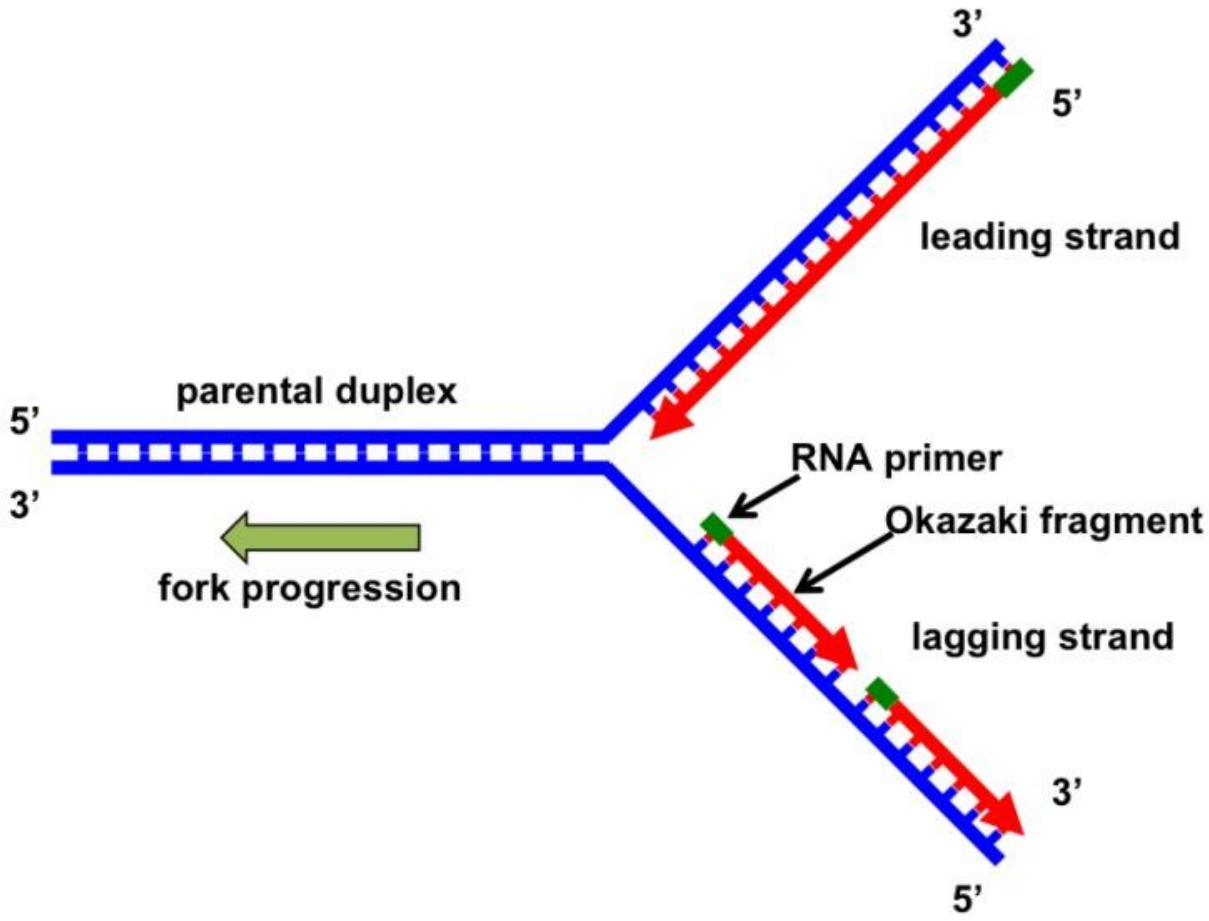
in vitro methods in studies of nucleic acids

Polymerase chain reaction (PCR)

