (nucleic acid), therefore stimulating formation of daughter cells.



TWO HIT HYPOTHESIS

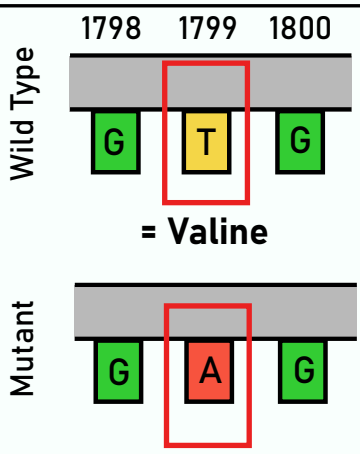
The two hit hypothesis is central to understanding how inherited mutations cause cancer. A cell may need to lose two copies of a gene for it to progress towards malignancy. In many cases, both these mutations arise as two separate somatic mutations. However, one mutation may be inherited, in which case only a single somatic mutation in one cell is required. In an individual who has inherited a mutation, progression to cancer is much more likely at an earlier age. This mechanism has been shown to be important in many cases such as familial retinoblastoma and inherited breast cancer.

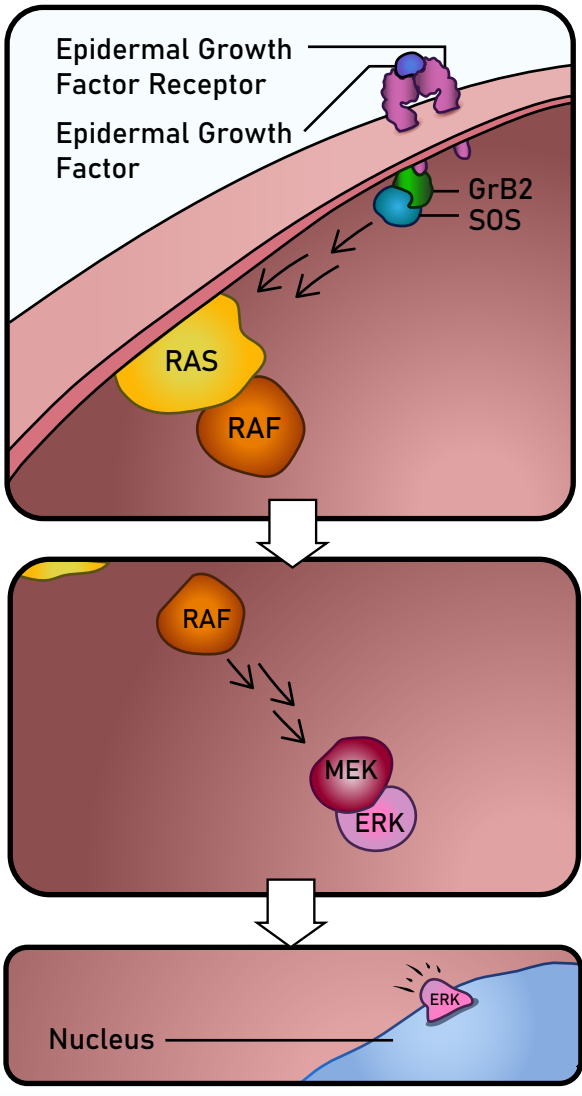
BRAF MUTATION

CANCER IN THE MAPK PATHWAY

Cancer cells acquire multiple mutations as one of the mechanisms of achieving the different hallmarks. An example is the V600E mutation in *BRAF*. This mutation changes a Valine to a Glutamic acid at position 500 in the protein, activating the *BRAF* protein. Activated BRAF drives the MAP kinase signalling pathway, increasing cell proliferation.

