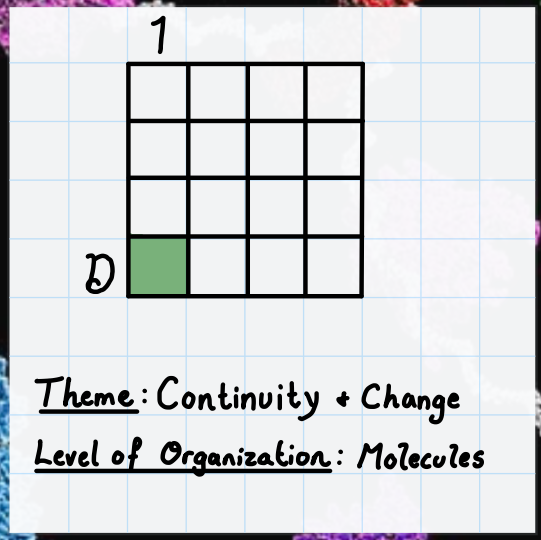
D1.1 DNA REPLICATION

Guiding Questions
How is new DNA produced?
How has knowledge of DNA replication enabled applications in biotechnology?

Linking Questions
How is genetic continuity ensured between generations?
What biological mechanisms rely on directionality?



Theme: Continuity + Change

Level of Organization: Molecules

Written and drawn by:

Peter Marier

NOTEWORTHY SCIENCE

MARIER

SL Learning Outcomes

D1.1.1	DNA replication as production of exact copies of DNA with identical base sequences	Students should appreciate that DNA replication is required for reproduction and for growth and tissue replacement in multicellular organisms.
D1.1.2	Semi-conservative nature of DNA replication and role of complementary base pairing	Students should understand how these processes allow a high degree of accuracy in copying base sequences.
D1.1.3	Role of helicase and DNA polymerase in DNA replication	Limit to the role of helicase in unwinding and breaking hydrogen bonds between DNA strands and the general role of DNA polymerase.
D1.1.4	Polymerase chain reaction and gel electrophoresis as tools for amplifying and separating DNA	Students should understand the use of primers, temperature changes and *Taq* polymerase in the polymerase chain reaction (PCR) and the basis of separation of DNA fragments in gel electrophoresis.
D1.1.5	Applications of polymerase chain reaction and gel electrophoresis	Students should appreciate the broad range of applications, including DNA profiling for paternity and forensic investigations. **NOS:** Reliability is enhanced by increasing the number of measurements in an experiment or test. In DNA profiling, increasing the number of markers used reduces the probability of a false match.

HL Learning Outcomes

D1.1.6	Directionality of DNA polymerases	Students should understand the difference between the 5' and 3' terminals of strands of nucleotides and that DNA polymerases add the 5' of a DNA nucleotide to the 3' end of a strand of nucleotides.
D1.1.7	Differences between replication on the leading strand and the lagging strand	Include the terms “continuous”, “discontinuous” and “Okazaki fragments”. Students should know that replication has to be initiated with RNA primer only once on the leading strand but repeatedly on the lagging strand.
D1.1.8	Functions of DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase in replication	Limit to the prokaryotic system.
D1.1.9	DNA proofreading	Limit to the action of DNA polymerase III in removing any nucleotide from the 3' terminal with a mismatched base, followed by replacement with a correctly matched nucleotide.

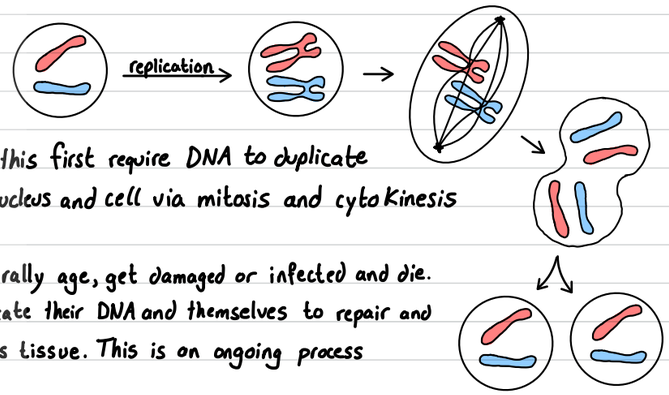
D1.1.1—DNA replication as production of exact copies of DNA with identical base sequences.
D1.1.2—Semi-conservative nature of DNA replication and role of complementary base pairing
D1.1.3—Role of helicase and DNA polymerase in DNA replication

DNA replication: production of exact copies of DNA with identical base sequences

Recall: DNA is a polynucleotide made up of 4 types of nucleotides which differ by their nitrogenous base

nucleic acids A1.2

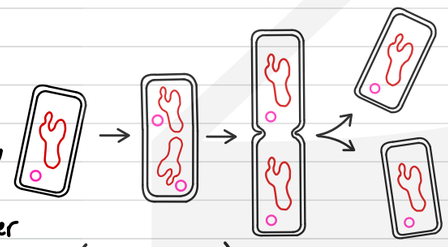
↳ DNA is integral for the functioning of cells and organisms thus, when more cells are produced, DNA must first be replicated completely so the new cell contains the same DNA