DNA replication

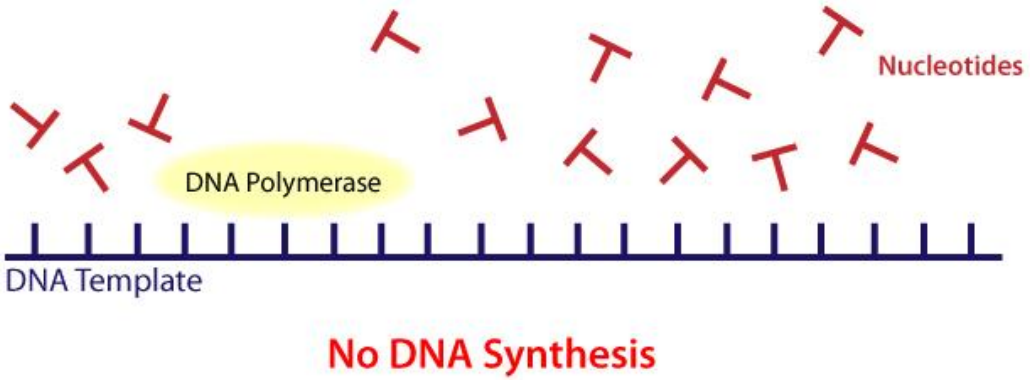


In vitro replication of DNA

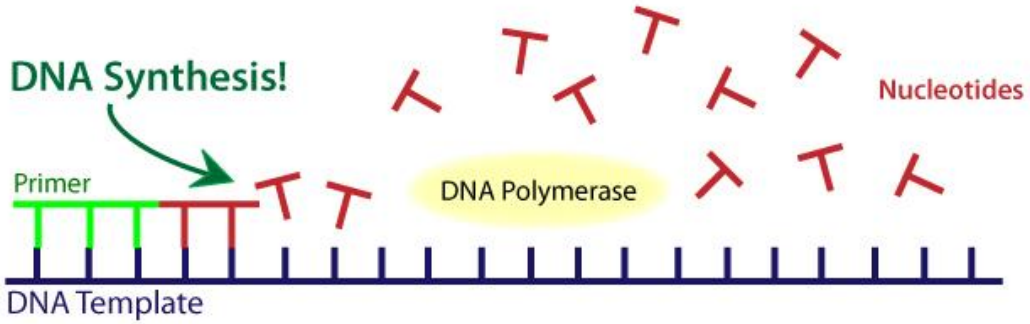
DNA can be replicated *in vitro*. The term *in vitro* (Latin “in glass”) indicates that the experiment is conducted in a test tube. The reaction requires:

- DNA template (single or double stranded)
- Sequence-specific primer
- Deoxyribonucleotide triphosphates (dNTP)
- DNA polymerase enzyme.
- Appropriate buffer (pH, ionic strength, Mg^{2+} etc)

Primer extension reaction.



