NÜKLEİK ASİT AMPLİFİKASYON TESTLERİ "NAAT"

Dr. Dilek Çolak

NAAT

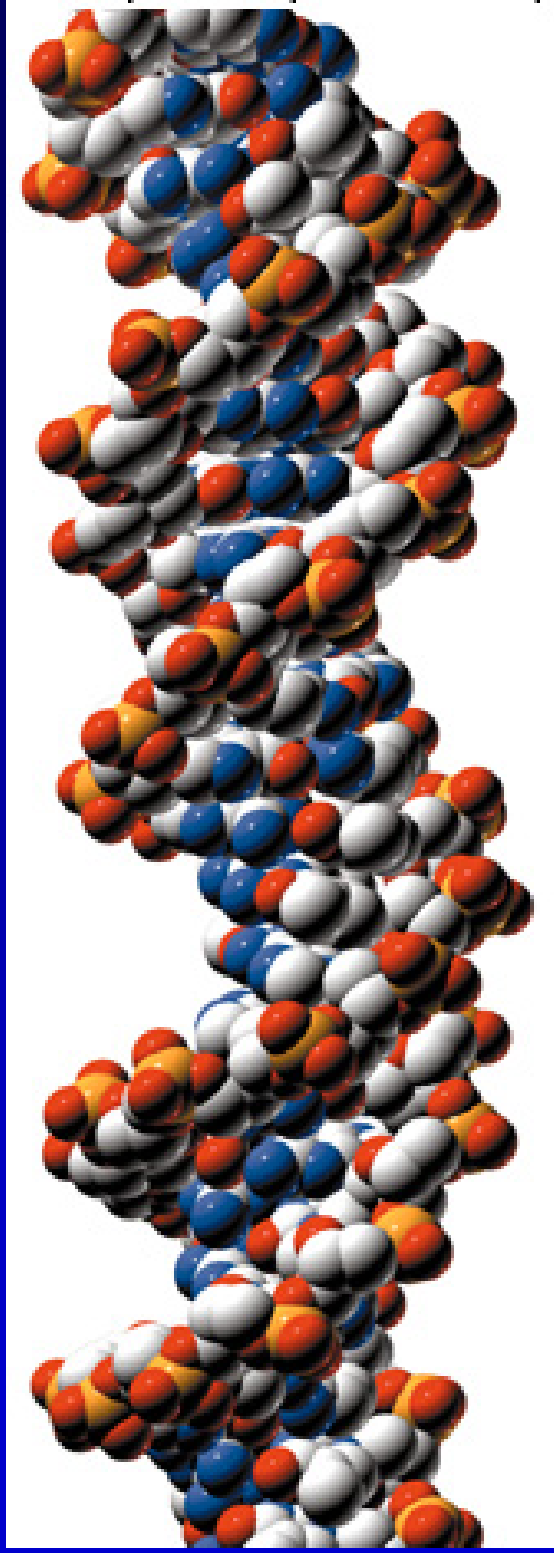
⇓

Mikroorganizmalara ait spesifik nükleik asit dizilerinin araştırılması

"İnsanlardaki parmak izi gibi"

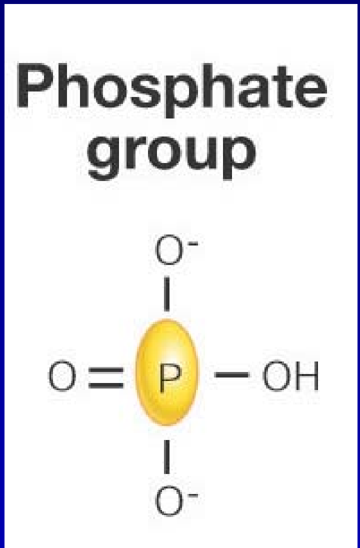
DNA YAPISI

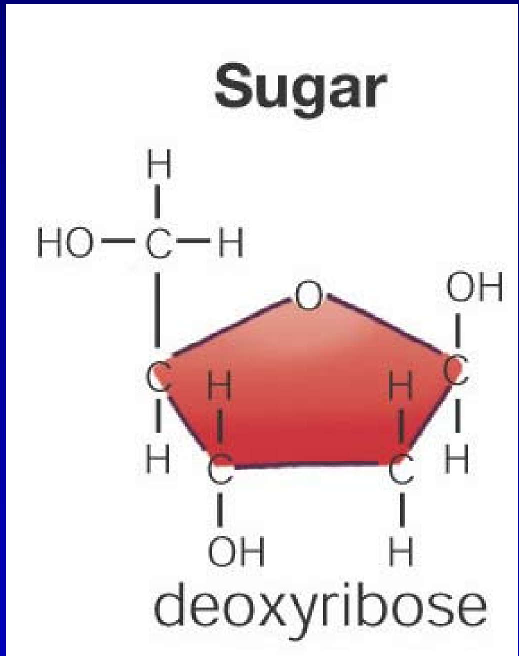
- Monoton yapıların peşpeşe dizilmesi ile oluşmuş dünyanın belki de en sıkıcı molekülü
- Herhangi bir reaksiyon katalize etmez
- Depolama ve kopyalama yapar: Basit kurallarla biraraya gelen tamamlayıcı bazlardan oluşan çift iplikli molekül!