↳ DNA replication is required for:

Reproduction in asexual reproduction a parental cell undergoes binary fission where DNA is first replicated and then the mother cell divides to produce two clones (daughter cells)

reproduction D3.1



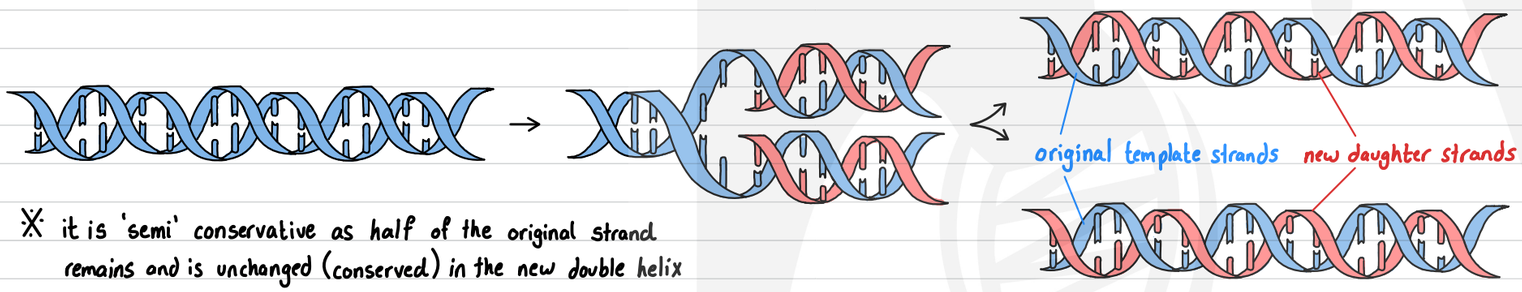
Growth and tissue replacement When multicellular organisms create new cells this first require DNA to duplicate (S phase of cell cycle) followed by division of nucleus and cell via mitosis and cytokinesis

cell + nuclear division D2.1

↳ When multicellular organisms grow, cells proliferate, all with identical DNA in their nucleus

↳ Cells naturally age, get damaged or infected and die. Cells duplicate their DNA and themselves to repair and replace this tissue. This is an ongoing process

DNA replication is **semi-conservative**: each strand of an existing DNA double helix (parent strand) acts as a template for the synthesis of a new strand (daughter strand) from free DNA nucleotides



↳ Due to complementary base pairing, the DNA sequence of one strand determines the other

nucleic acids A1.2

Adenine	Thymine
Guanine	Cytosine

? → TAAGGCGTA

ATTCCGCAT → ATTCCGCAT

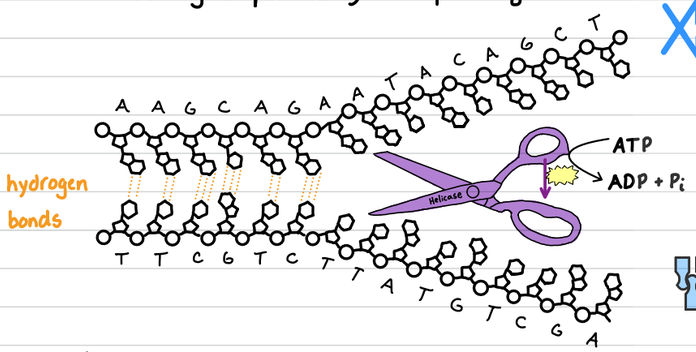
※ it is 'semi' conservative as half of the original strand remains and is unchanged (conserved) in the new double helix

↳ As A always pairs with T and G with C, the newly synthesized base sequence will be identical and be easily checked for errors in pairing - ensuring a high degree of accuracy

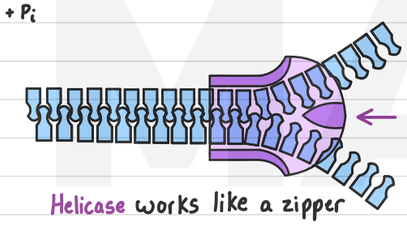
Helicase: enzyme responsible for unwinding and separating DNA double helix thereby exposing the nitrogenous bases

