İLERİ ARAŞTIRMA YÖNTEMLERİ (ADVANCED RESEARCH TECHNIQUES)

2020-21 BAHAR

DR. GÜNSELİ ÇUBUKÇUOĞLU DENİZ

POLİMERAZ ZİNCİR REAKSİYONU (PCR)

DERS 1

MART 2021

PCR TARİHİ

Chemistry | The Nobel Prize in Chemistry 1993 | Kary B. Mullis - Facts

The Nobel Prize in Chemistry 1993

Kary B. Mullis
Michael Smith

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Kary B. Mullis
Facts

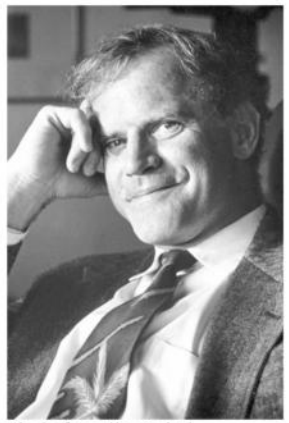


Photo from the Nobel Foundation archive.

Kary B. Mullis
The Nobel Prize in Chemistry 1993

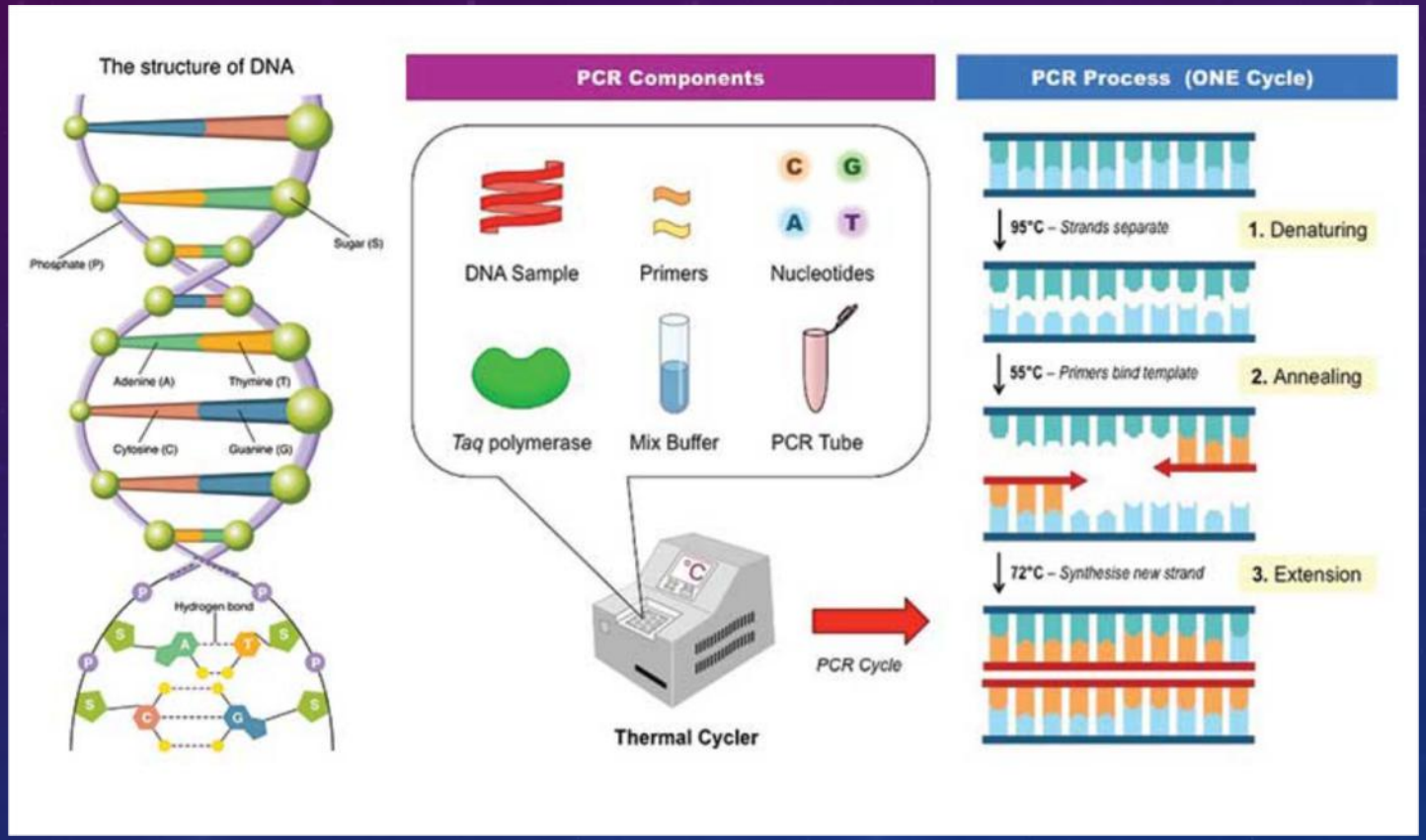
Born: 28 December 1944, Lenoir, NC, USA

Died: 7 August 2019, Newport Beach, CA, USA

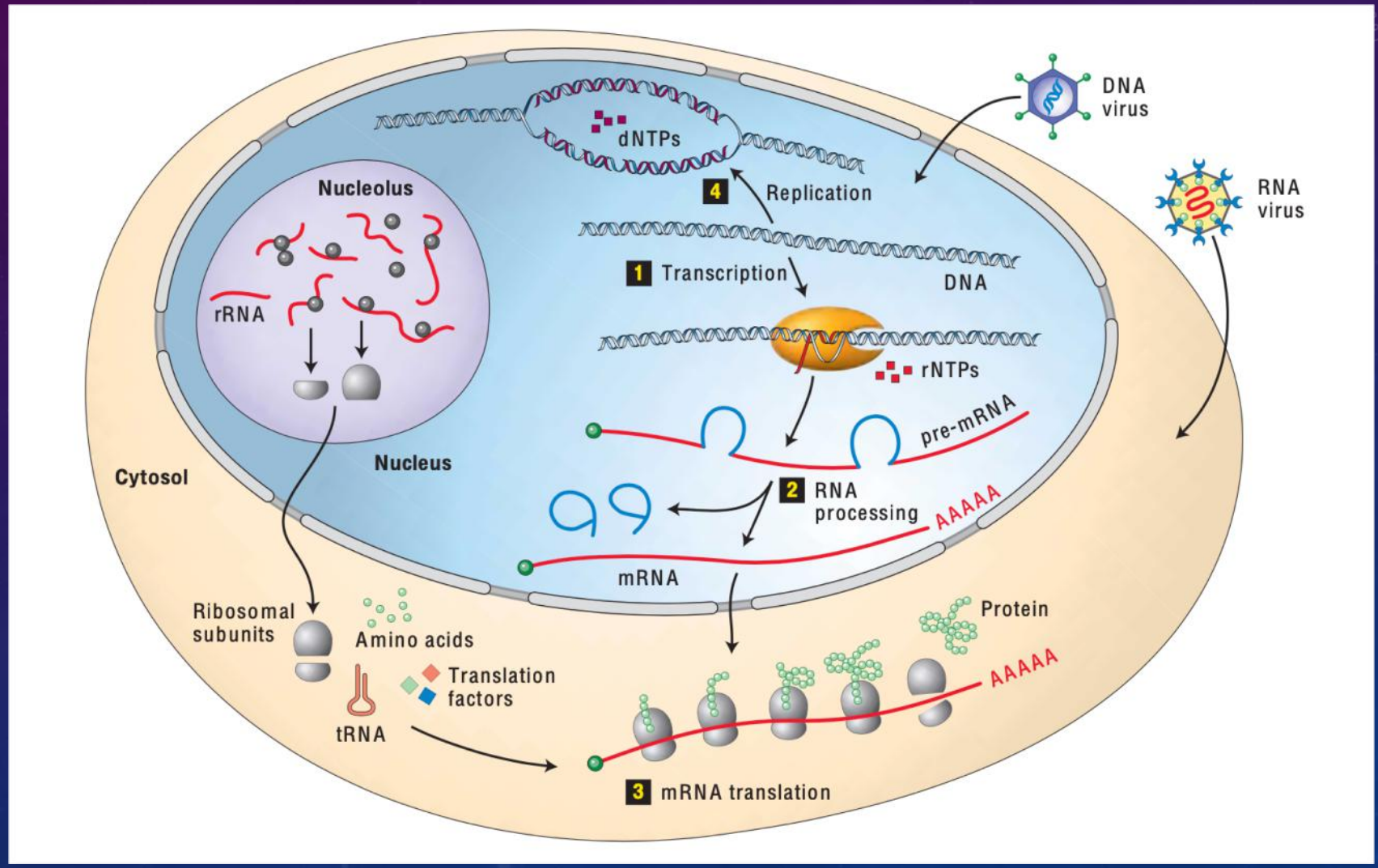
Affiliation at the time of the award: , La Jolla, CA, USA

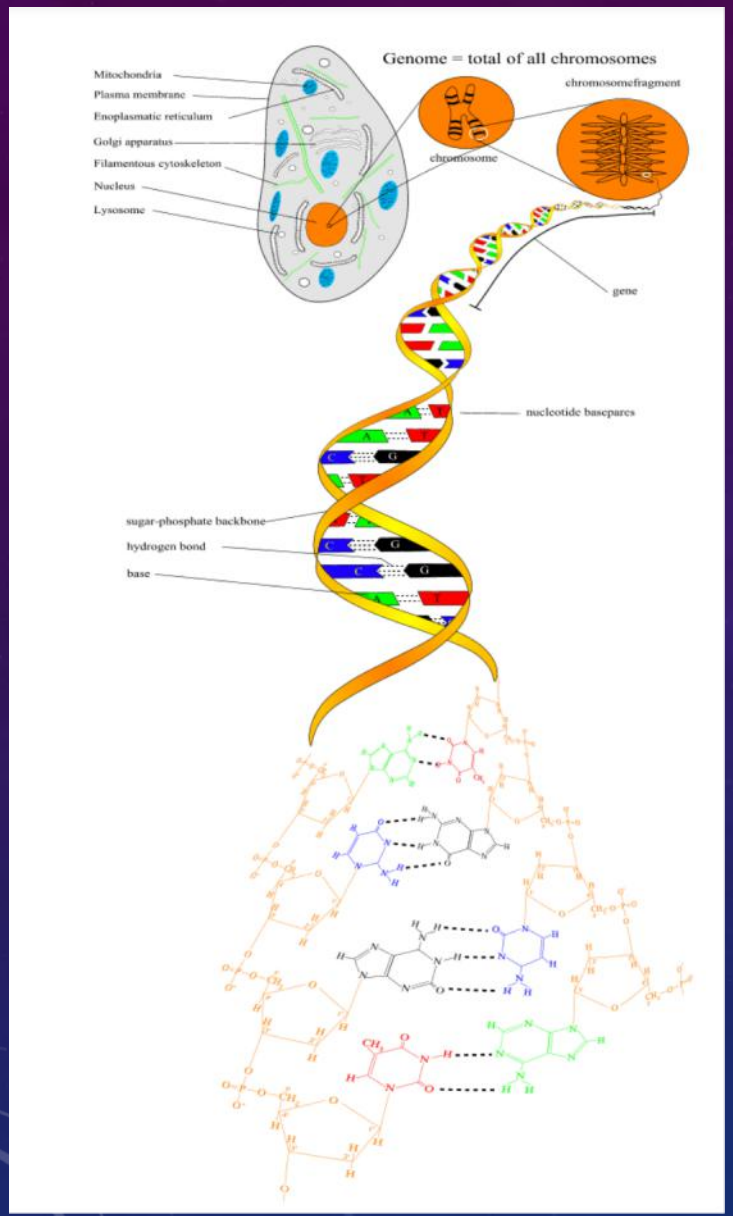
Prize motivation: "for his invention of the polymerase chain reaction (PCR) method."