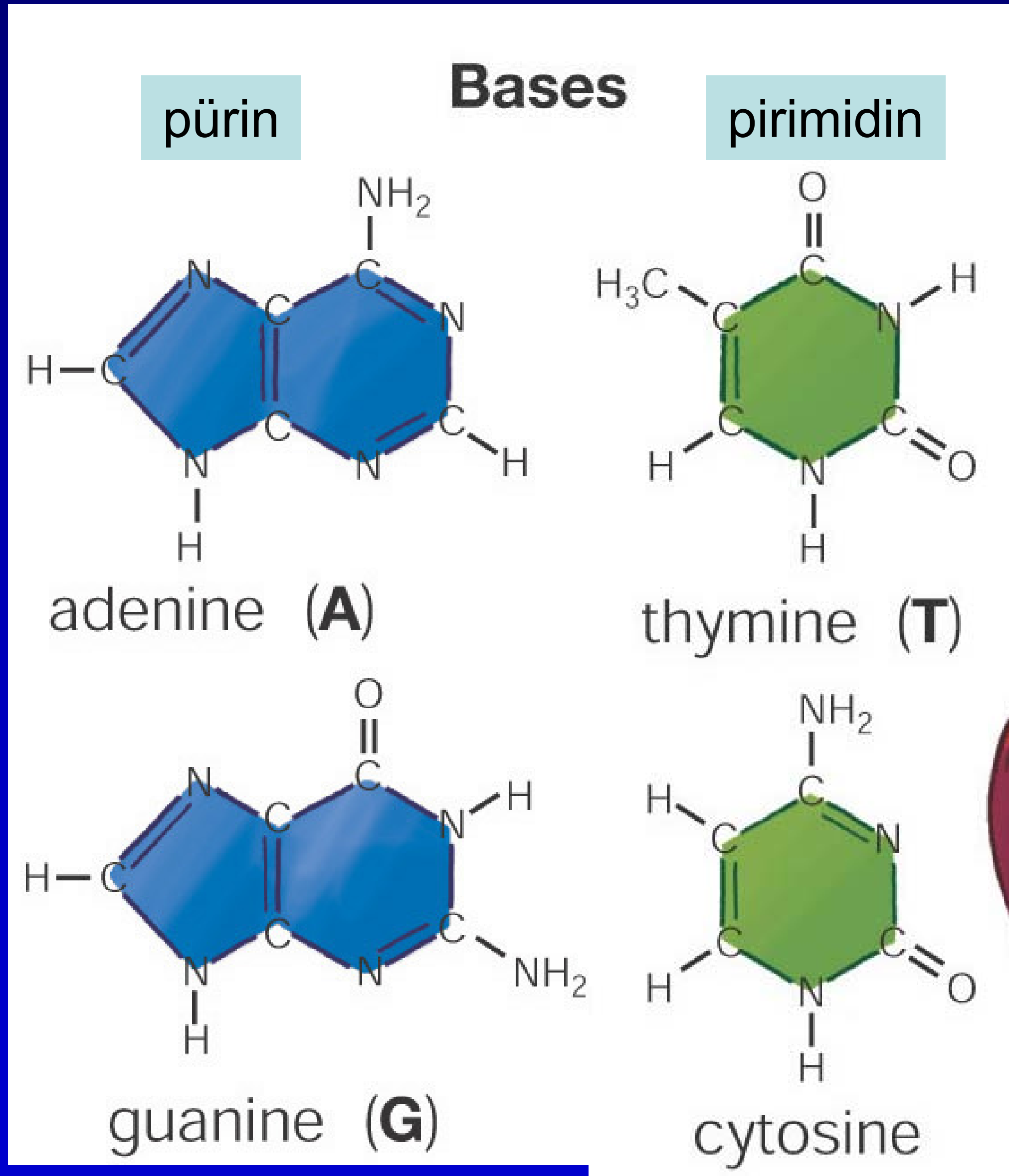


DNA Yapısı – 1953 James Watson and Francis Crick
1962 Nobel Ödülü (Fizyoloji/Tıp)

