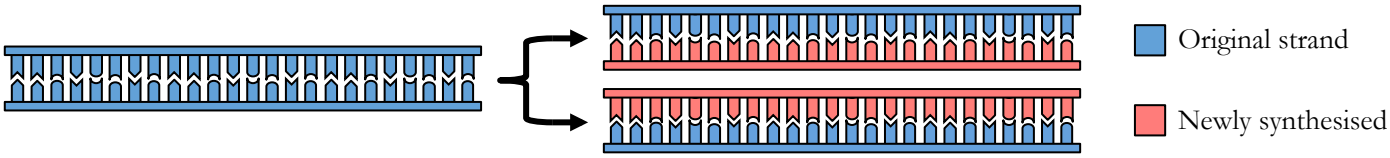
TOPIC 2.7: DNA REPLICATION

Semi-Conservative

DNA replication is semi-conservative – one strand is from an original template molecule and one strand is newly synthesised

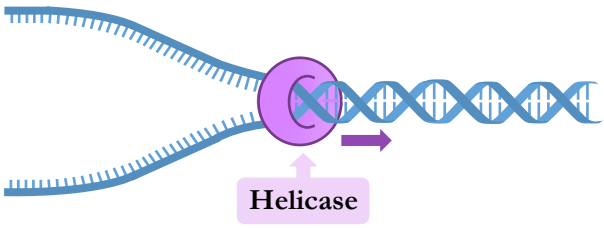
- This occurs because each base will only pair with its complementary partner and thus ensure the sequence is conserved



DNA Replication

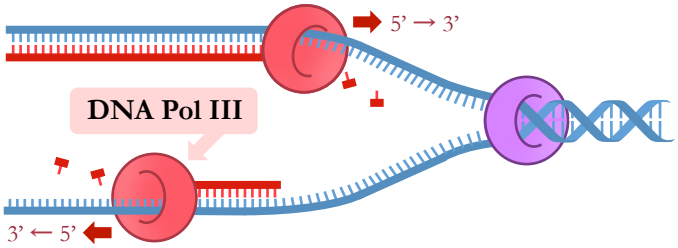
Helicase:

- Unwinds and separates the double stranded DNA
- Breaks the hydrogen bonds between the base pairs



DNA Polymerase III

- Free nucleotides line up opposite complementary partners
- DNA Pol III covalently joins the free nucleotides together



Meselson-Stahl Experiment

The Meselson-Stahl experiment supported the theory that DNA replication occurred via a semi-conservative process

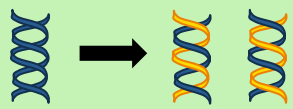
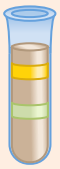
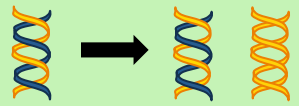
They incorporated radioactive nitrogen isotopes into DNA

- Templates were prepared with heavier ^{15}N
- New sequences were replicated with lighter ^{14}N

The DNA was then separated via centrifugation in order to determine its composition of radioisotopes

- **1st division:** DNA had ^{15}N and ^{14}N (i.e. mixed)
- **2nd division:** DNA is mixed or has ^{14}N only

The results were consistent with a semi-conservative model

	ACTUAL RESULT	SEMI-CONSERVATIVE
1st Division	 N-15 / N-14	 N-15 → Mix (×2)
2nd Division	 N-14 only N-15 / N-14	 Mix → Mix N-14

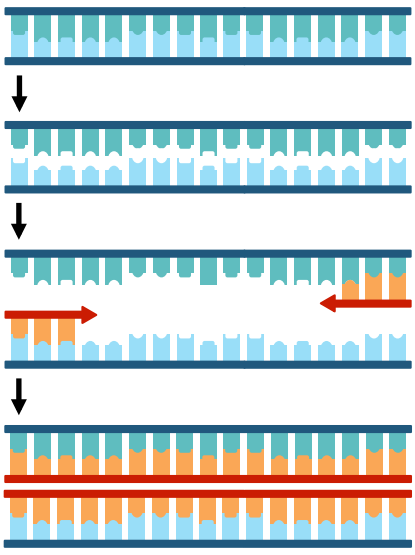
Polymerase Chain Reaction

The polymerase chain reaction (PCR) is an artificial method of DNA replication that is used to rapidly copy sequences

PCR occurs in a thermal cycler over three repeating steps:

- **Denaturation:** DNA heated in order to separate strands
- **Annealing:** Primers attach to ends of a target sequence
- **Elongation:** A heat-tolerant polymerase copies strands

A standard reaction of 30 cycles would generate 2^{30} copies of the target DNA sequence (i.e. >1 billion copies of DNA)



1. Denaturing

Strands separate

(95°C)

2. Annealing

Primers bind

(55°C)

3. Extension

Taq polymerase

(72°C)