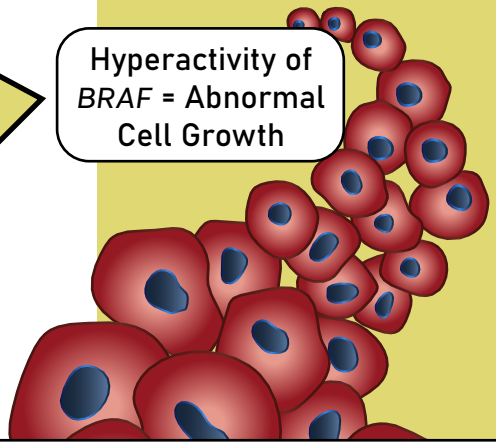
BRAF Exon 15 Sequence V600E



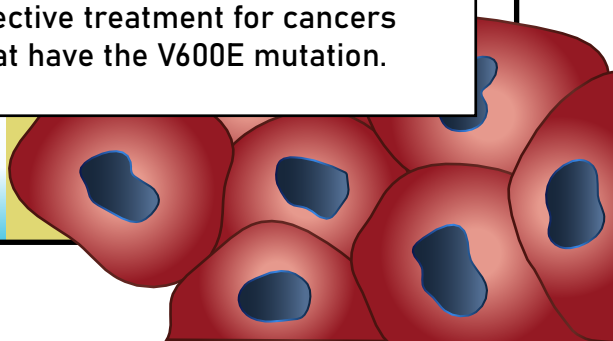


Once the epidermal growth factor attaches to the cell receptors, proteins are recruited to the receptor cytoplasmic tail. The proteins interact with each other and eventually ERK enters the nucleus to activate transcription.

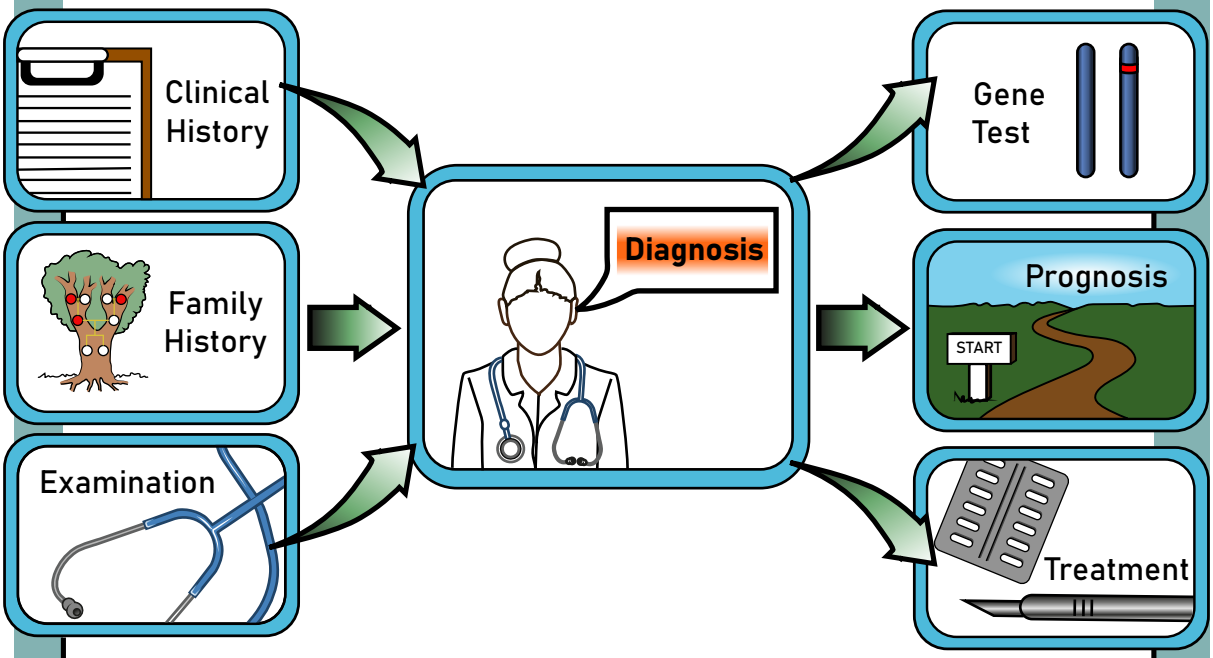
Hyperactivity of *BRAF* = Abnormal Cell Growth



The drug Vemurafenib inhibits the activated *BRAF*, and can be an effective treatment for cancers that have the V600E mutation.