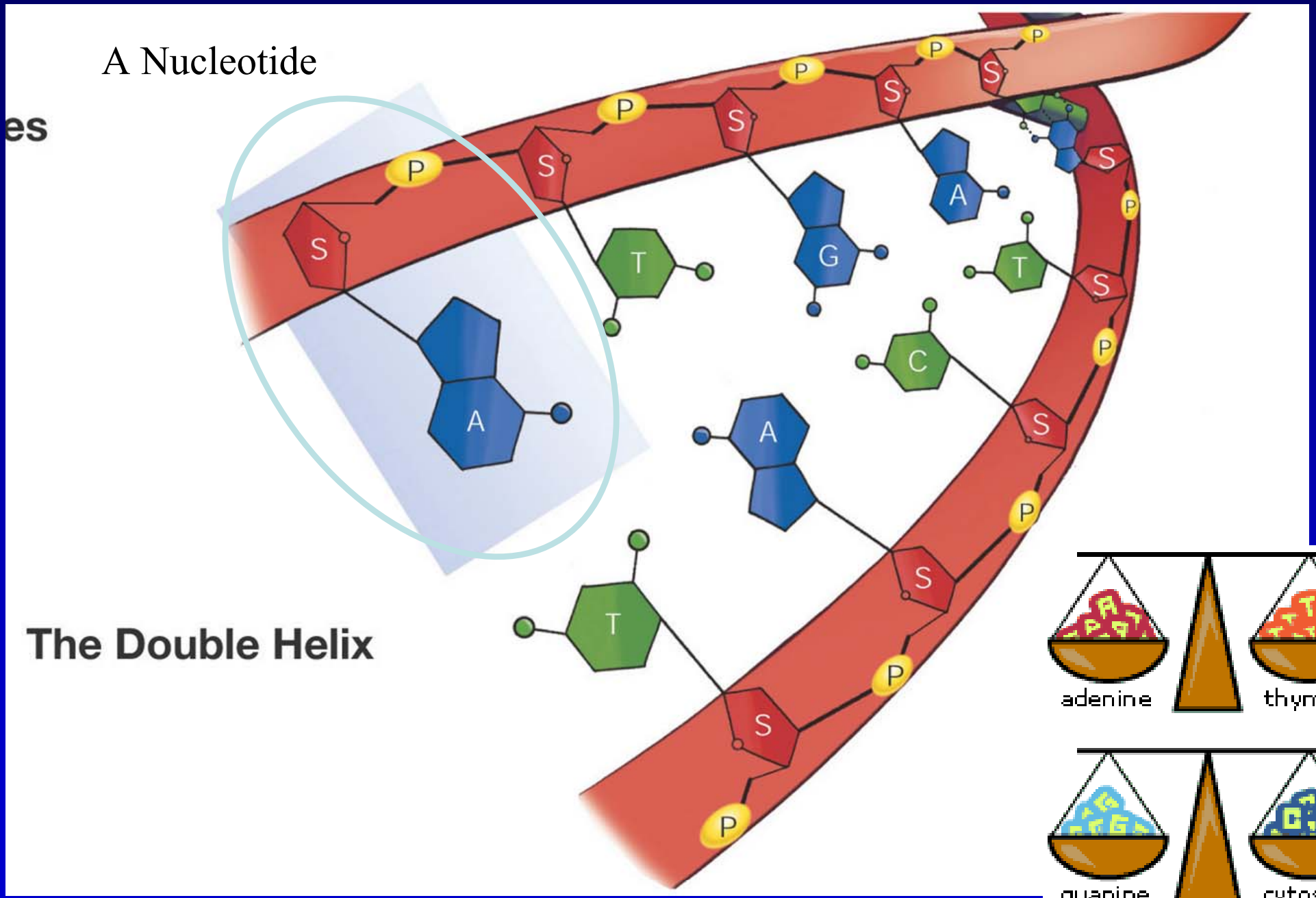
DNA YAPISI


Phosphate group
O-
O
P
OH
O-

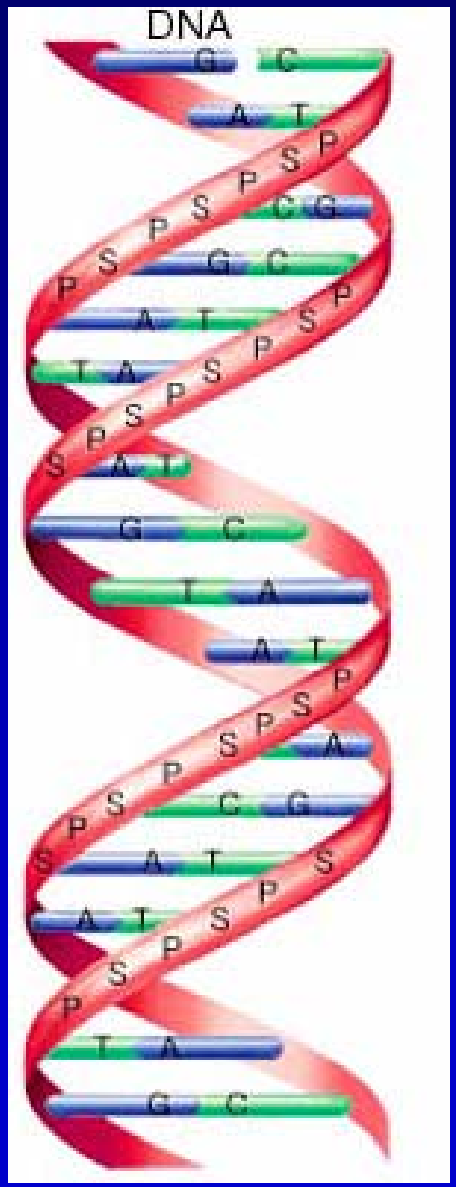

Sugar
H
HO
C
H
O
OH
C
H
H
C
H
H
C
H
C
OH
H
deoxyribose

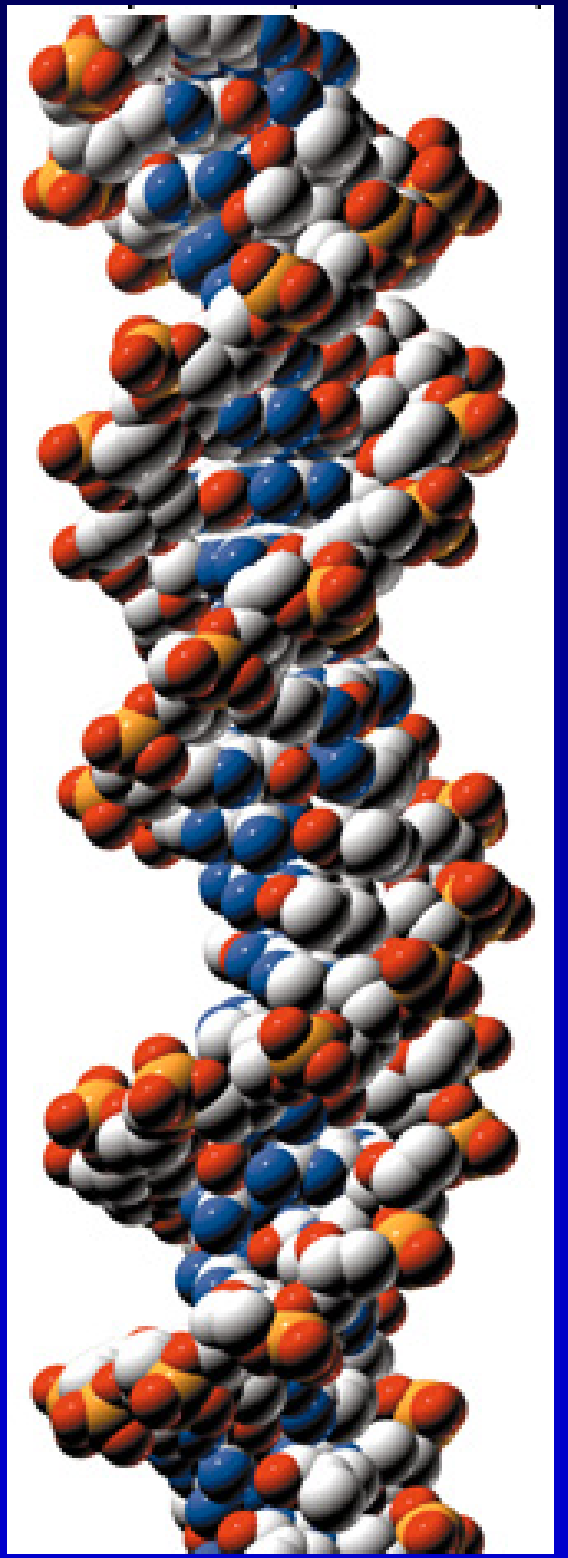

Bases
pürin
pirimidin
NH_2
adenine (A)
H_3C
thymine (T)
O
guanine (G)
NH_2
cytosine