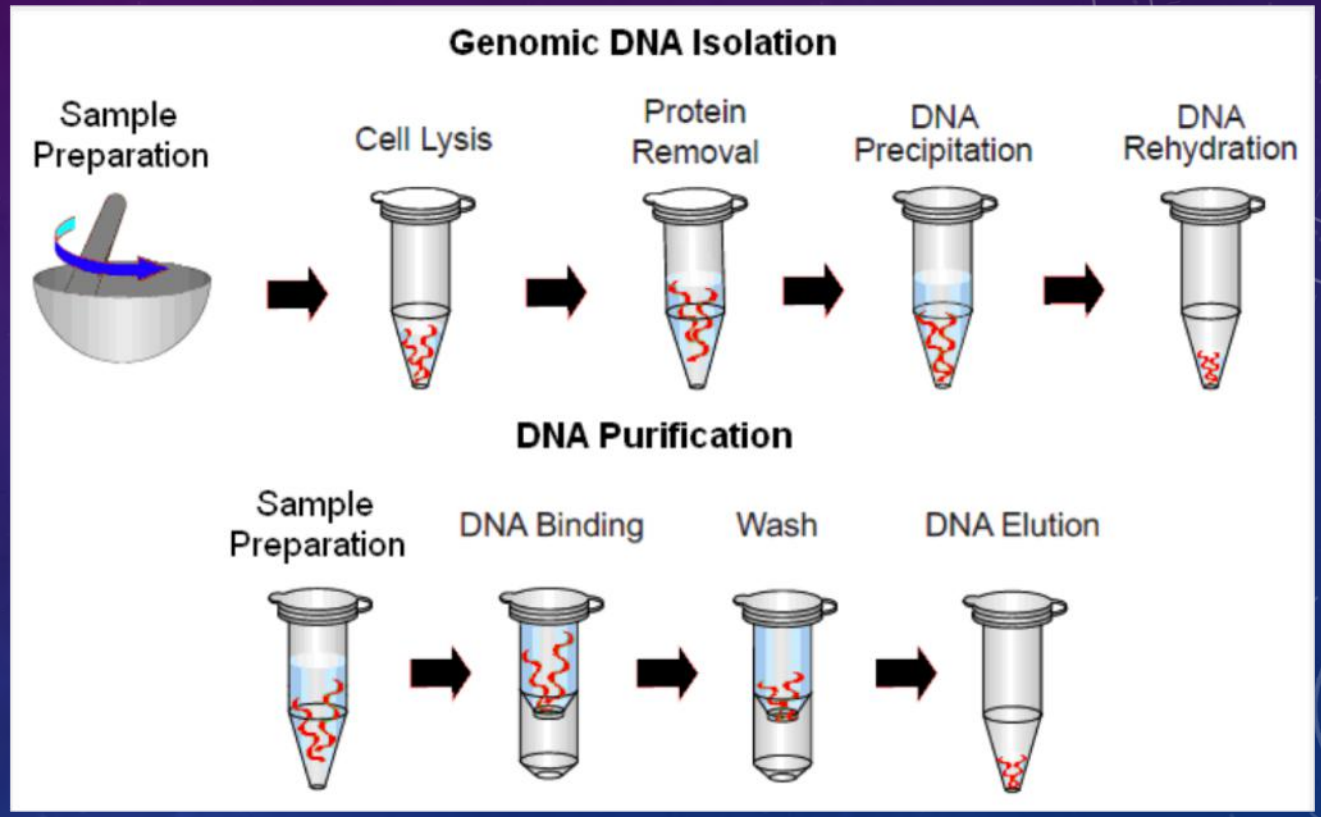
Prize share: 1/2


The structure of DNA
Phosphate (P)
Sugar (S)
Adenine (A)
Thymine (T)
Cytosine (C)
Guanine (G)
Hydrogen bond
PCR Components
DNA Sample
Primers
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
Thermal Cycler
PCR Cycle
PCR Process (ONE Cycle)
95°C – Strands separate
1. Denaturing
55°C – Primers bind template
2. Annealing
72°C – Synthesise new strand
3. Extension