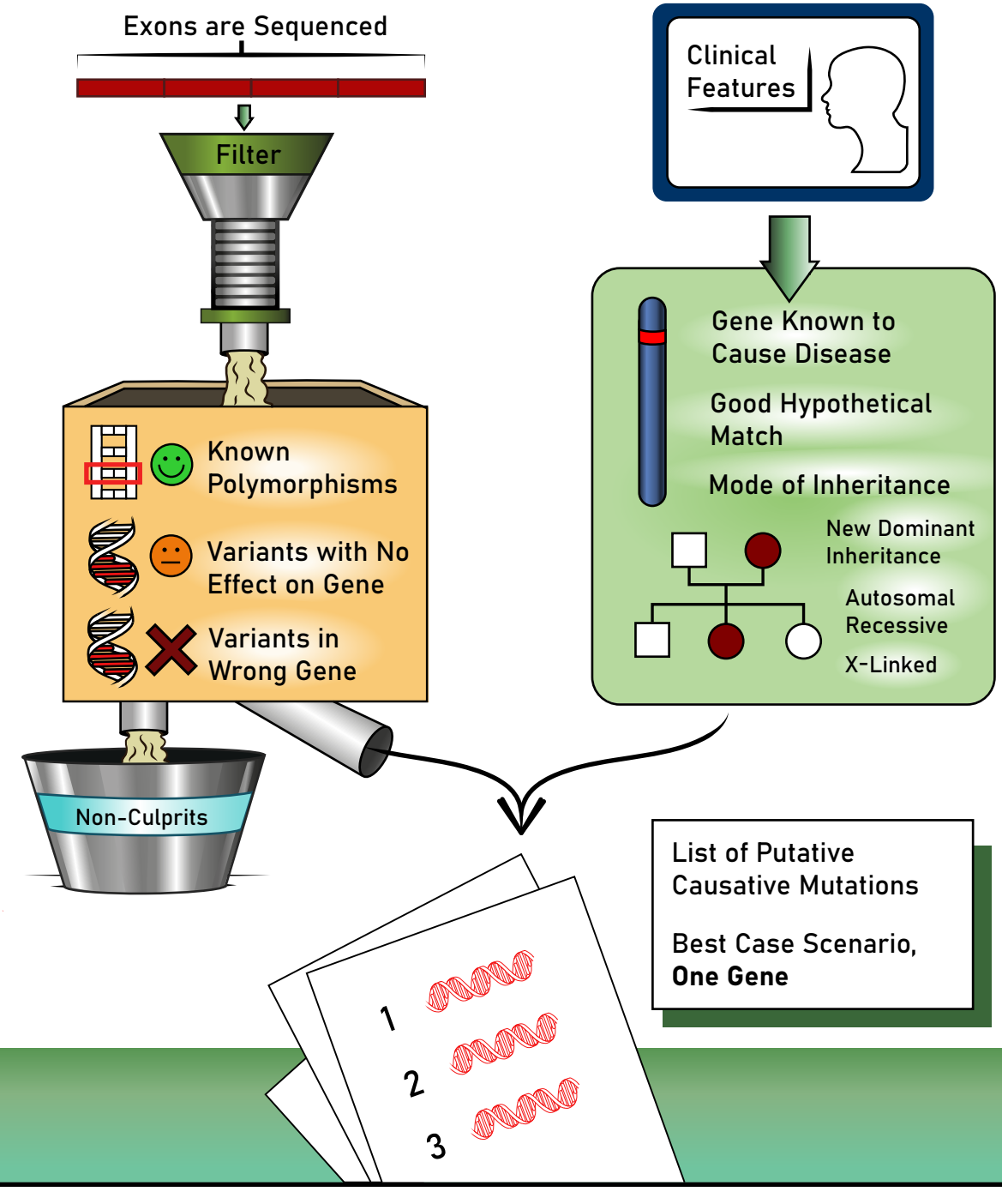
CLASSIC CLINICAL PRACTICE

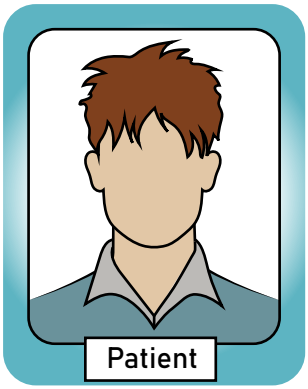


In a Clinical Genetics consultation, the first task is to establish a diagnosis for the patient. As for any branch of medicine, this is done using a combination of clinical history, family history and any findings on clinical examination. The possible diagnosis is essential in guiding genetic testing, as determining treatment, and providing information for the patient.

INTEGRATING