No DNA Synthesis



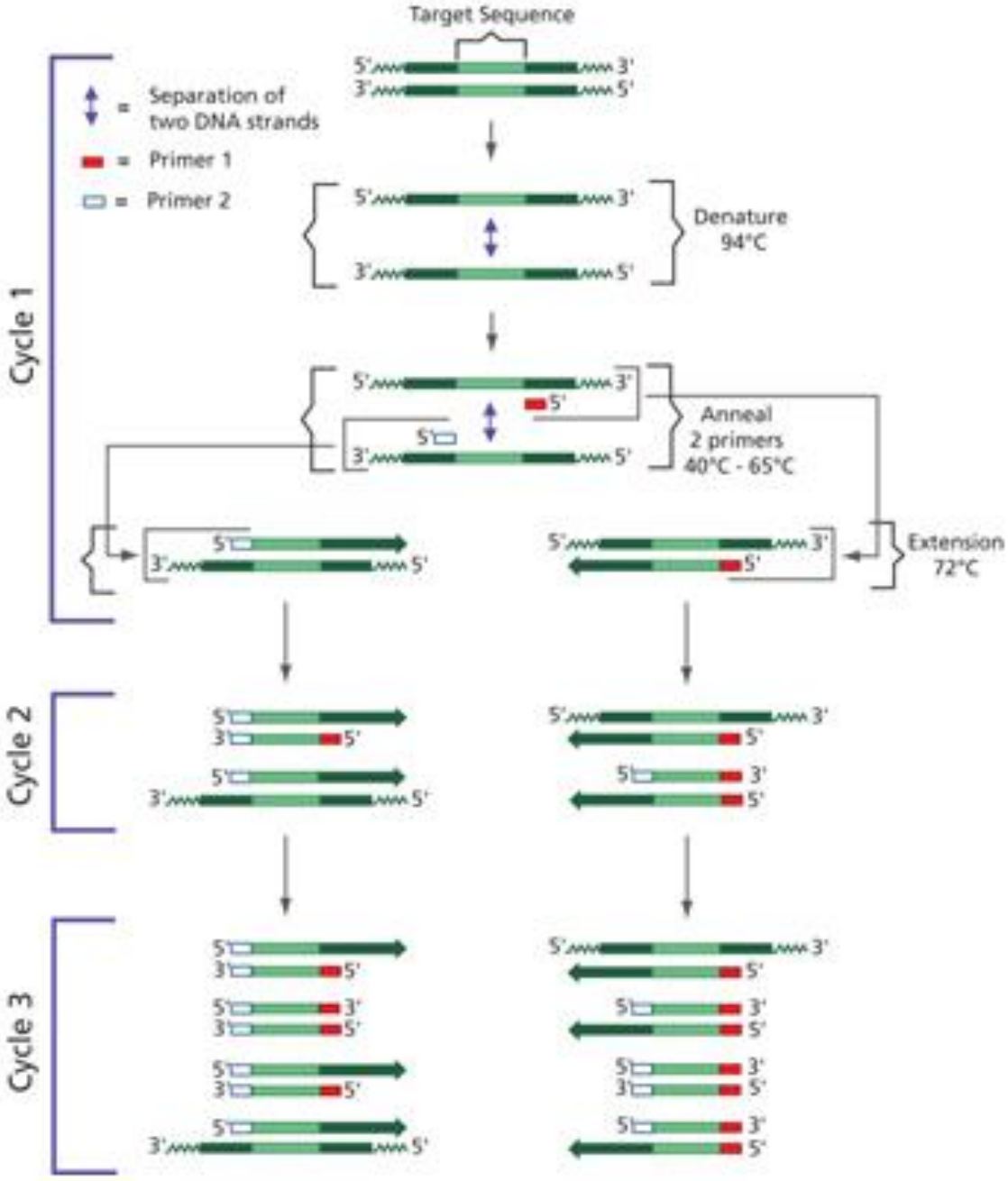
Primer annealing to template DNA

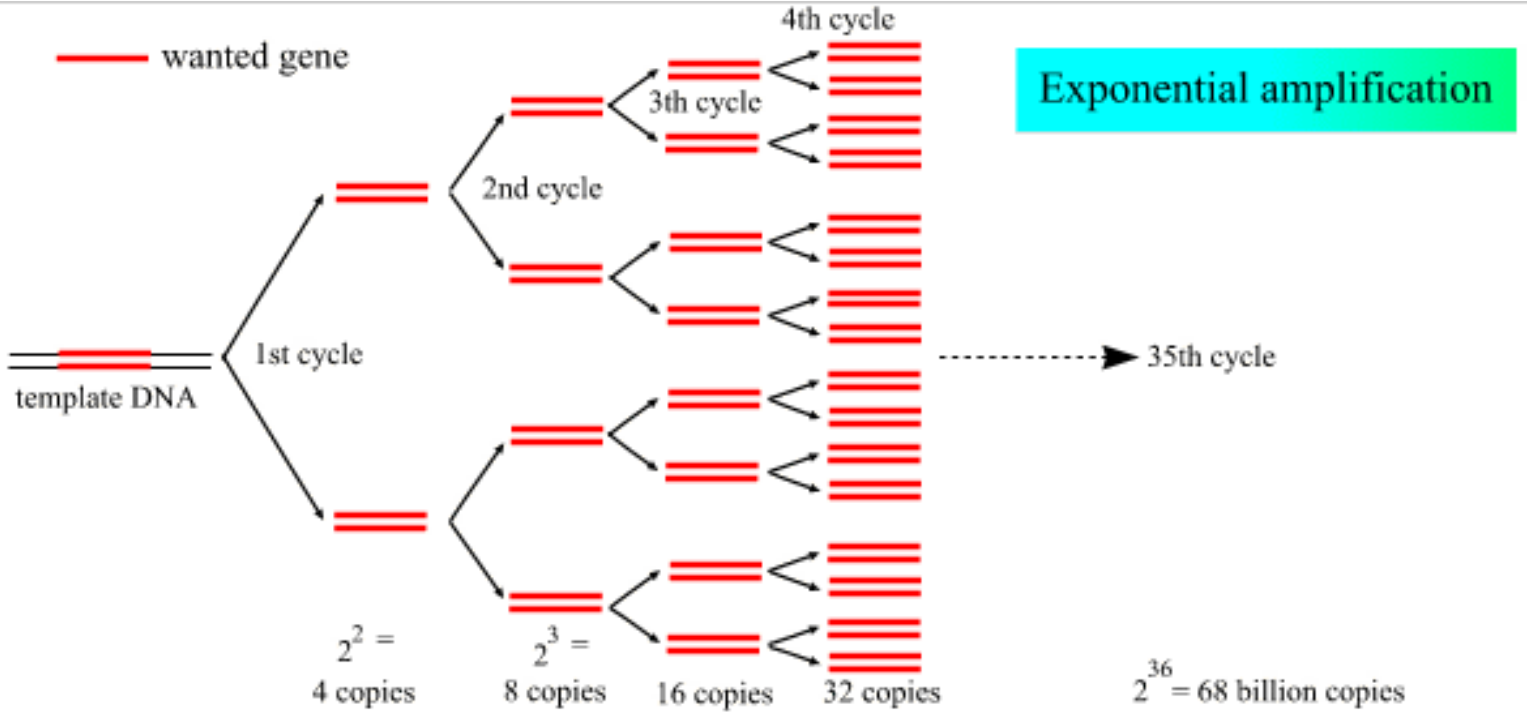
- Prior to the primer extension reaction the template is denatured usually with heat.
- When temperature is lowered sequence-specific primer can anneal (hybridize) to the template where it finds complementary sequence.
- Primer can anneal non-specifically to the template region with only partially matching sequence
- The specificity of the primer annealing is defined by the stringency of the annealing conditions.