DNA hidrojen bağları ile birleşmiş iki uzun nükleotid zincirinden oluşur



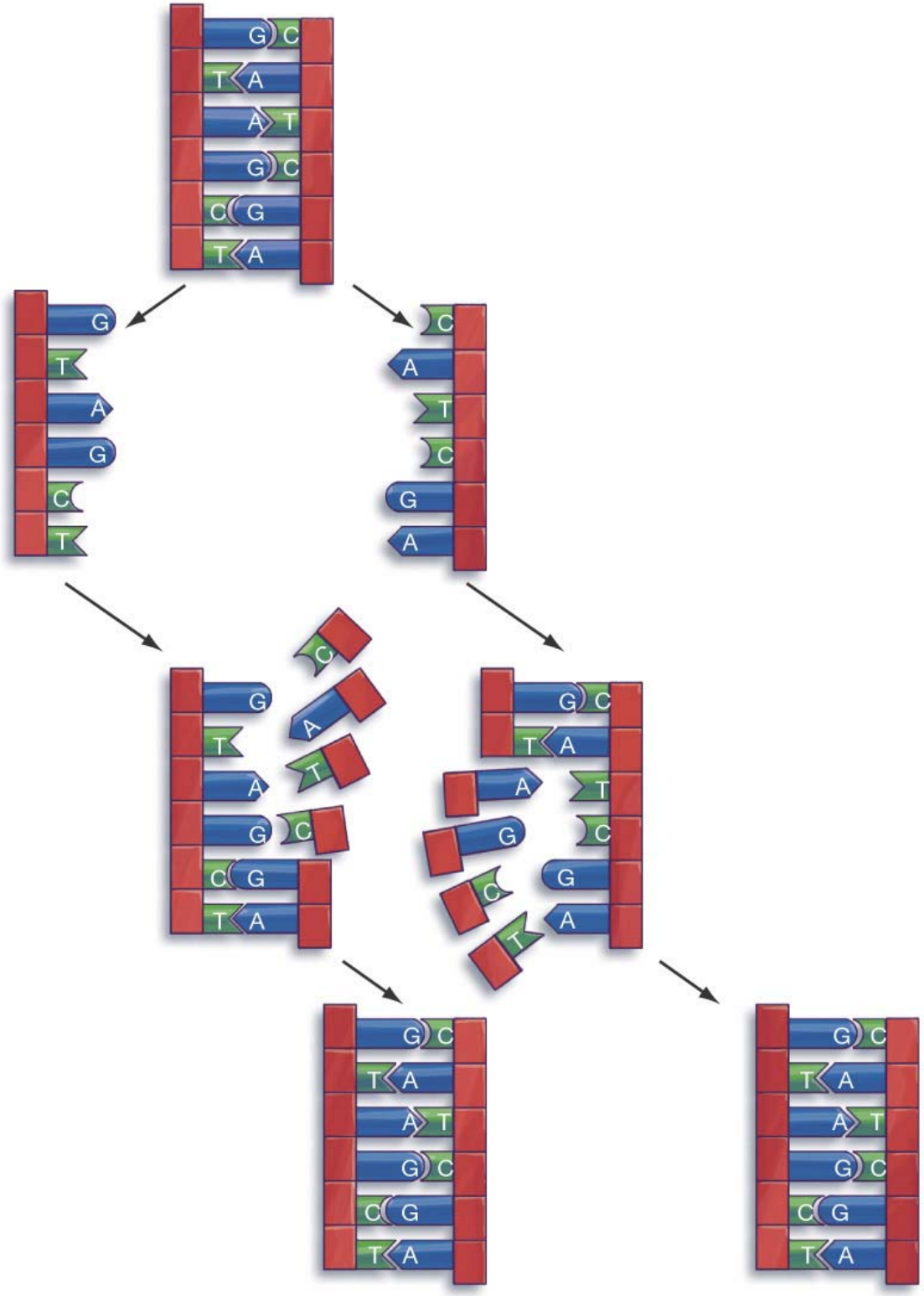
Çift iplikli DNA yapısı



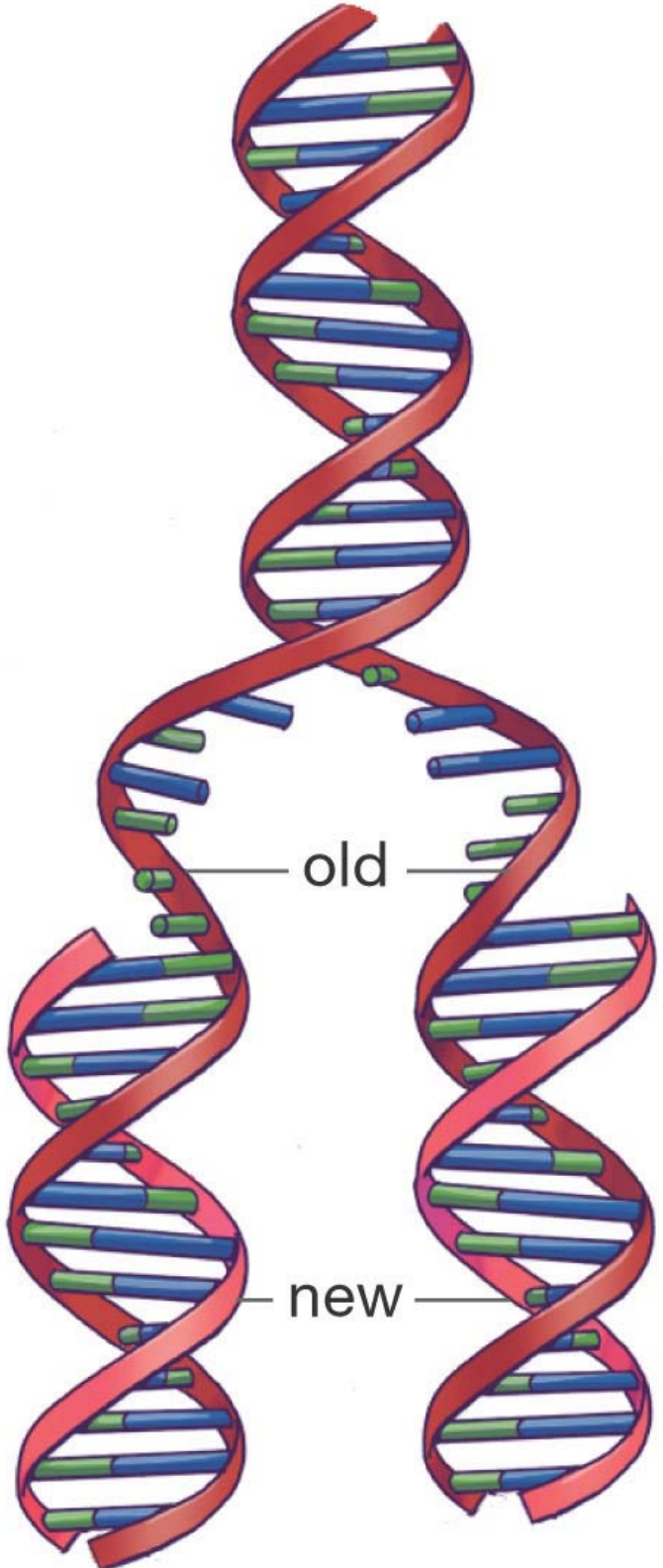


DNA Replikasyonu

Tamamlayıcı baz çiftleri replikasyonda her ipliğin kalıp olmasını sağlar

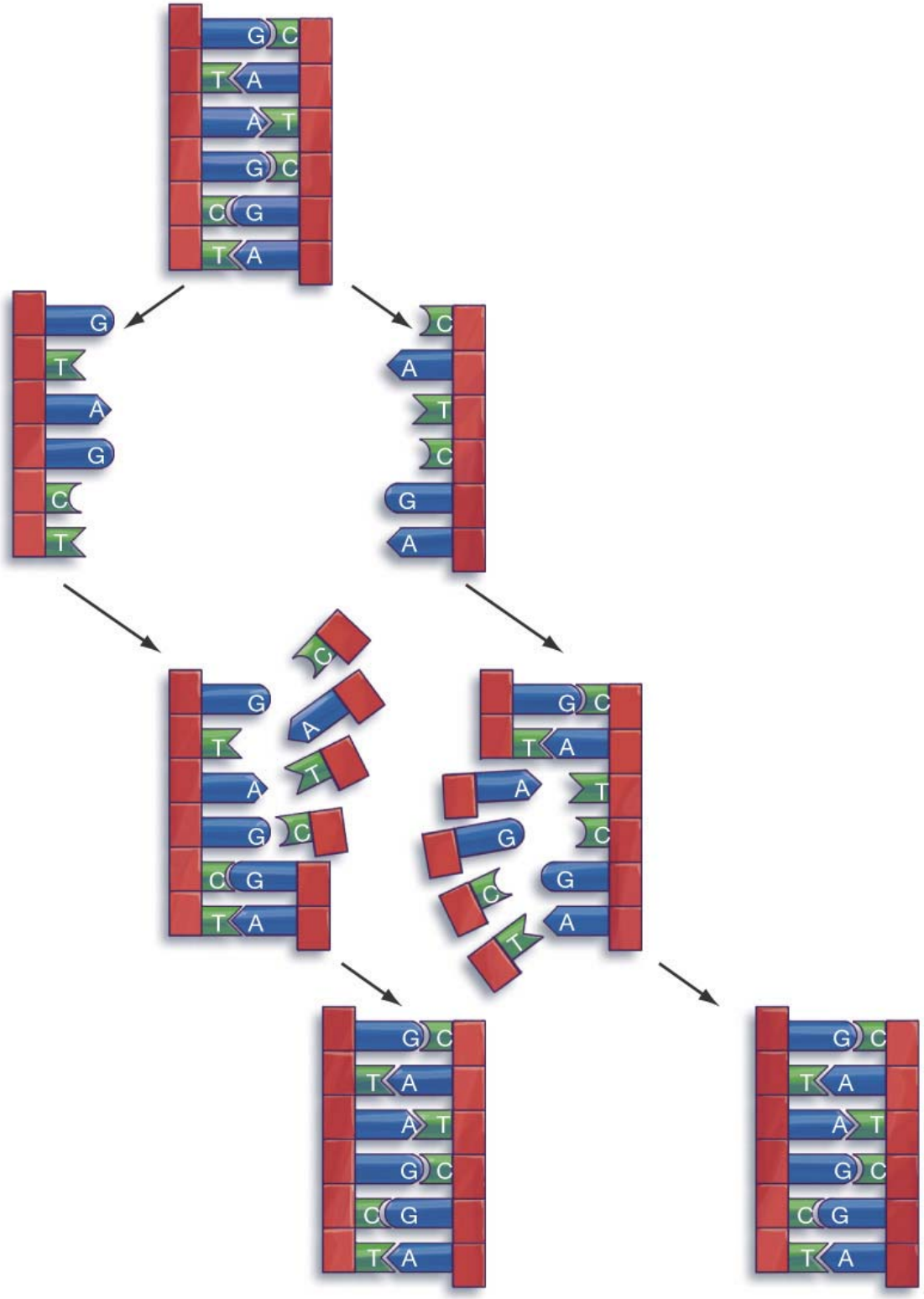


