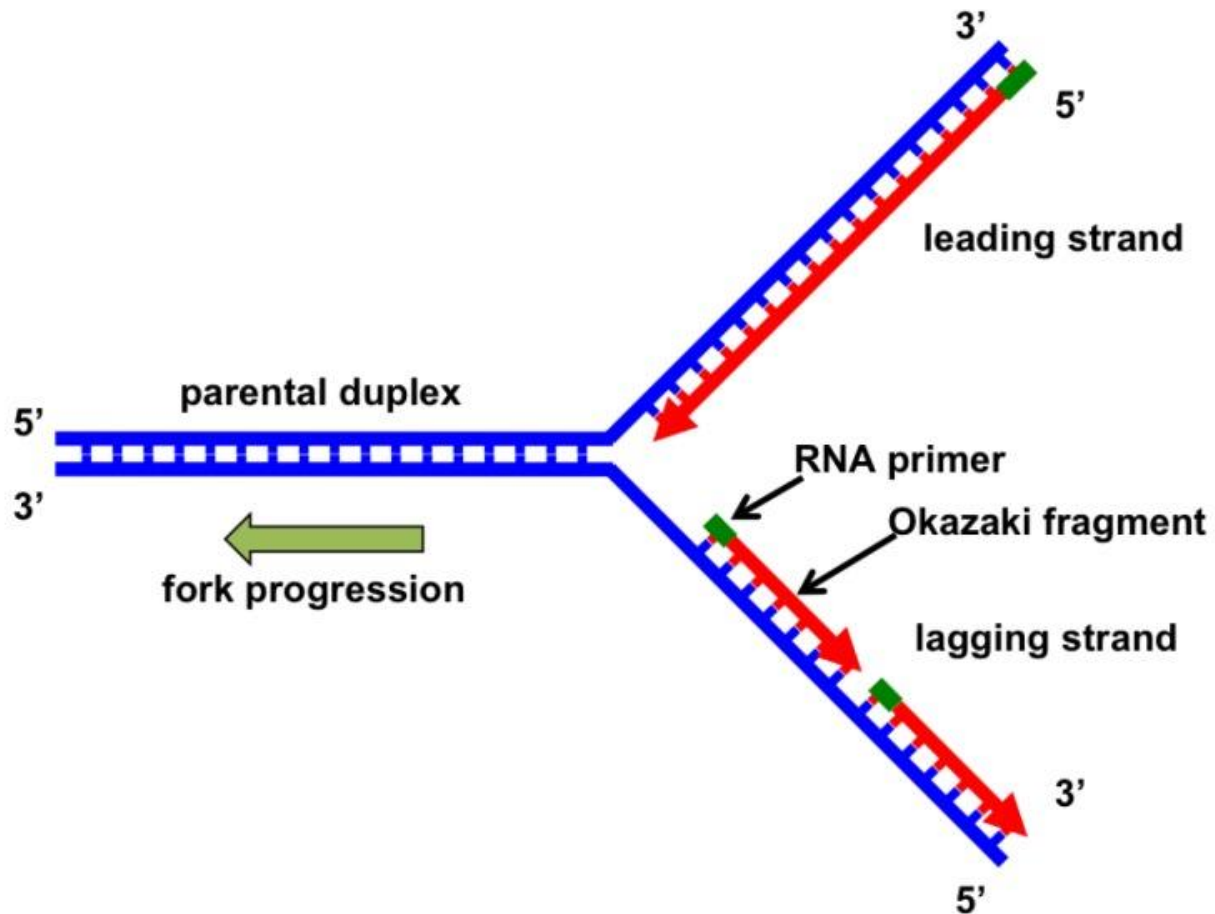


# **Polymerase chain reaction (PCR)**

# DNA replication

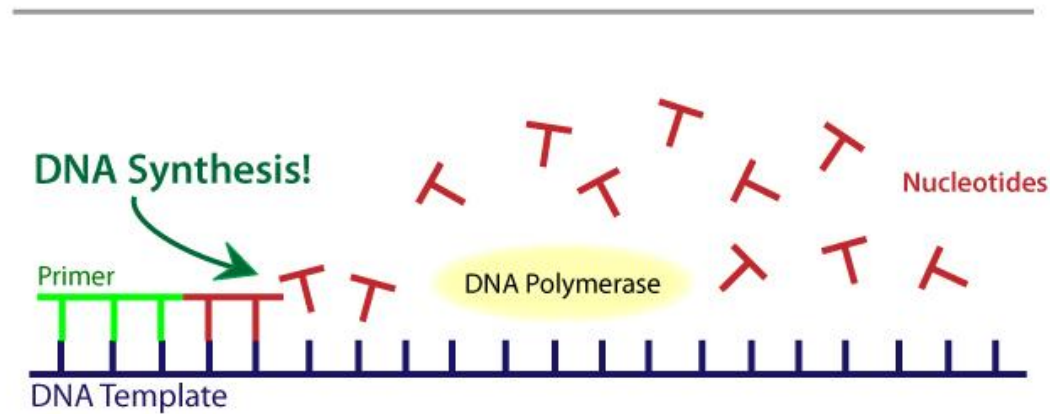
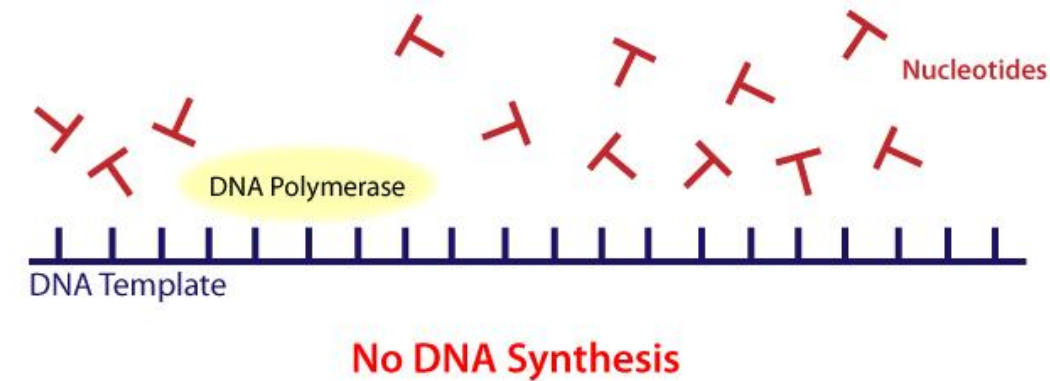


# *In vitro* replication of DNA

DNA can be replicated *in vitro*. 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.