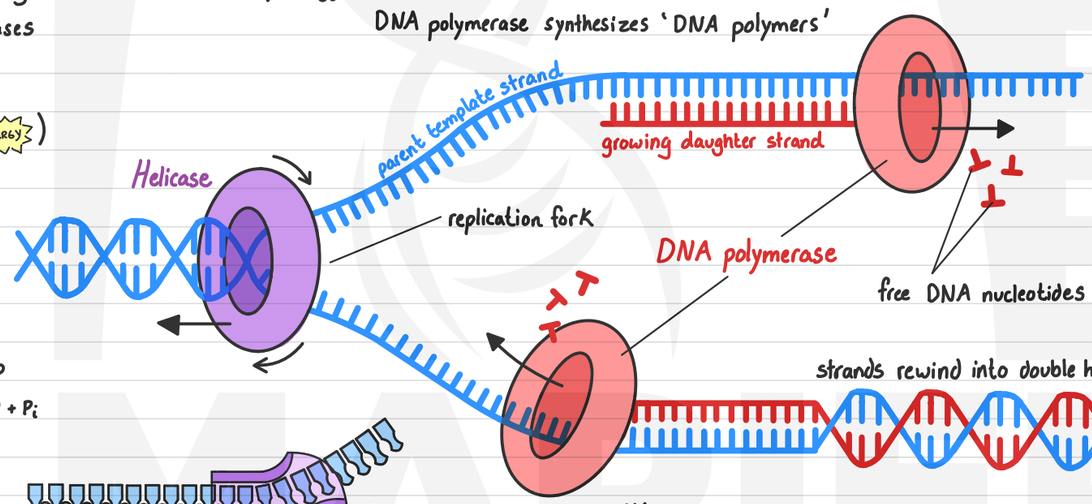
※ etymology: Helicase works on 'helix'
DNA polymerase synthesizes 'DNA polymers'

↳ helicase binds to DNA and using the energy from ATP-hydrolysis ($ATP \rightarrow ADP + P_i + ENERGY$) it catalyzes the breaking of hydrogen bonds holding complementary base pairs together



↳ the action of helicase results in two single-stranded DNA strands which will act as templates



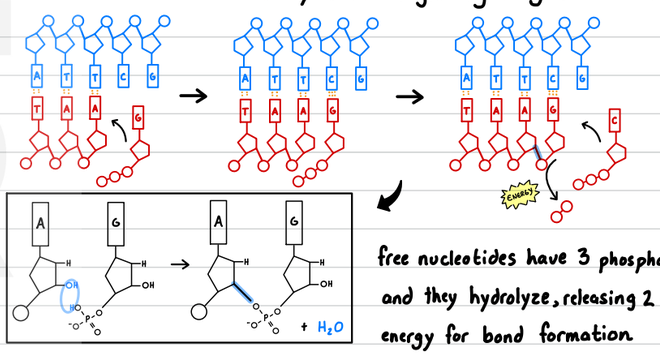


※ In addition, DNA polymerase acts as a 'proofreader' where it can remove and replace bases added incorrectly → more in HL (an uncorrected error results in a mutation)

DNA polymerase: enzyme responsible for synthesizing a new complementary DNA strand using free DNA nucleotides

↳ helps bring free DNA nucleotides close to exposed bases on the DNA template strand, allowing hydrogen bonds to form

↳ DNA polymerase catalyzes condensation reactions: catalyzing covalent phosphodiester bond between the phosphate of a free nucleotide to the deoxyribose of growing daughter strand



free nucleotides have 3 phosphates and they hydrolyze, releasing 2 and energy for bond formation

D1.1.4 — Polymerase chain reaction and gel electrophoresis as tools for amplifying and separating DNA

Polymerase Chain Reaction (PCR): automated technique for amplifying selected regions of DNA through multiple cycles of DNA synthesis

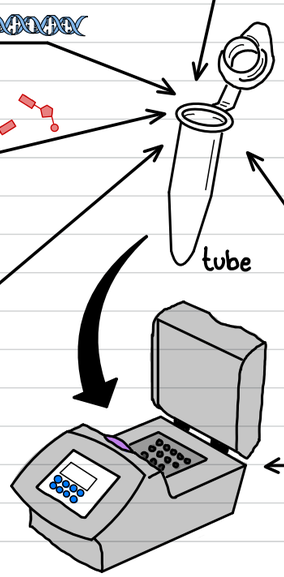
↳ with every cycle, the DNA sample is doubled, thus 30 cycles results in >1 billion copies (2^{30})

↳ what is required:

target DNA sample
extracted sequence of DNA

free DNA nucleotides
A, T, C, G nucleotides for use in DNA synthesis

Buffer solution
provides optimal pH conditions for Taq Polymerase activity



Taq Polymerase
heat-tolerant polymerase enzyme which synthesizes complementary DNA strands using free nucleotides. Enzyme is extracted from *Thermus aquaticus* (Taq) - a hot spring bacterium which has a high optimum temperature (72°C) and can withstand high heat without denaturing

DNA primers
short, single-stranded DNA sequences which are specific to the target sequence - binding complementarily to 3' end of each sequence, allowing Taq Polymerase to bind and begin replicating

Thermal cycler
machine which automatically cycles between specific temperatures required for the reaction

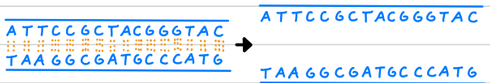
↳ process entails cycling through the following 3 steps over and over until the desired amount is produced

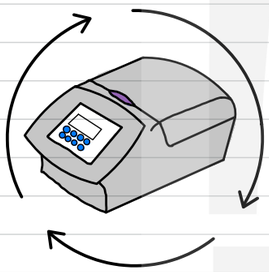
1 Denaturation

thermal cycler set to 95°C

↳ high heat breaks the hydrogen bonds between complementary base pairs, separating DNA helices