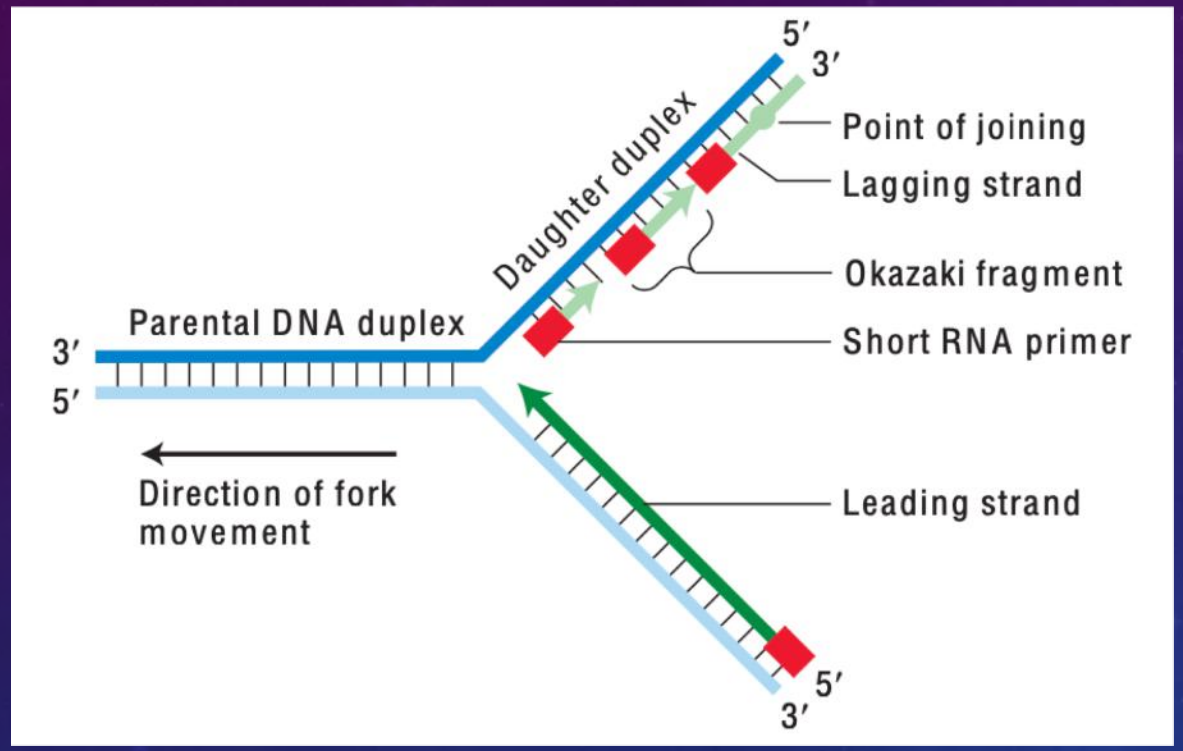
TEMEL MOLEKÜLER GENETİK MEKANİZMALAR

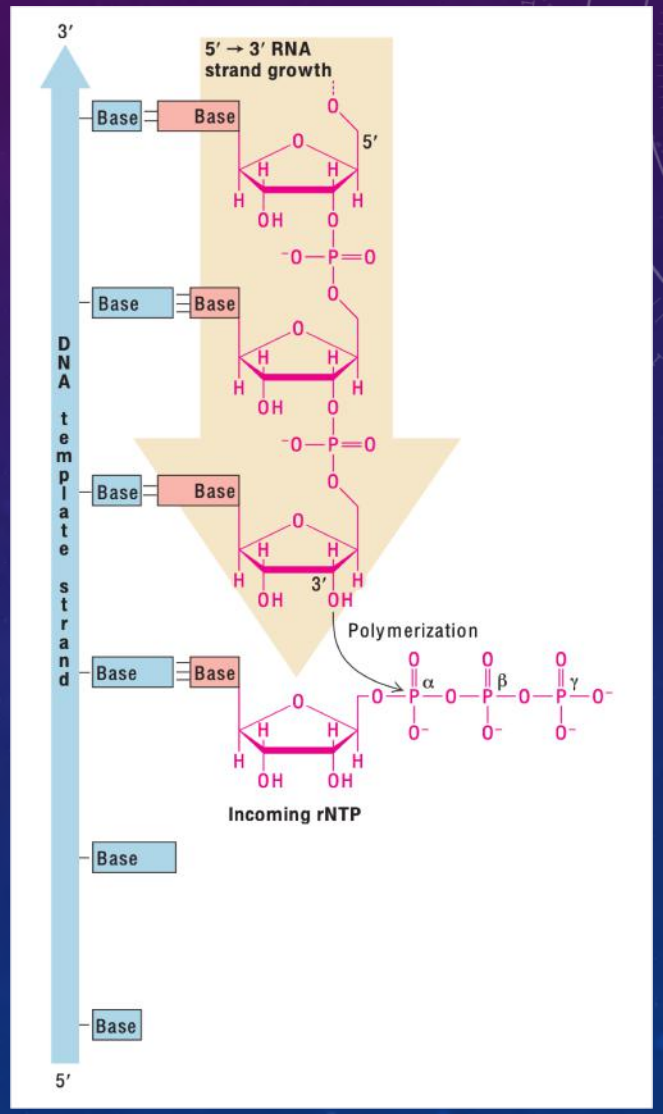



Genome = total of all chromosomes
Mitochondria
Plasma membrane
Enoplasmatic reticulum
Golgi apparatus
Filamentous cytoskeleton
Nucleus
Lysosome
chromosomefragment
chromosome
gene
nucleotide basepares
sugar-phosphate backbone
hydrogen bond
base

