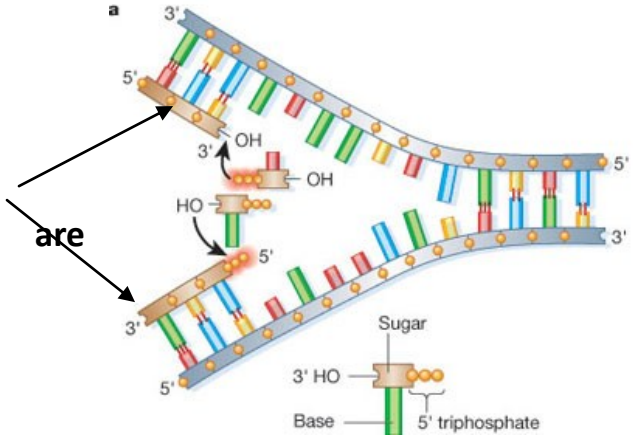
Key Area 2 Replication of DNA

Prior to cell division, DNA is replicated by **DNA Polymerase.**

DNA Polymerase needs PRIMERS to start replication. A Primer is a short strand of nucleotides which binds to the 3' end of the template DNA strand allowing the DNA Polymerase to add DNA Nucleotides.

Primers **are**



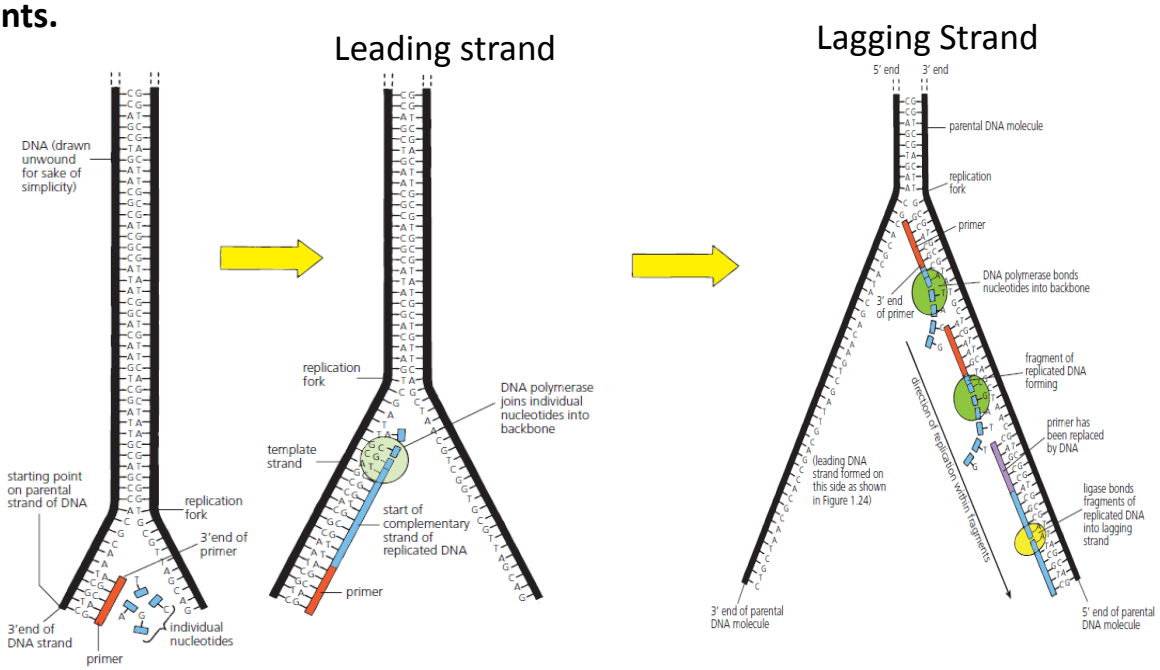
DNA is **unwound** (by DNA Polymerase) and **Hydrogen bonds between the bases broken** to form 2 template strands.

DNA Polymerase adds DNA Nucleotides, using complimentary base pairing, to **the deoxyribose (3') end of the new DNA strand** which is forming.