- High stringency conditions – high temperature, lower ionic strength, denaturing agents present increase the specificity of primer annealing
- On the other hand, high stringency conditions reduce efficiency of annealing.

Polymerase chain reaction (PCR)

- PCR is used to amplify (increase number of) DNA fragments.
- PCR reaction is performed in several cycles each consisting of following steps:
  1. DNA denaturation
  2. Primers annealing
  3. Primer extension

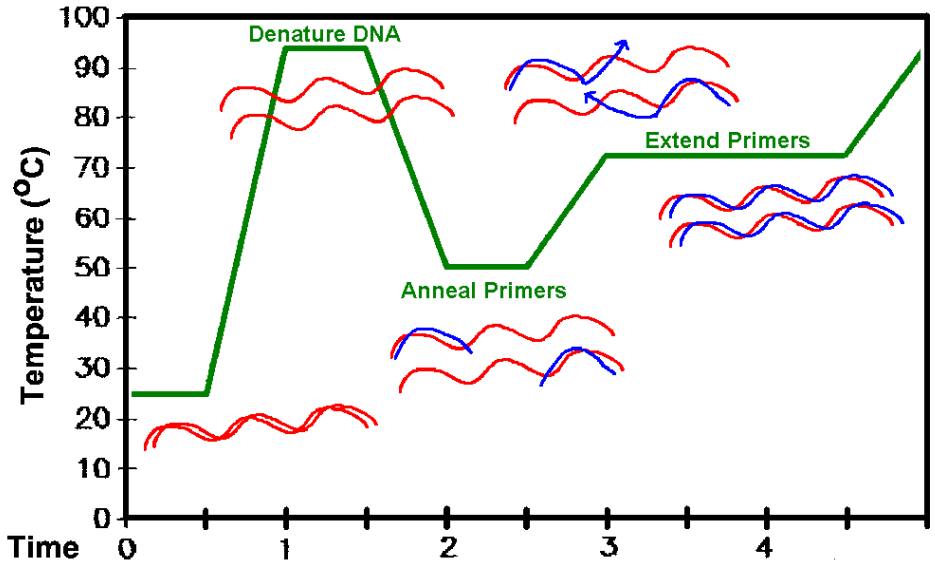

Target Sequence
5'
3'
3'
5'
= Separation of two DNA strands
= Primer 1
= Primer 2
Cycle 1
Denature 94°C
Anneal 2 primers 40°C - 65°C
Extension 72°C
Cycle 2
Cycle 3

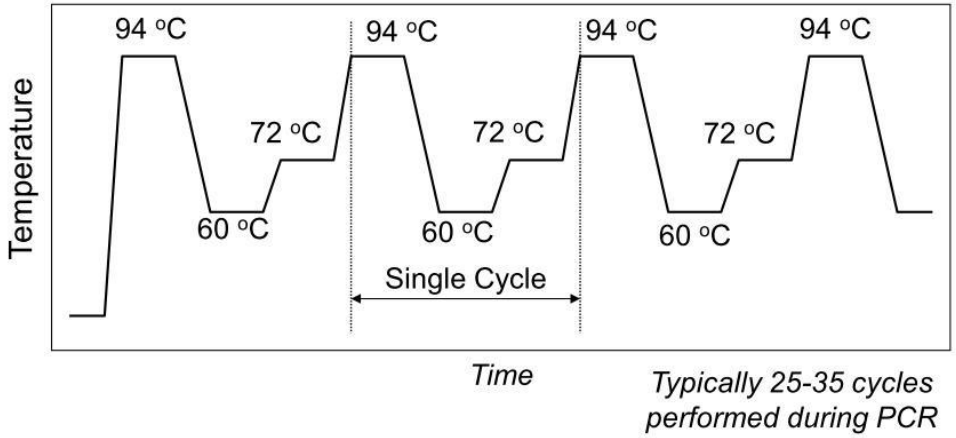

wanted gene
4th cycle
Exponential amplification
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies

Taq polymerase

- PCR reactions could be conveniently performed using DNA polymerase from thermophilic microorganism *Thermus Aquaticus*. The important property of the enzyme is its stability at high temperatures – up to 100^{o}C. Thus, the enzyme survives the repeated denaturation stage during each cycle of the PCR.
- Many other thermo stable DNA polymerases were discovered and used in the PCR reactions

PCR thermal cycling





Typically 25-35 cycles performed during PCR

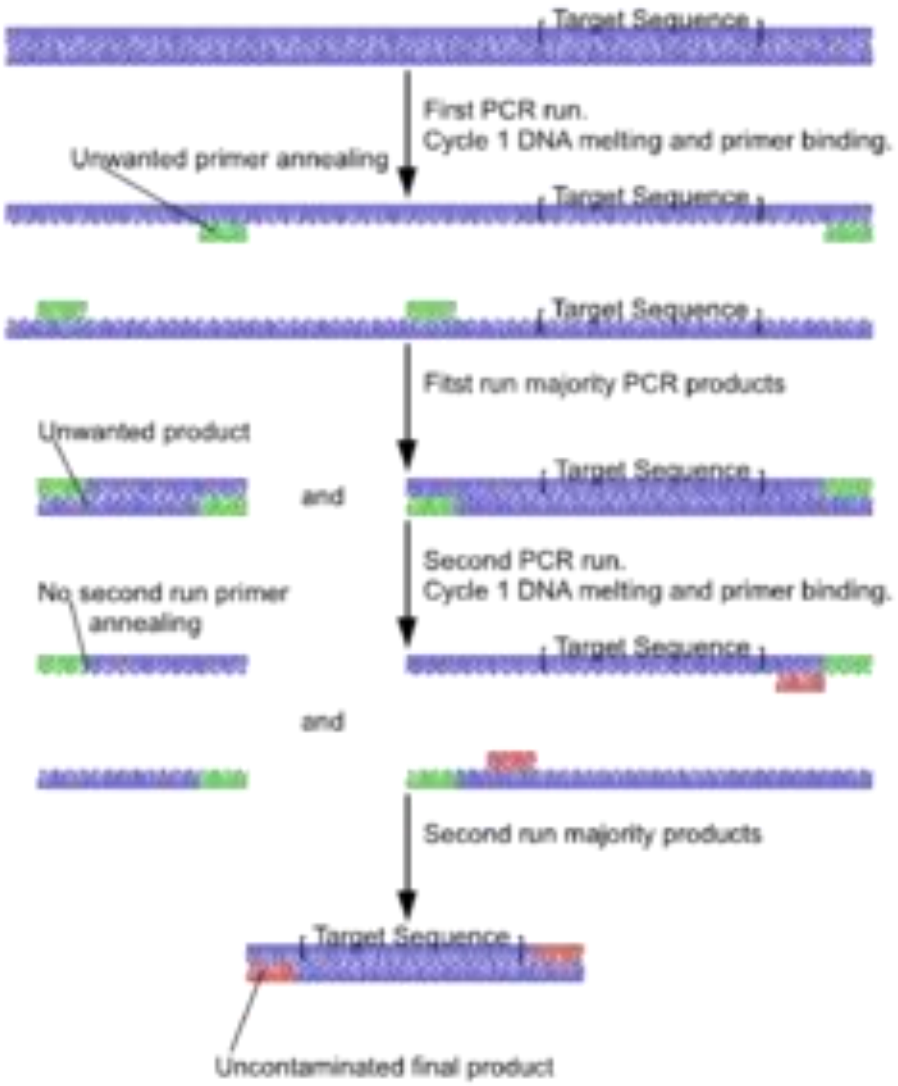
Optimizing PCR reaction

- In selecting the conditions for PCR two goals must be achieved:

1. High yield of the product
2. Specificity of the product amplification

- The balance between the goals is achieved mainly by selecting the stringency of the primer annealing conditions

Nested PCR


Target Sequence
First PCR run.
Cycle 1 DNA melting and primer binding.
Unwanted primer annealing
Target Sequence
Target Sequence
Fitst run majority PCR products
Unwanted product
and
Target Sequence
Second PCR run.
Cycle 1 DNA melting and primer binding.
No second run primer annealing
Target Sequence
and
Second run majority products
Target Sequence
Uncontaminated final product