DNA Replikasyonu



DNA replikasyonunda pek çok enzim görev alır

-DNA polimeraz



Klinik Mikrobiyoloji Laboratuvarı'nda NAAT Kullanımı

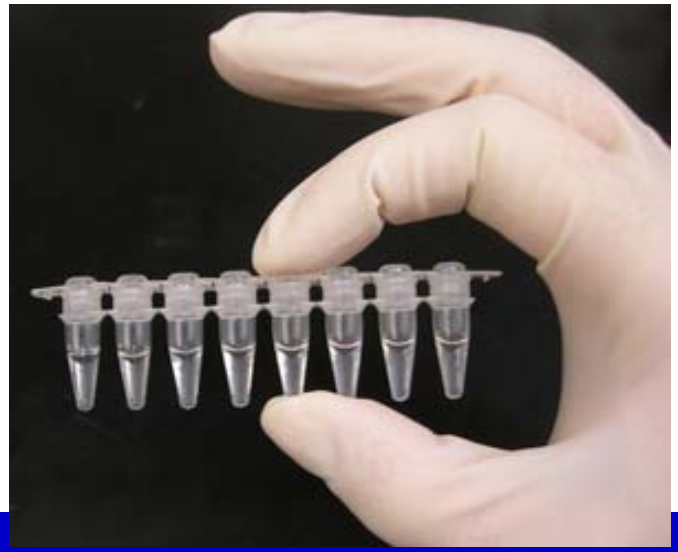
- Hedef Amplifikasyonu (Çoğaltma)
  - Polimeraz zincir reaksiyonu (PCR)
  - Ligaz zincir reaksiyonu (LCR)