DNA Polymerase can only add DNA Nucleotides in one direction, resulting in the **Leading Strand being replicated continuously** and the **Lagging Strand being replicated in Fragments.**

Leading strand

Lagging Strand



Fragments of DNA on the Lagging strand are joined together by **LIGASE.**

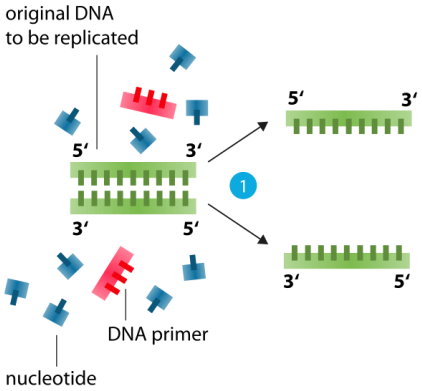
Polymerase Chain Reaction (PCR)

PCR **AMPLIFIES DNA** using complimentary primers for specific target sequences.

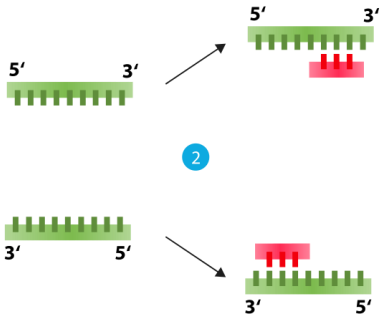
In PCR, primers are short strands of nucleotides which are complimentary to specific target sequences at the 2 ends of the region of DNA to be amplified.

Repeated cycles of **HEATING & COOLING** amplify the target region of DNA.

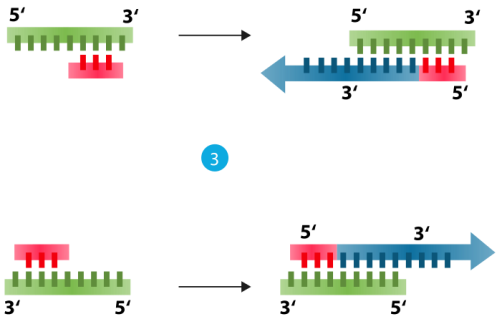
1. **DNA is heated to between 92 and 98°C to separate the strands**.



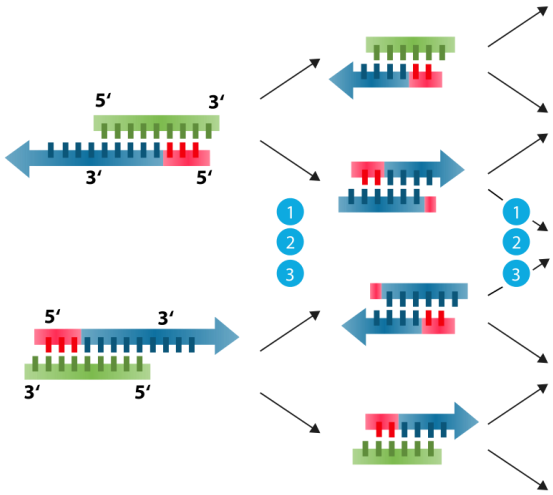
2. **It is then cooled to between 50 and 65°C to allow Primers to bind to target sequences.**



3. **It is then heated to between 70 and 80°C for HEAT-TOLERANT DNA Polymerase to replicate the region of DNA.**



4. The cycle is then repeated.



Each cycle DOUBLES the amount of DNA present.

Example:

1 copy	→	2	→	4	→	8	→	16	→	32	→	64	→	128 copies
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6		Cycle 7	

After 7 PCR Cycles, 128 copies of the original DNA target sequence are produced.

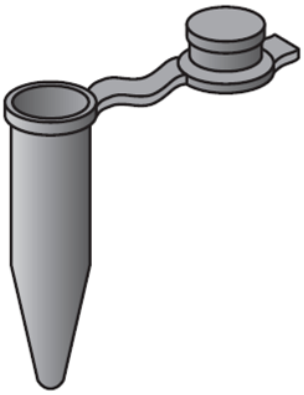
Requirements for PCR

PCR requires:

1. **A DNA Template**
2. **A Supply of the 4 types of DNA Nucleotides (A,T,C &G)**
3. **Primers**
4. **Heat-tolerant DNA Polymerase (enzyme)**
5. **A pH Buffer (to create optimum conditions for enzyme activity)**

Contents of tube

– DNA
– DNA nucleotides
– primers
– enzyme and buffer



Practical Applications of PCR

PCR can amplify DNA for use in the following applications:

1. To help **SOLVE CRIMES** (Forensic evidence).
2. Settle **PATERNITY SUITS**
3. **Diagnose Genetic Disorders.**