※ Taq Polymerase does not denature





2 Annealing

thermal cycler set to 45°C - 60°C

↳ lower temperature allows DNA primers to bind to the start of the target sequence on each separated strands

※ large number of primers ensure that they bind to DNA, preventing strands rejoining

※ two different primers are used - one complementary to each strand

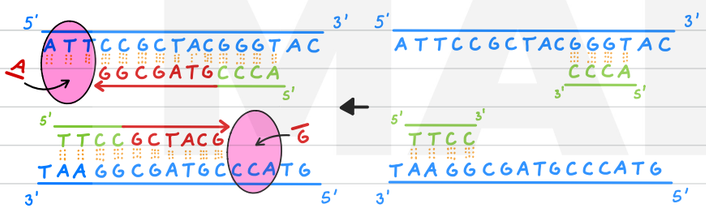
3 Elongation

thermal cycler set to 72°C

↳ Taq Polymerase binds onto DNA primers and synthesizes complementary DNA strands using free nucleotides

↳ amount of DNA has doubled

※ 72°C is optimum for Taq Polymerase

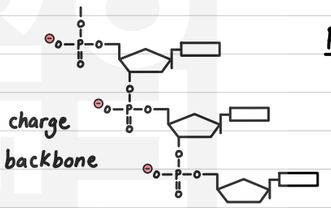


Gel electrophoresis: process used to separate fragments of DNA or proteins on a polymer gel, according to size and overall charge

※ electrophoresis: movement of charged particles in a fluid/gel in an electric field

↳ In order to be separated, samples must ① have uniform charge (-) and ② be small enough to move through gel

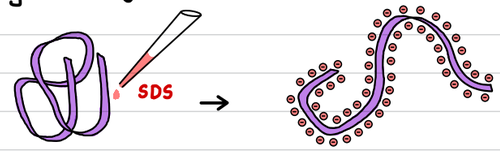
DNA sample preparation



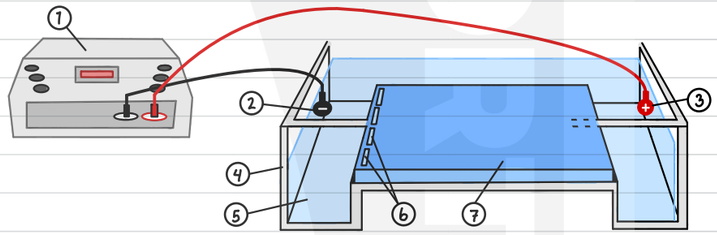
- DNA has an overall negative charge due to ⊖ phosphate groups on backbone
- DNA is a very long molecule, too large to be separated so **restriction endonuclease** enzymes are used to cut DNA at specific base sequences, producing different DNA fragments

Protein sample preparation

- Proteins do not have overall charge and can be large due to their many different R groups and how they fold, so a detergent (SDS) is applied to denature (unfold) and give the proteins an overall negative charge

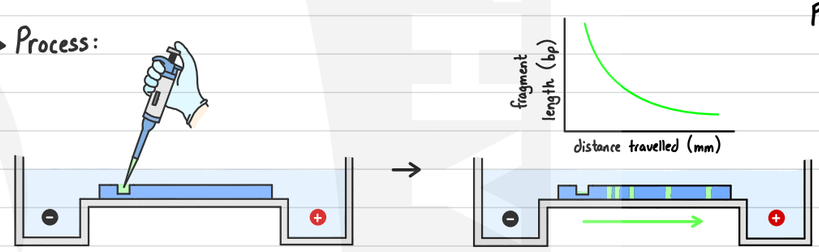


↳ Gel electrophoresis setup:



① power supply
② cathode (-)
③ anode (+)
} creates electric field: ⊖ at sample wells, ⊕ at opposite end
④ gel chamber - holds gel
⑤ buffer solution - electrolyte solution to conduct electricity
⑥ sample wells - indents where samples are loaded
⑦ gel - made of agarose (from seaweed) or polyacrylamide, both clear and porous

↳ Process:



1 Sample is injected into the well at the top of the gel using a micropipette. Additionally, a sample of known lengths/mass is placed (ladder) where this can be used as a comparison

※ for DNA, many copies are made and used via PCR

2 Molecules migrate down towards attractive anode ⊕ at different rates through the pores of the gel: smaller molecules pass through easier ∴ travel faster and further down

※ Molecules of the same length/mass will travel at the same rate and group together in a band

3 After smallest molecules have reached the bottom, voltage is turned off and gel removed and stained with a dye (which in the case of DNA, fluoresces under UV light)

Bands are compared with ladder and with each other

D1.1.5—Applications of polymerase chain reaction and gel electrophoresis

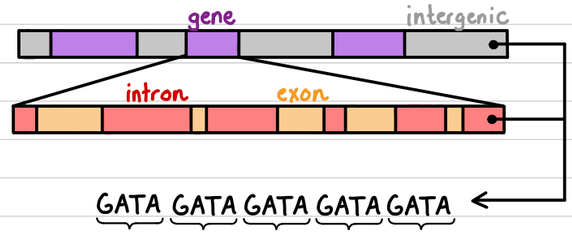
PCR and gel electrophoresis have many applications:

DNA profiling (fingerprinting): the identification of individual organisms or species using DNA

↳ this technique compares DNA base sequences for different individuals - looking for similarities and differences
∴ base sequences used must be unique between individuals

↳ When using DNA to identify individuals, it is not feasible or necessary to compare the entire genome - instead only select variable regions are amplified and compared → STR sequences

※ Eukaryotic DNA is primarily composed of non-coding sections, i.e. sequences which do not code for proteins including those in between genes (intergenic) and within genes (introns)

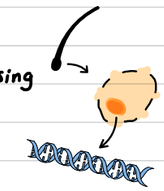


↳ Some of these sequences are **Short-Tandem Repeats (STRs)** which are sections 2-7 base pairs which are repeated consecutively one after another. The amount of repeats vary between individuals

ex: this sequence has 5 repeats in one individual but could have 8 repeats in another - showing a difference

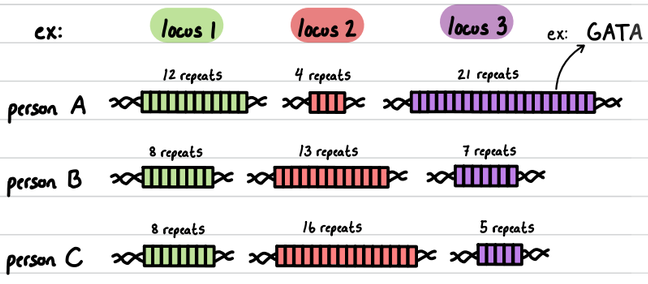
Procedure

1 Collect cell sample from individuals. Using detergents, cells are lysed and DNA within cell is extracted for analysis



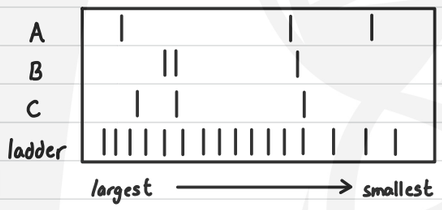
2 Several STR sequences (loci) are selected for analysis
↳ the FBI's DNA database - CODIS (Combined DNA Index System) uses 20 STR loci
※ note: like most genes, individuals inherit 2 STR alleles per loci - one from each parent

ex:



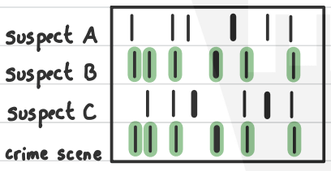
3 STR sequences are amplified using PCR
↳ DNA Primers specific to the STR sequences are used as initiators to replication
↳ PCR is important as very little of the subject's DNA needs to be used and it allows clear differences in size to be determined as many copies are made

4 Gel electrophoresis separates the tested STR fragments by size (fragments with fewer repeats are smaller and travel further down gel) - producing a banding pattern and a DNA profile



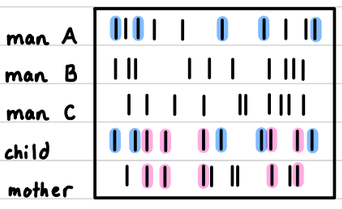
Applications of DNA profiling

① **Forensics analysis** - DNA can be obtained from crime scenes (or on a victim of a crime) and compared with suspects to determine a probability of guilt or innocence - 100% match is required. Samples can come from any cells with DNA (blood, skin, hair, sperm, saliva, etc.)



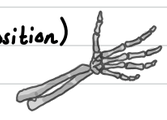
Suspect B's DNA profile 100% matches DNA from crime scene / evidence
∴ very strong probability

② **Paternity test** - In cases where the father of a child is unknown, DNA from the mother, child, and potential fathers are compared
※ child's DNA pattern is a combination of the mother's and father's - resulting in ~50% match

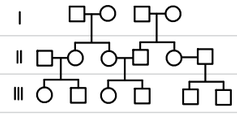


child inherits all their bands from parents:
1- match all bands with child and mother
2- father matchs the rest

③ **Corpse identification** - DNA from corpses whose identities are unknown (due to damage or decomposition) can be compared with others to determine identity

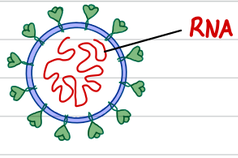


④ **Familial relationships** - DNA from different individuals can be compared to determine relatedness such as siblings, half-siblings and cousins

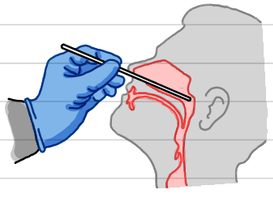


Reverse-Transcriptase (RT)-PCR diagnostic technique used to assess the presence of a specific RNA such as those in a virus

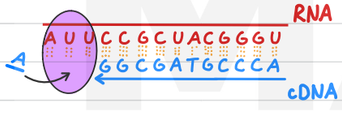
↳ SARS-CoV2 virus uses RNA as its genetic material and travels in droplets in the air