  - Strand displacement amplification (SDA)
  - Transkripsiyon temelli amplifikasyon (TMA, NASBA)
- Sinyal amplifikasyonu (Çoğaltma)
  - bDNA
  - Direkt hibridizasyon

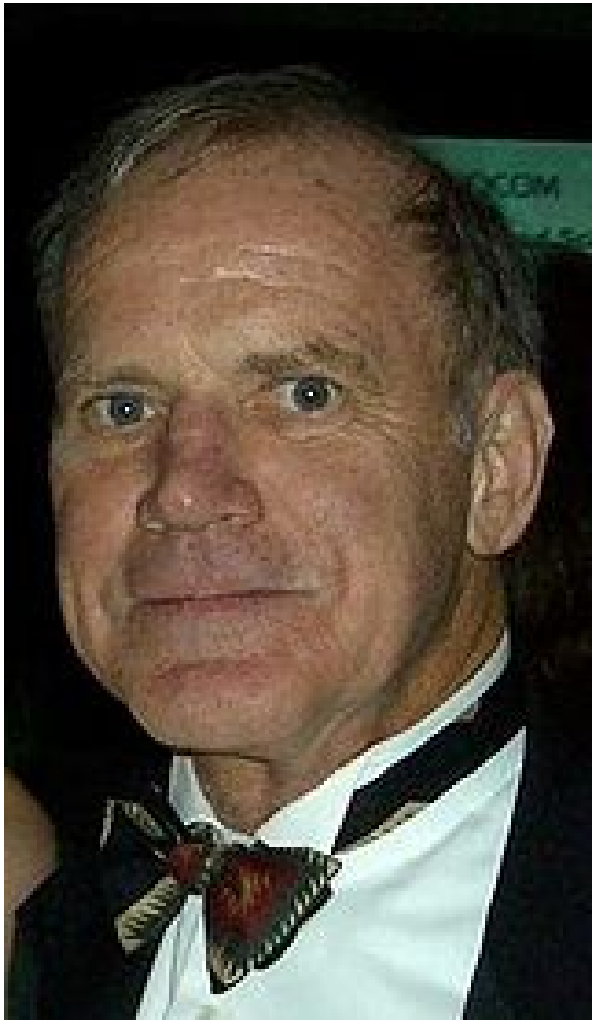
Hedef Çoğaltma

Polimeraz Zincir Reaksiyonu

- 1983 Kary Mullis

  1993'de Nobel ödülü (kimya)
- Milyon kopya DNA!