NEXT GENERATION SEQUENCING

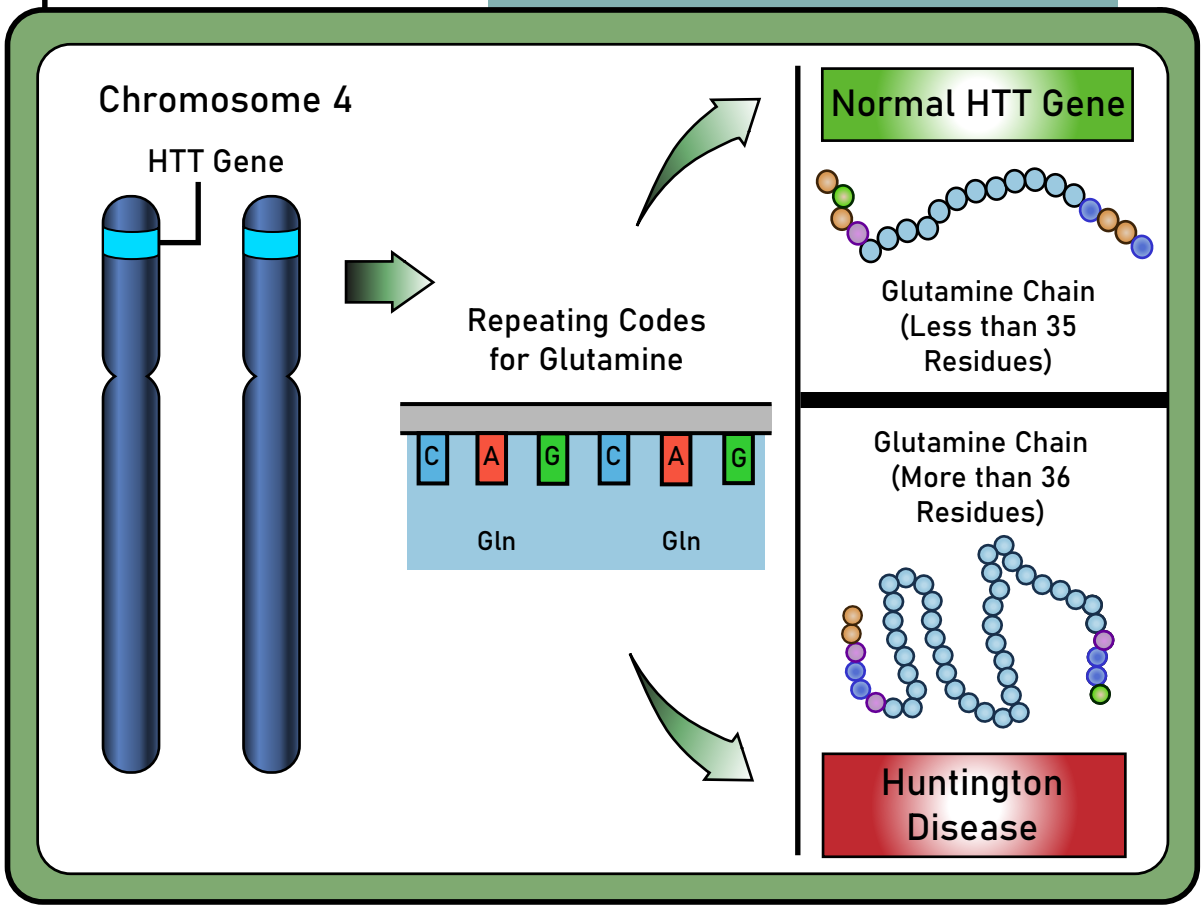
With the ability to sequence the entire genome, clinical genetic practice is changing. Gene sequencing and clinical assessment are integrated to find the genetic cause of a rare disease. Clinical presentation identifies the relevant genes to be included in the analysis. Once a list of possible pathogenic variants is created, clinical and laboratory information are both used to attempt to find the single causative pathogenic variant.