1 Sample is collected where virus may be located (ex: nose)



2 Sample exposed to reverse transcriptase which creates complementary DNA (cDNA) from RNA (if present)



3 PCR is done using fluorescent primers, specific to the virus' gene sequence

4 After many cycles the DNA is analyzed

\+ Positive test
fluorescence indicates presence of viral RNA

− negative test
no fluorescence indicates no viral RNA present

※ PCR ensures that even a small amount of viral RNA will be detected

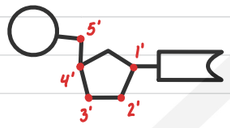
NOS **reliability**: the trustworthy nature of a result in science is largely a factor of how consistently the same result can be obtained if a method is repeated over and over

※ reliability is enhanced by increasing the number of measurements in a test

↳ it is possible that two individuals share the same STR at a locus - this can be determined by the frequency of that sequence in a population. If a person's DNA matched another, this is a false positive and could lead to incorrect conclusions
↳ By comparing many different STR markers (previously 13 now 20) the points of comparison increases and the likelihood 2 individuals match on all loci is near zero (note: monozygotic twins have identical DNA profiles)

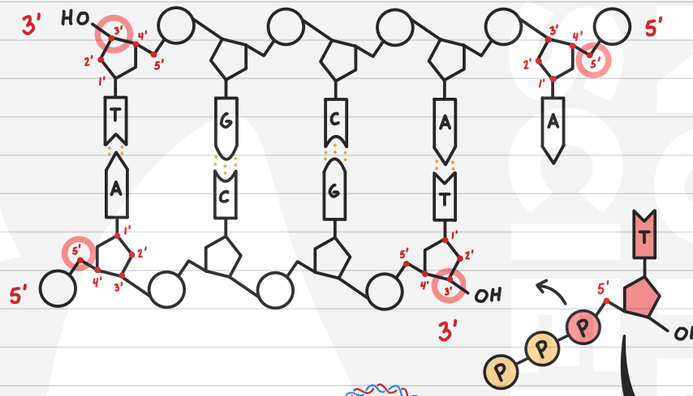
HL D1.1.6—Directionality of DNA polymerases. D1.1.7—Differences between replication on the leading strand and the lagging strand. D1.1.8—Functions of DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase in replication. D1.1.9—DNA proofreading

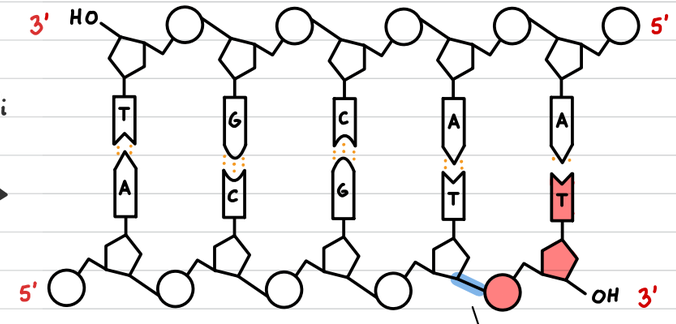
nucleic acids AI.2 DNA strand orientation/direction can be determined by the carbon position



↳ DNA polymerase III requires free 3'-OH group to start synthesis of DNA, where it catalyzes a condensation reaction between the phosphate of the 5' end of a free deoxynucleotide triphosphate to the OH on the 3' end of the new strand, forming a phosphodiester bond This is possible from the energy released from cleaving the phosphate
∴ it can only function in one direction:

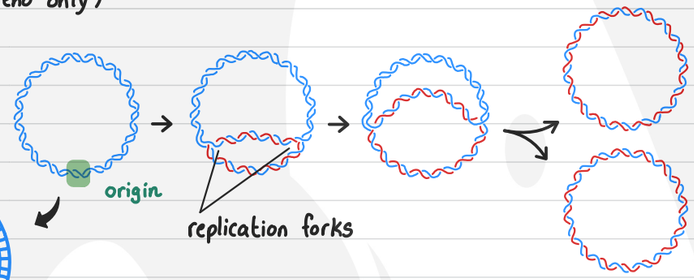
- moving along template strand in a 3' to 5' direction
- synthesizing DNA in a 5' to 3' direction (adding to 3' end only)