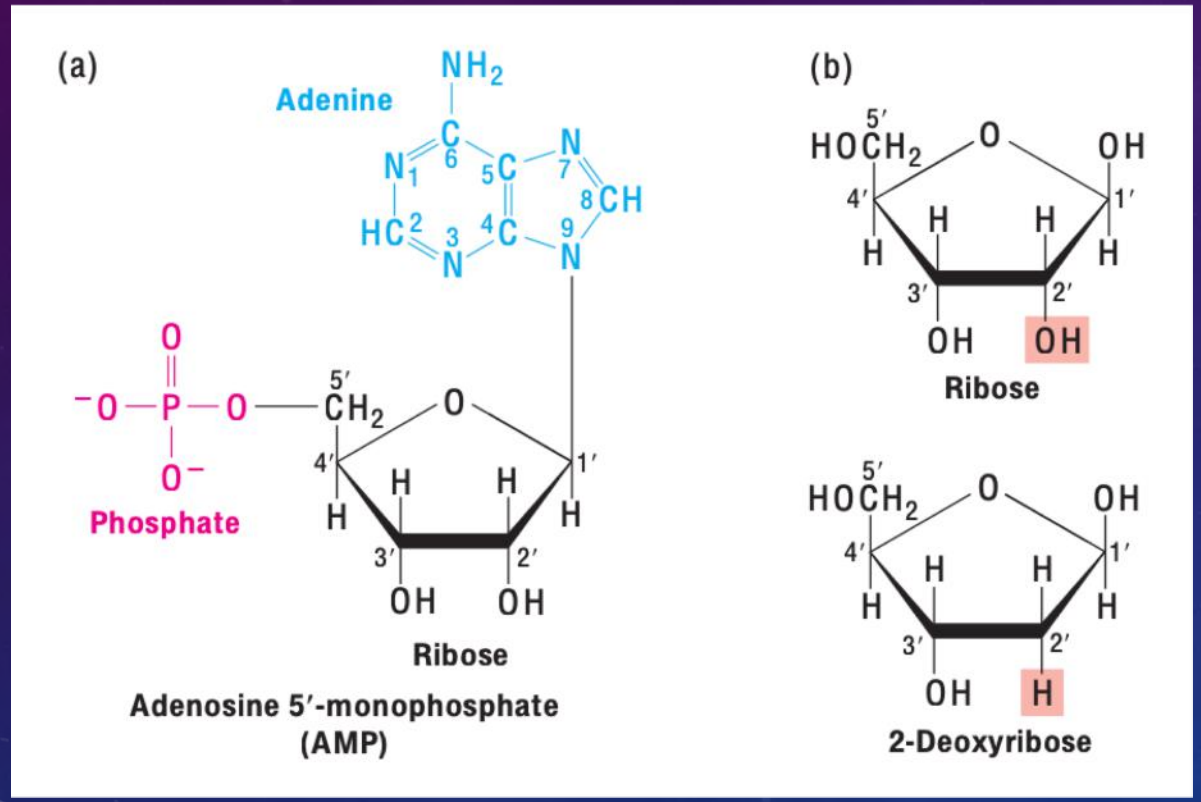

Genomic DNA Isolation
Sample Preparation
Cell Lysis
Protein Removal
DNA Precipitation
DNA Rehydration
DNA Purification
Sample Preparation
DNA Binding
Wash
DNA Elution