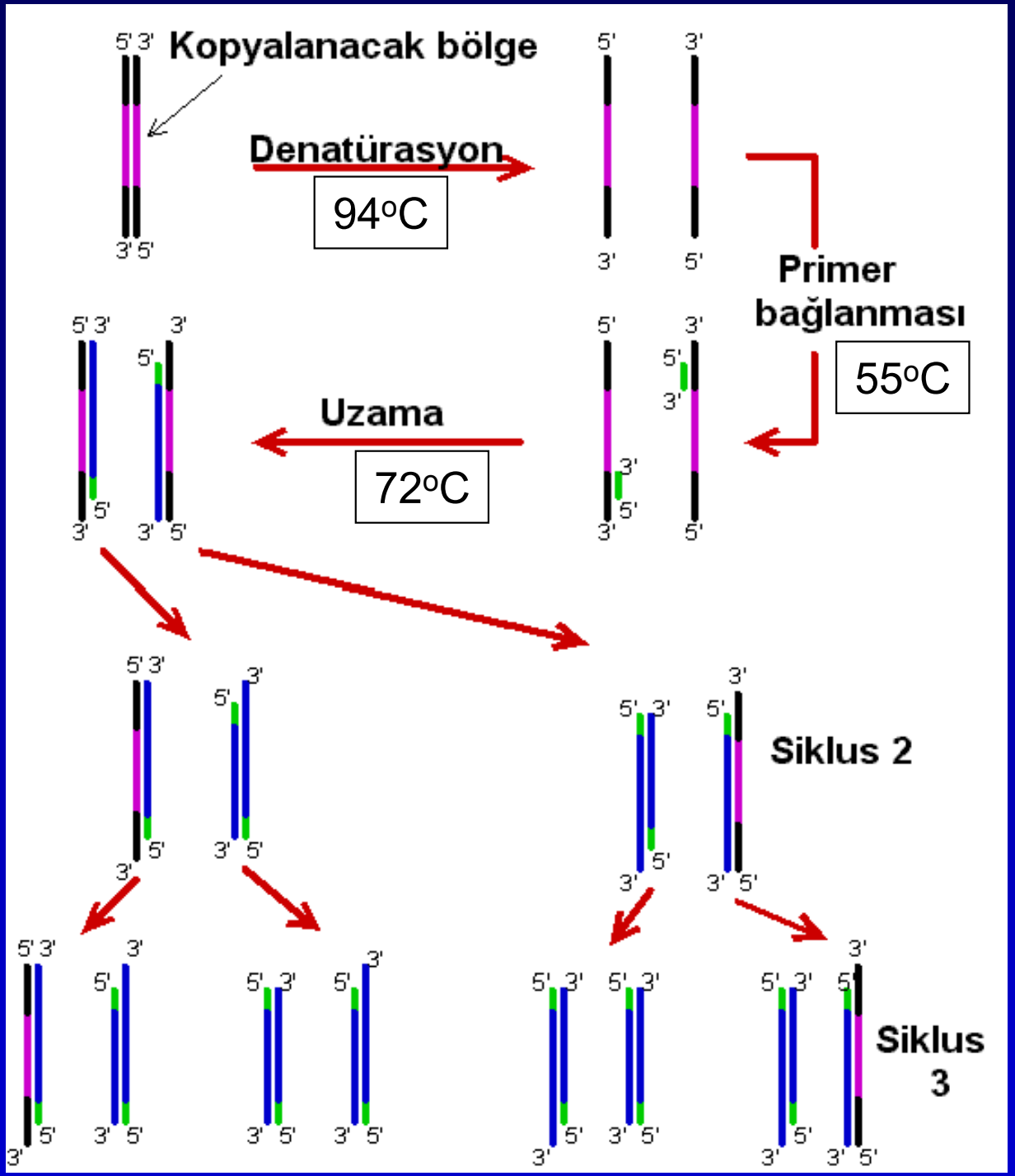
PCR:

1. Nükleik asit ekstraksiyonu
2. Amplifikasyon (Çoğaltma=PCR)
    - Denatürasyon
    - Bağlanma
    - Uzama
3. PCR ürününün saptanması
    - Jel elektroforezi
    - Hibridizasyon
    - Diğer

Polimeraz Zincir Reaksiyonu

1) DNA örneği
2) Çoğaltılacak olan bölgeyi sağdan ve soldan çevreleyen bir çift sentetik primer
3) dNTP'ler (A,T,C,G),
4) Isıya dayanıklı DNA-Polimeraz enzimi
   Taq polimeraz (Thermus aquaticus)
5) Uygun pH ve iyon koşullarını (Mg^{+2}) sağlayan tampon karışımı

PCR


Kopyalanacak bölge
Denatürasyon
94°C
Primer bağlanması
55°C
Uzama
72°C
Siklus 2
Siklus 3