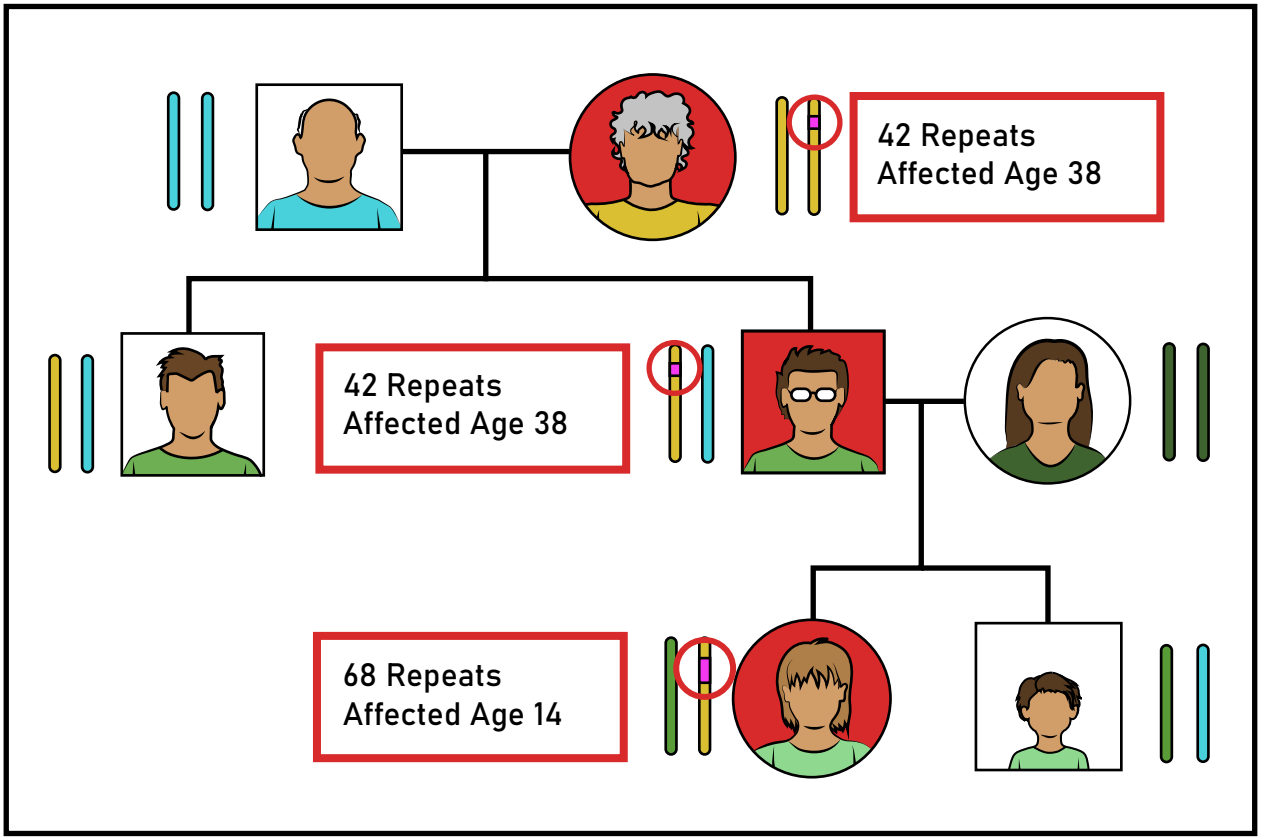


Pre-Symptomatic Testing

In Clinical Genetics, it is possible to test an individual before they have a disease. The patient shown is healthy, but at 50% risk of having a Huntington disease mutation, and therefore of developing a fatal neurodegenerative disease. This pre-symptomatic (or predictive) testing raises a number of issues. The patient will have to consider different issues before deciding whether or not to have the test.

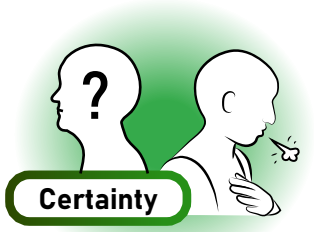


Huntington Disease Genetic Inheritance