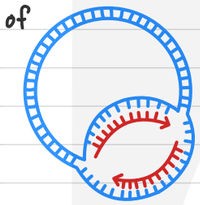




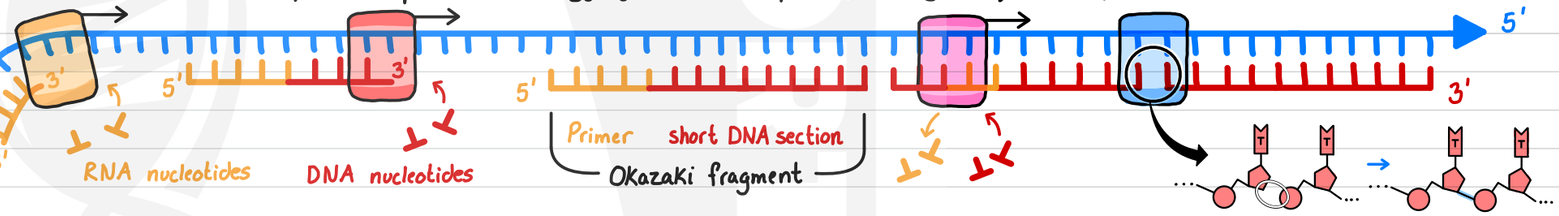
DNA replication in prokaryotes

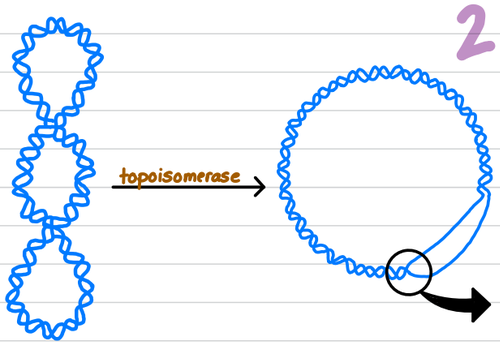
↳ DNA in prokaryotes is arranged as a single, looped chromosome
↳ when they replicate their DNA, there is one origin of replication, producing two replication forks where replication proceeds in either direction - 5' to 3'
↳ the process is carried out by several different enzymes working together



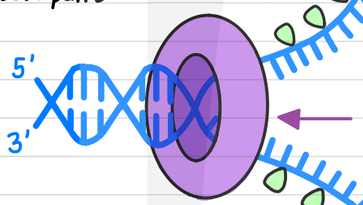


Discontinuous DNA replication produces the lagging strand, in pieces, moving away from replication fork - slower process



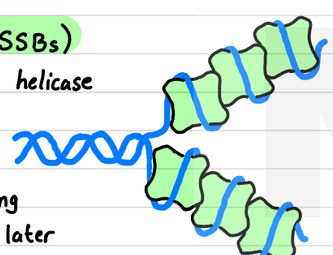


2 Helicase separates DNA strands by breaking hydrogen bonds between complementary base pairs



1 topoisomerase enzymes (ex: gyrase) relieve stress in DNA and unwinds DNA from supercoiled state ahead of helicase

3 single-stranded binding proteins (SSBs) bind to single-stranded DNA after helicase has separated strand in order to stabilize strand and prevent DNA from reannealing (rejoining), allowing free nucleotides to bind. They are later removed as polymerases start replicating

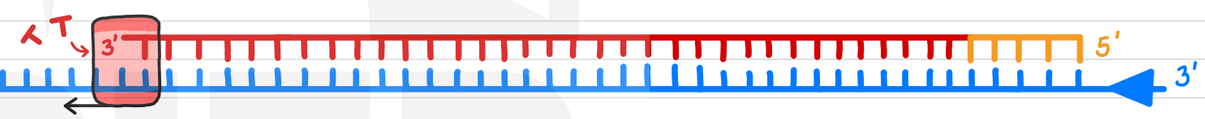


4 DNA Primase synthesizes a complementary RNA primer strand by catalyzing phosphodiester bonds between free RNA nucleotides. This RNA primer acts as starting point for DNA polymerase III

5 DNA polymerase III binds to RNA primer and synthesizes a complementary DNA strand by joining nucleotides to 3' -OH end (5' to 3')

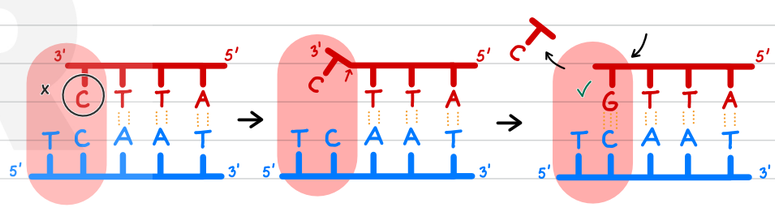
6 DNA polymerase I Removes RNA nucleotides of primer from 5' end and replaces them with DNA added to the 3' end of next fragment, leaving a gap

7 DNA Ligase joins Okazaki fragments together by catalyzing phosphodiester bonds between gap in fragments, forming a continuous strand
※ ligation = join together



Continuous DNA replication produces the leading strand continuously and uninterrupted, towards the replication fork - faster process

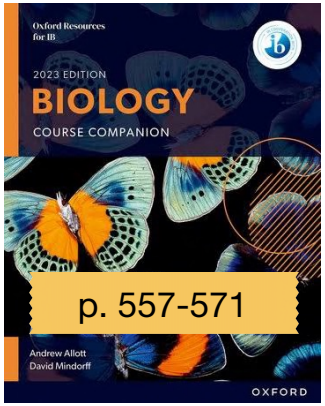
※ DNA polymerase III also acts as a proofreader as it moves along and synthesizes daughter strand:
① identifies mispaired nucleotide in daughter strand
② removes incorrect nucleotide from 3' terminal
③ replaces with correctly matched nucleotide