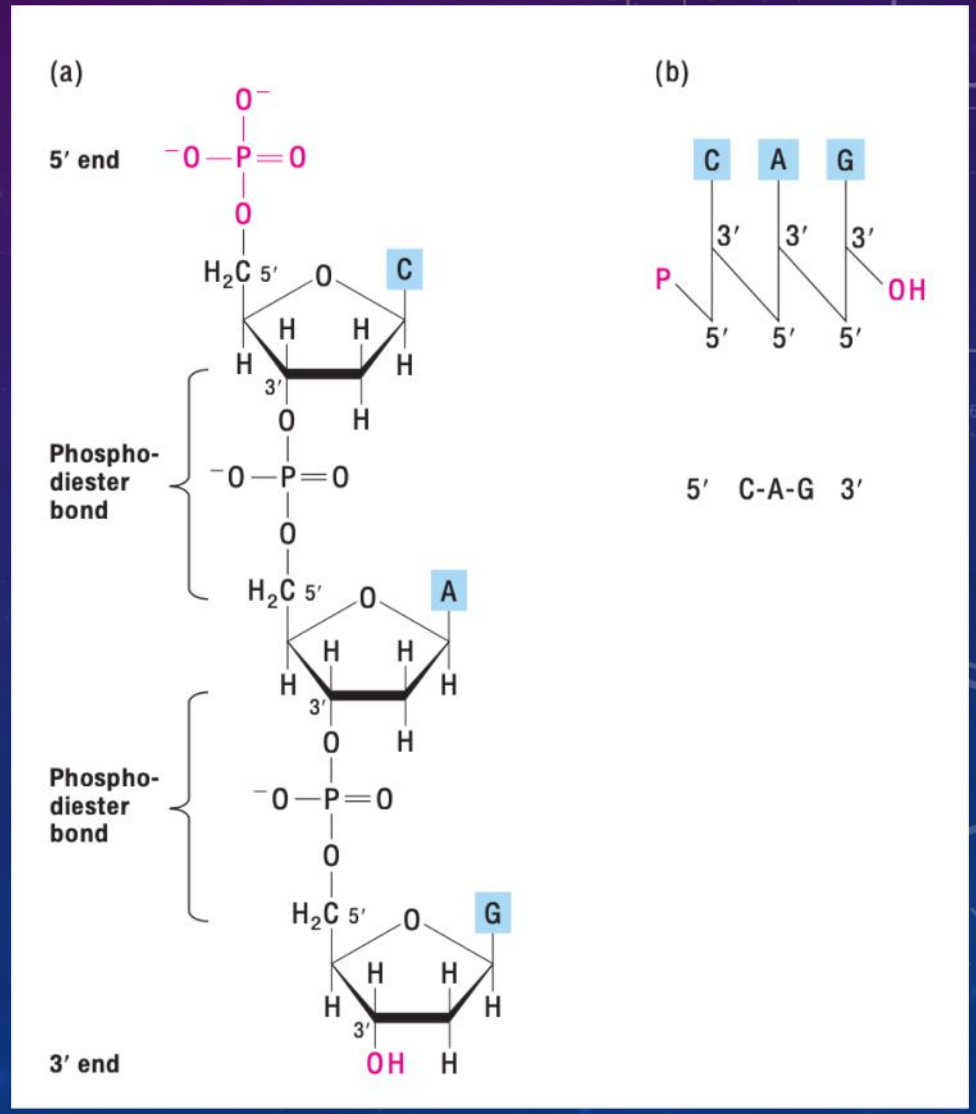
DNA REPLİKASYONU



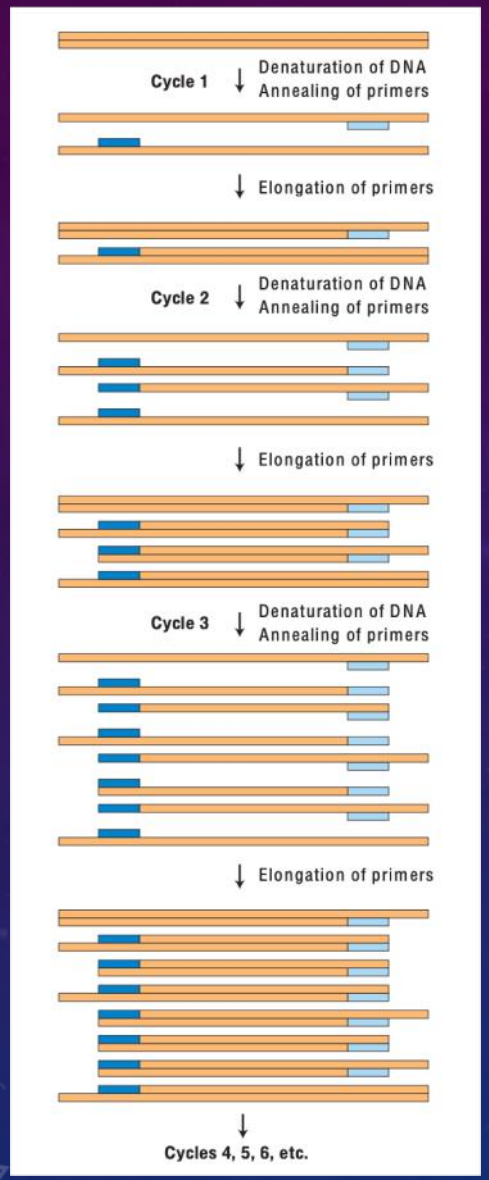


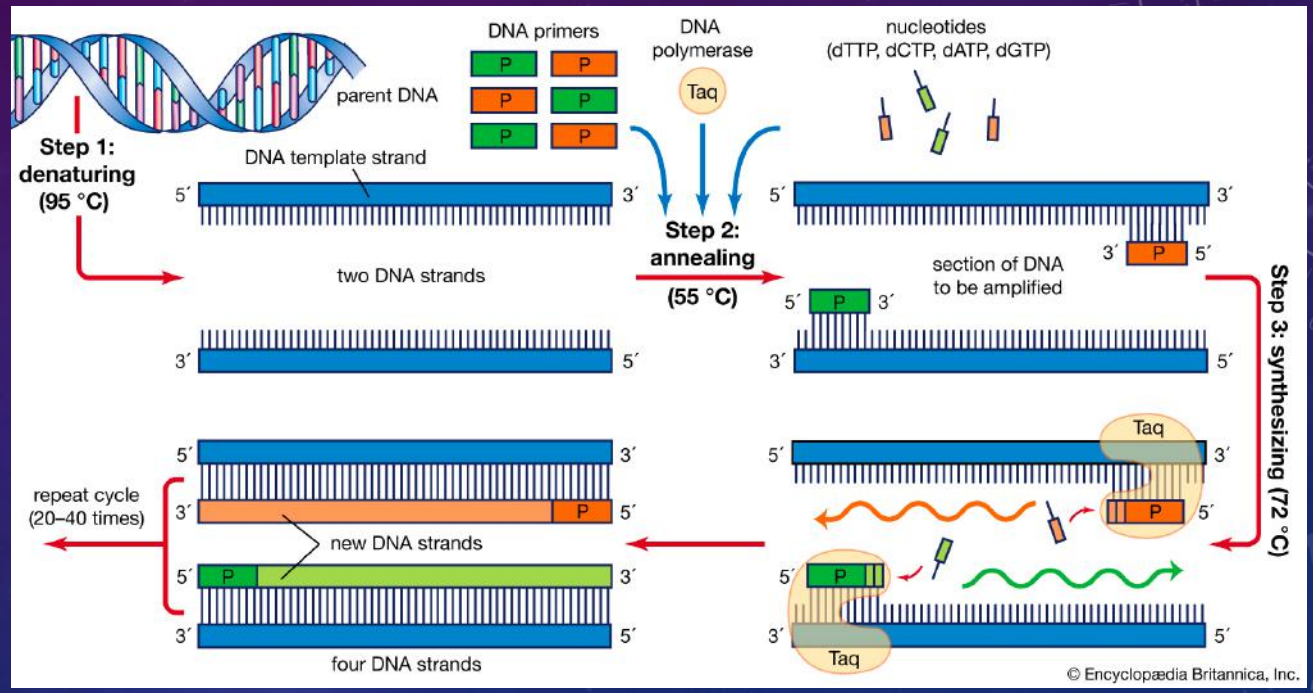
DNA KİMYASI


(a)
Adenine
Phosphate
Ribose
Adenosine 5′-monophosphate
(AMP)
(b)
Ribose
2-Deoxyribose


(a)
5′ end
Phospho-
diester
bond
Phospho-
diester
bond
3′ end
(b)
5′ C-A-G 3′

	Bases			
	Purines		Pyrimidines	
	Adenine (A)	Guanine (G)	Cytosine (C)	Uracil (U) Thymine [T]
Nucleosides — in RNA	Adenosine	Guanosine	Cytidine	Uridine
Nucleosides — in DNA	Deoxyadenosine	Deoxyguanosine	Deoxycytidine	Deoxythymidine
Nucleotides — in RNA	Adenylate	Guanylate	Cytidylate	Uridylate
Nucleotides — in DNA	Deoxyadenylate	Deoxyguanylate	Deoxycytidylate	Deoxythymidylate
Nucleoside monophosphates	AMP	GMP	CMP	UMP
Nucleoside diphosphates	ADP	GDP	CDP	UDP
Nucleoside triphosphates	ATP	GTP	CTP	UTP
Deoxynucleoside mono-, di-, and triphosphates	dAMP, etc.			


