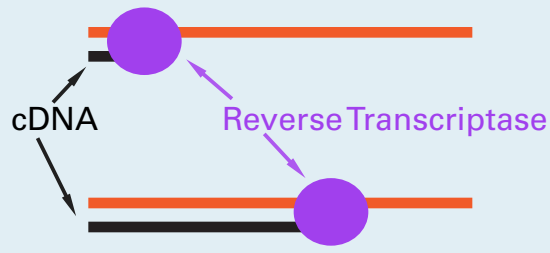
RT-PCR & PCR: Polymerase Chain Reaction

REVERSE TRANSCRIPTASE STEP

A. Anneal anchored oligo (dT) primers, random primers, or specific primers.

Viral RNA

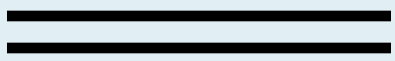
B. First strand synthesis.



C. RNase H function degrades the RNA portion leaving the cDNA.

D. Goto first cycle PCR.

cDNA



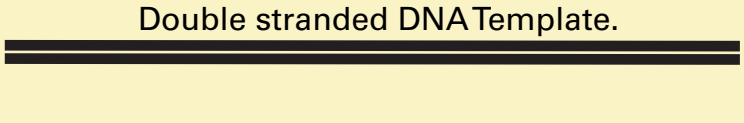
PCR INGREDIENTS

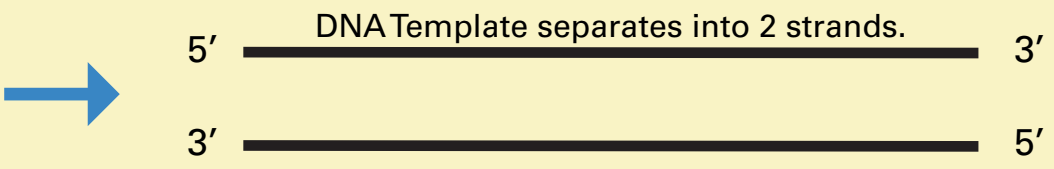
- DNA template (this is what you want to copy).
- Primers that start the PCR reaction. These primers are specific for the piece of DNA you want to copy.
- You need the building blocks Adenine, Cytosine, Guanine, Thymine, so that you can make more copies of the DNA.
- Thermostable DNA polymerase, Taq polymerase (Thermus aquaticus) enzyme. Taq polymerase comes from the bacteria Thermus aquaticus or T. aquaticus. Thermostability is important because the PCR process involves repeated cycles (20-40 cycles) of heating and cooling.
- Buffer to help stabilize/maintain conditions for the enzyme to work to build copies that you want.

1. DENATURING

Heat to 96° C. This breaks the hydrogen bonds and separates the double-helical DNA strand into two strands. This takes 15-30 seconds.

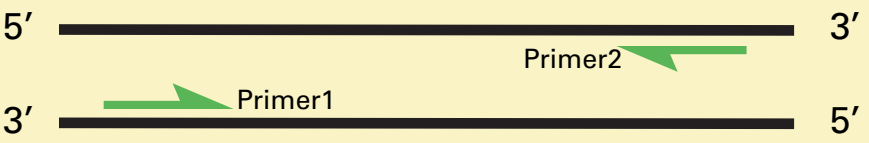
Double stranded DNA Template.





2. ANNEALING

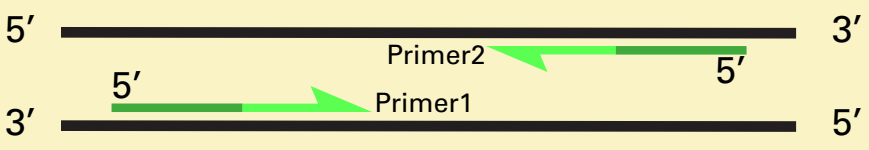
Primers 1 and 2 bind to each strand of the original DNA template.



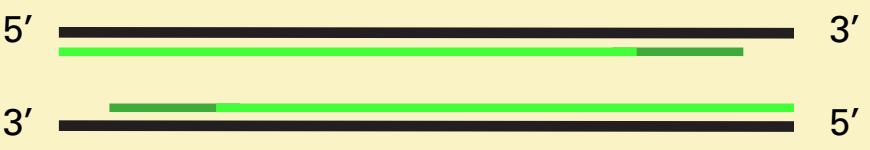
Cool to 55-65° C. Lowering the temperature allows primers bind to each strand via hydrogen bonds. This takes 10-30 seconds.

3. EXTENDING

Taq polymerase extend the primers.



Result after one cycle. The cycle repeats 20-40 times.



Heat to 72° C. Optimum temperature allows *Taq* polymerase to extend the primers from 3′ to 5′ thereby building a new DNA strand off the original template. Approximate rate is 1000 DNA bases (1Kb)/min.