Huntington disease (HD) shows a phenomenon called anticipation. The trinucleotide repeat mutation can get longer when transmitted at male meiosis. Disease onset is usually in adult life. Rarely, a child will inherit a very large expansion mutation in the gene, and be affected during childhood.

PRE-SYMPTOMATIC ADVANTAGES



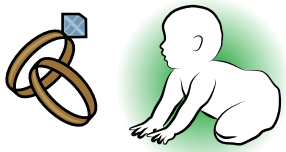
Certainty

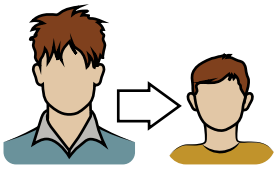
- Potentially obtain reassurance

Potential Future Medical Action/ Stabilization

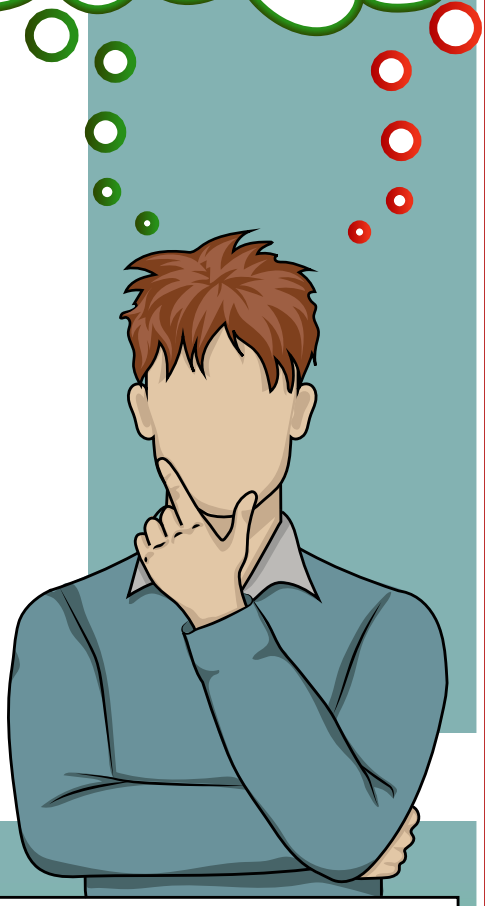
- HD Symptoms can be kept under control with medication, but not cured