Cycle 1
Denaturation of DNA
Annealing of primers
Elongation of primers
Cycle 2
Denaturation of DNA
Annealing of primers
Elongation of primers
Cycle 3
Denaturation of DNA
Annealing of primers
Elongation of primers
Cycles 4, 5, 6, etc.


DNA primers
DNA polymerase
Taq
nucleotides
(dTTP, dCTP, dATP, dGTP)
parent DNA
Step 1:
denaturing
(95 °C)
DNA template strand
two DNA strands
Step 2:
annealing
(55 °C)
section of DNA
to be amplified
Step 3: synthesizing (72 °C)
repeat cycle
(20–40 times)
new DNA strands
four DNA strands
© Encyclopædia Britannica, Inc.

https://www.sciencelearn.org.nz/resources/2347-what-is-pcr

PCR

- PCR Buffer: Ortam pH kontrol altında tutar
- Primerler: f/r primer çifti
- Kalıp (template) DNA
- Sıcaklığa dayanıklı DNA polimeraz: *Thermus aquaticus* (Taq pol), *Pyrococcus furiosus* (Pfu pol), *Thermus thermophilus* (Tth pol), *Thermus flavus* (Tfl pol), *Thermococcus litoralis* (Tli pol/vent pol), *Pyrococcus species* (deep vent pol)
- $MgCl_2$: DNA polimeraz enzimi kofaktörü
- dNTPler: dGTP, dCTP, dATP, dTTP

PRİMER

- Özgüllük
- Komplementerlik
- 18-25 bp
- Erime Sıcaklığı (Tm): 55-65 °C, f ve r primerlerin Tm'leri yakın max 5 °C
- Hairpin (saç tokası) oluşturma
- Dimer oluşturma
- GC yüzdesi: %40-60 self-complementarity!

- Primer-BLAST
- PCR ürünü uzunluğu: 100-200 bp
- IDT Oligoanalyzer

DENEY TASARIMI

- Bağlanma (annealing) sıcaklığı (T_a): Tm'den ~5 °C düşüktür.

- Başlama (95 °C) 2-10 dk
- Denatürasyon (95 °C) 20-30 s
- Bağlanma (55-65 °C) 20-40 s
- Uzama-Sentez (72 °C) 30-40 s
- Final uzama (72-75 °C) 5-15 dk

DENEY TASARIMI

- 2 (siklus sayısı)

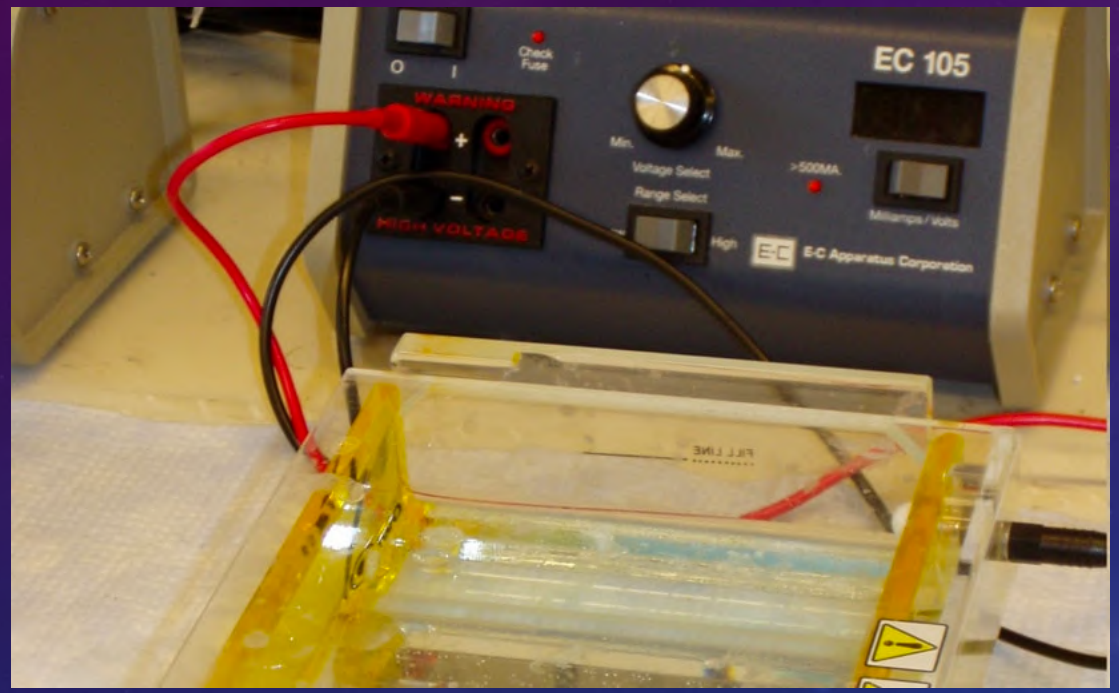
- **Positive control**: A sample of DNA or cDNA known to contain the target sequence of interest. If the amplicon is short (<130 bases), an artificial oligo that matches the target of interest can be used as a positive control.