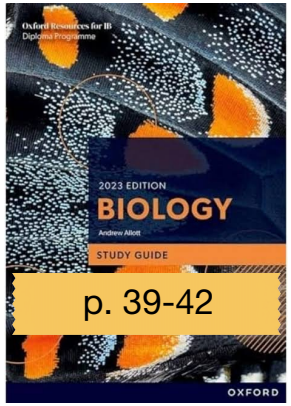
Resource Links

each resource is hyperlinked

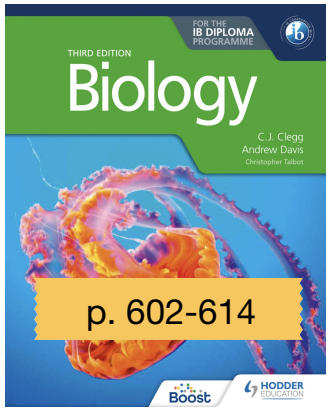
Textbooks



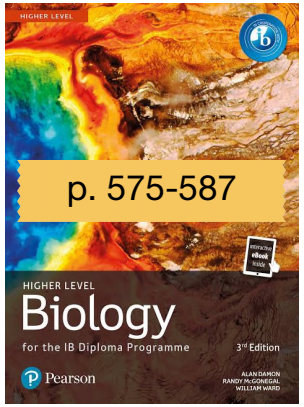
p. 557-571



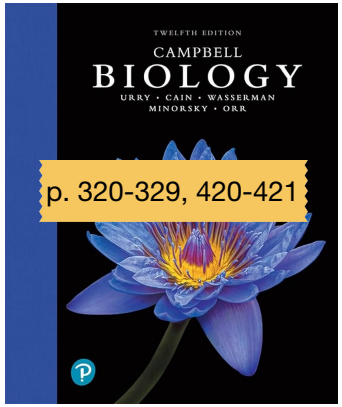
p. 39-42



p. 602-614

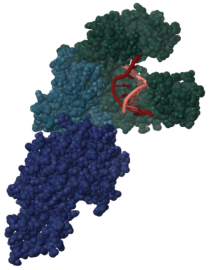


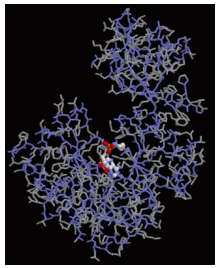
p. 575-587

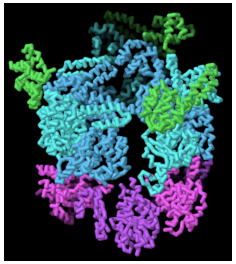


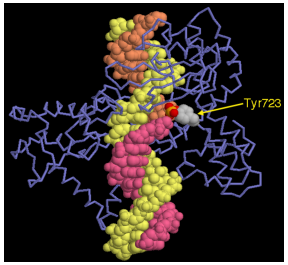
p. 320-329, 420-421

3D models

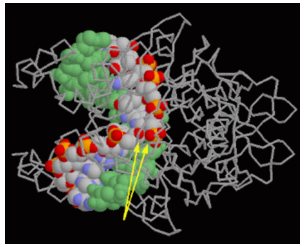

DNA polymerase


DNA Ligase


DNA Helicase



Topoisomerases


Restriction enzymes

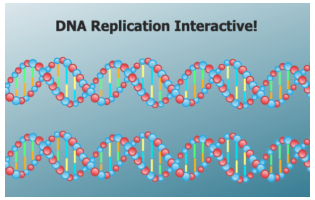
Articles

Alamoudi, E., Mehmood, R., Albeshri, A., & Gojobori, T. (2018). DNA Profiling Methods and Tools: a review. Springer eBooks, 216–231. https://doi.org/10.1007/978-3-319-94180-6_22

Meselson, M., & Stahl, F. W. (1958). The replication of DNA in Escherichia coli. Proceedings of the National Academy of Sciences, 44(7), 671–682. https://doi.org/10.1073/pnas.44.7.671

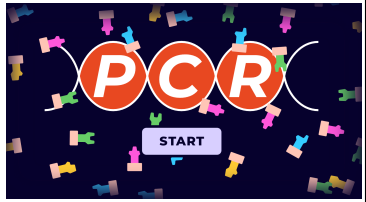
Zhu, H., Zhang, H., Xu, Y., Laššáková, S., Korabečná, M., & Neužil, P. (2020). PCR Past, present and future. BioTechniques, 69(4), 317–325. https://doi.org/10.2144/btn-2020-0057

Simulators / Interactives

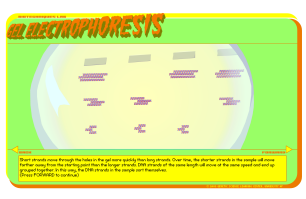


DNA replication

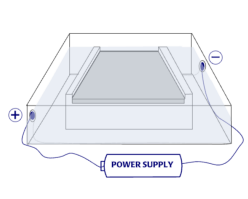
DNA replication enzymes



PCR



Gel electrophoresis



Gel electrophoresis