Planning for the Future





Inform Children of Genetic Risk



Patient

PRE-SYMPTOMATIC DISADVANTAGES

Mental Health Impact

- Depression
- Survivor guilt
- Future life plans

Social and Work Related Problems

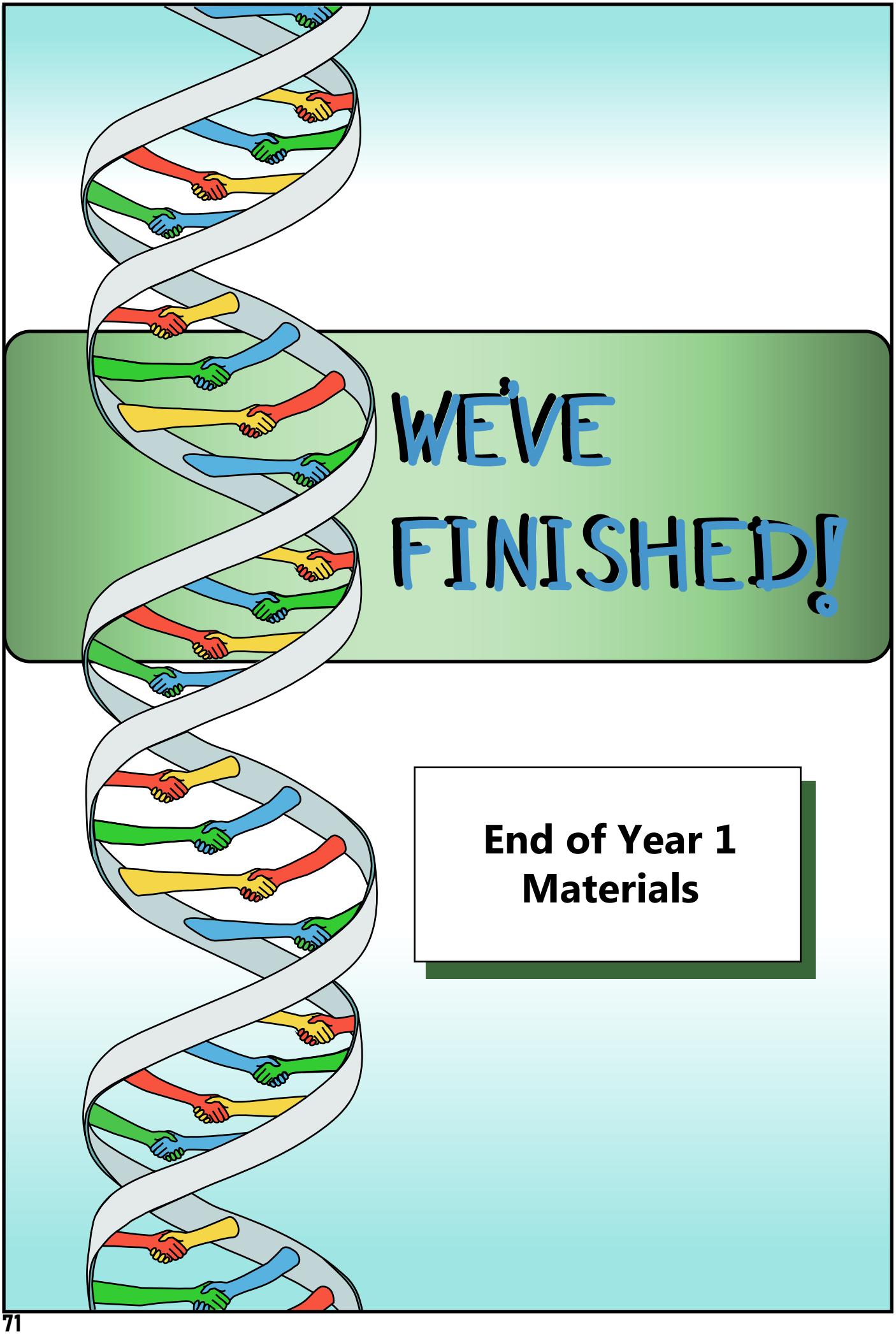
- Disclosure of results
- Discrimination
- Exacerbated stress

Family Issues

- Family skeletons (E.G adoption, non paternity)
- Implications on other family members

Financial Planning

- For example, mortgages



WE'VE FINISHED!

End of Year 1 Materials