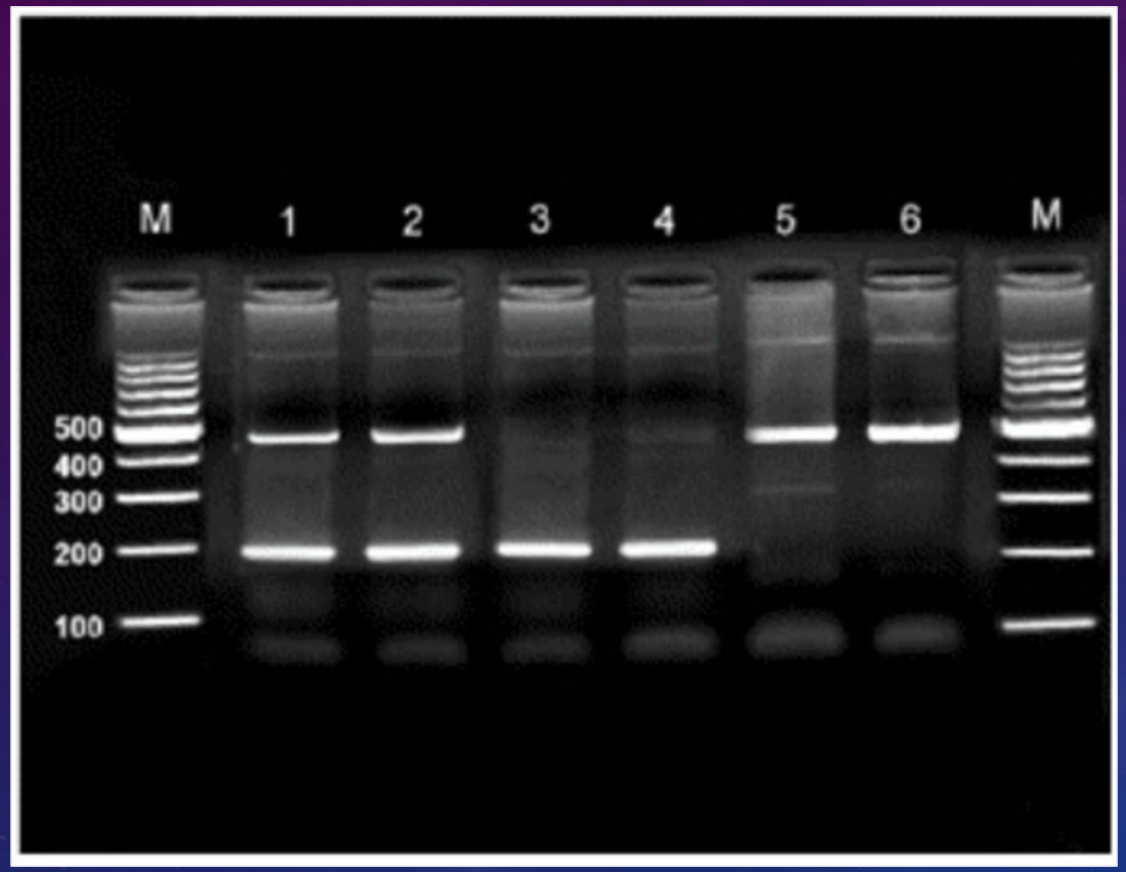
- **Negative control**: A sample of DNA or cDNA that does not contain the target sequence of interest.

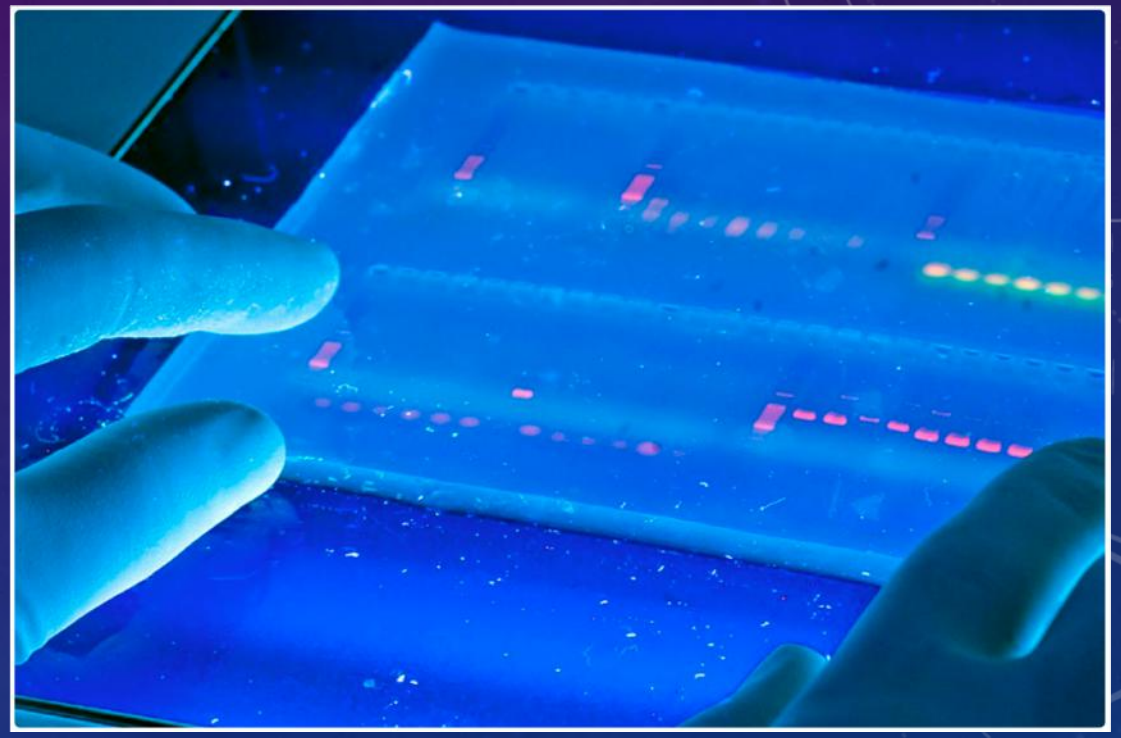
- **Water/contamination control or No Template Control (NTC)**: A water or NTC is a useful control to add to the plate as a monitor of potential contamination of the reactions with template.

- **RT minus control**: To identify contaminating genomic DNA (gDNA) in mRNA samples, each RNA sample is incubated in a RT reaction mix that does not contain RT enzyme.

JEL ELEKTROFOREZİ




M
1
2
3
4
5
6
M
500
400
300
200
100



OPTİMİZASYON

- $MgCl_2$ konsantrasyonu
- T_a varyasyonları