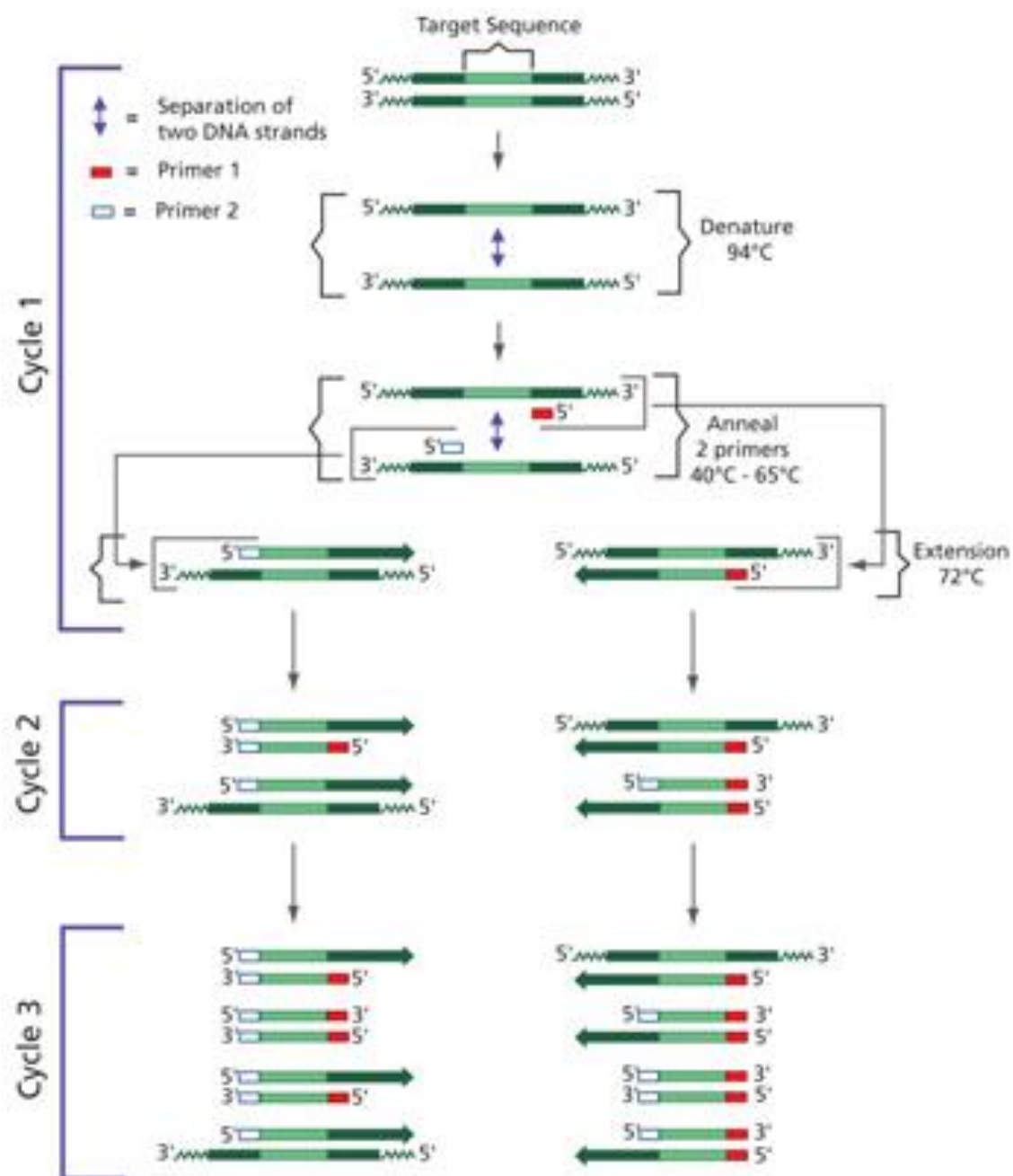
# Primer annealing to template DNA

- 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.

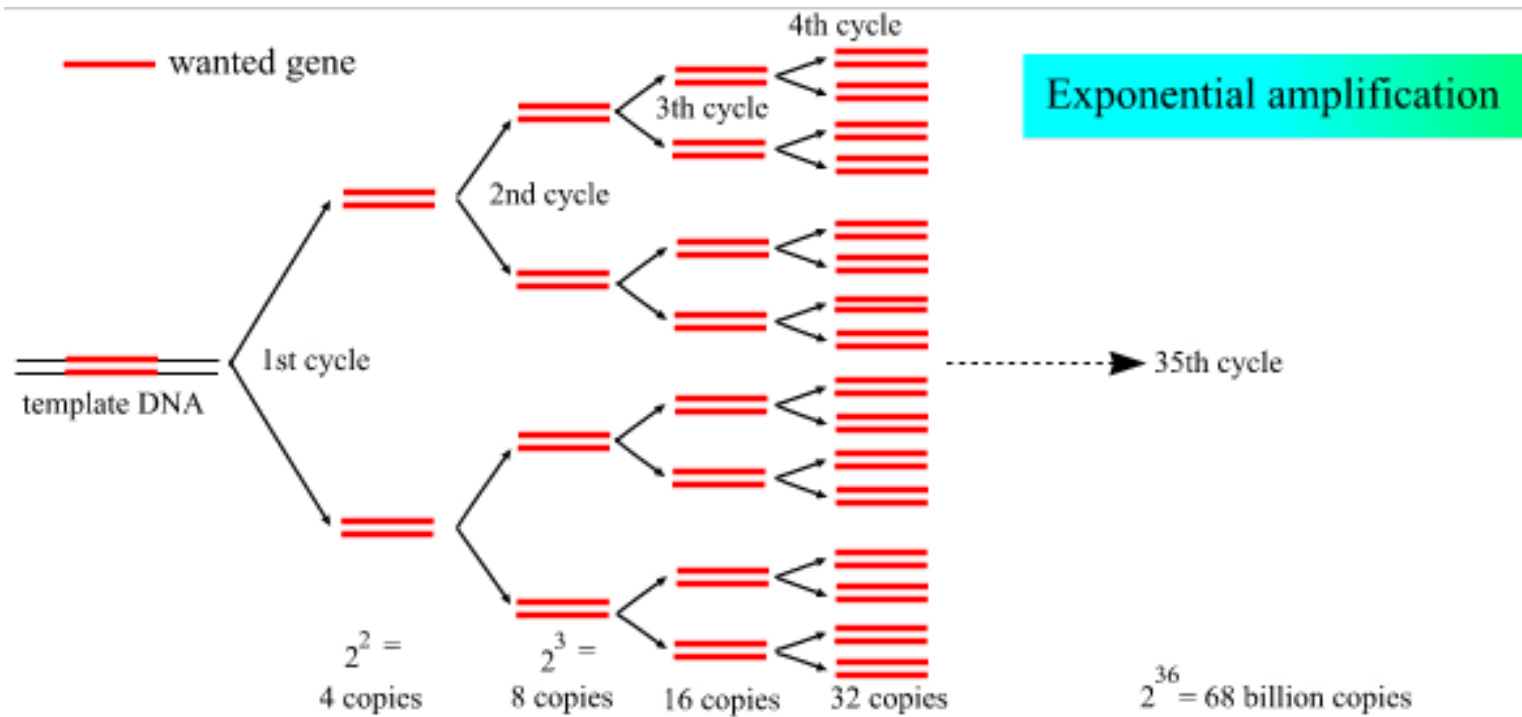
- High stringency conditions – high temperature, lower ionic strength, denaturing agents present increase the specificity of primer annealing
- On the other hand, high stringency conditions reduce efficiency of annealing.

# 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







# 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°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

# 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