PCR

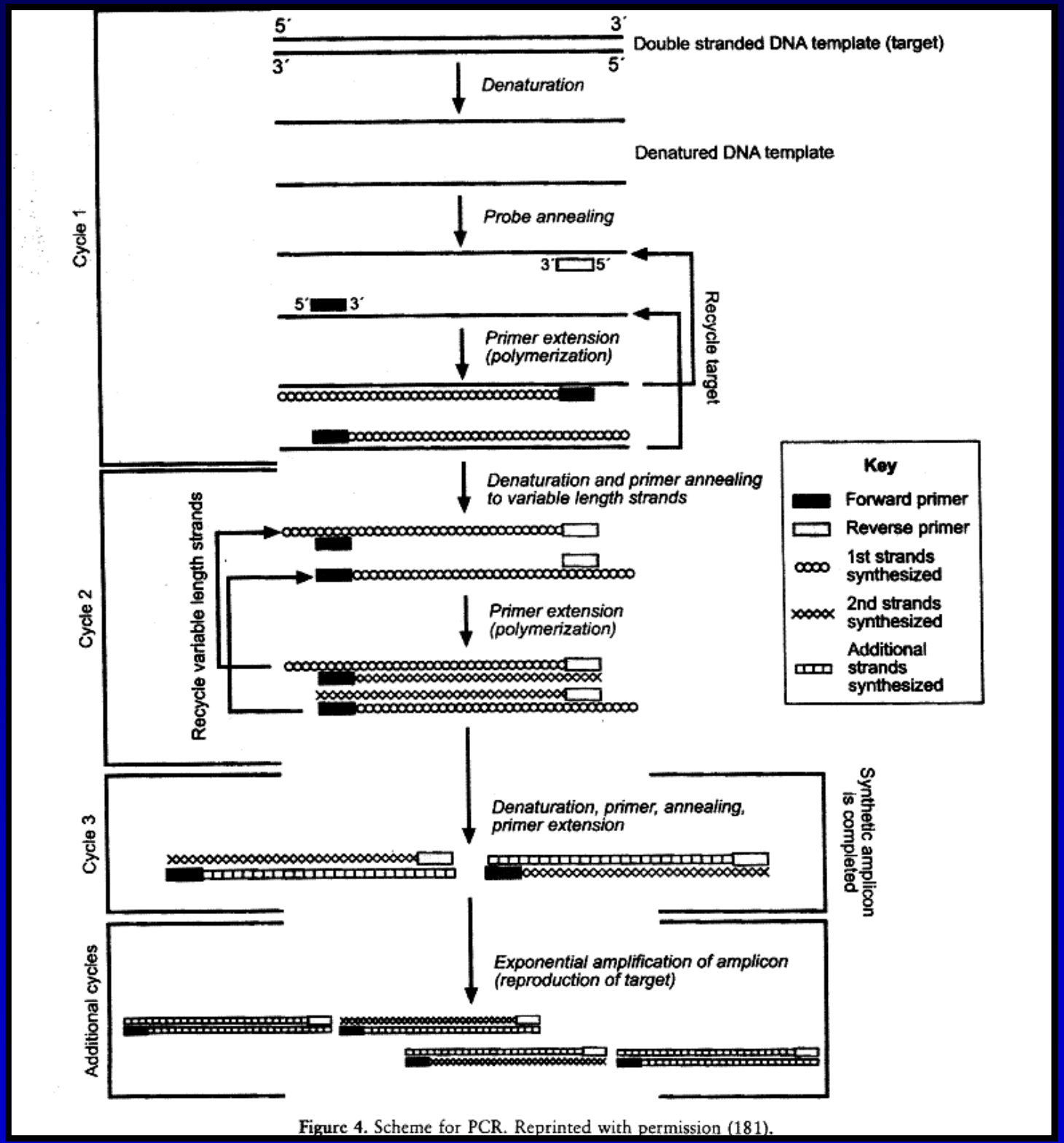


Figure 4. Scheme for PCR. Reprinted with permission (181).

Polimeraz Zincir Reaksiyonu

- Thermal cycler

Eski

Yeni

Polimeraz Zincir Reaksiyonu

Çoğaltma sonrası saptama

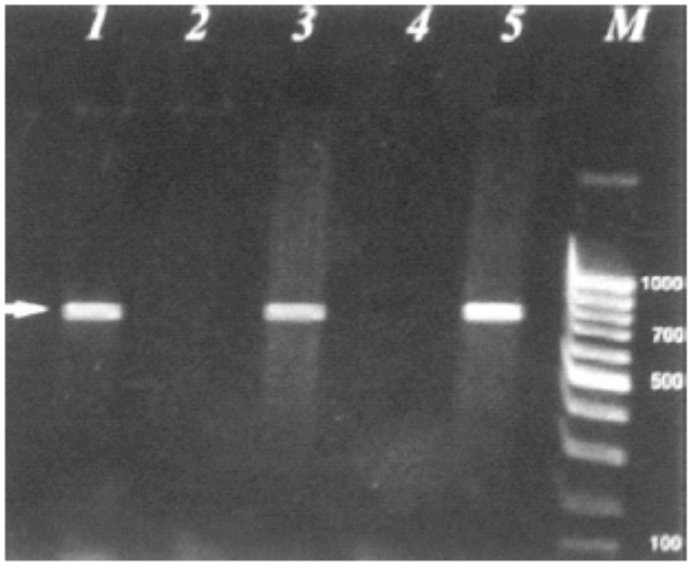


Figure 1. Agarose gel electrophoresis of polymerase chain reaction products (PCR) products. Lane 1; a 760 base pair PCR product amplified from DNA obtained from standard cultures, Lane 2; PCR product of DNA obtained from the control group rat, Lane 3; a 760 base pair PCR product amplified from DNA of the rat treated with EGb761, Lane 4; PCR control containing dH_2O. Lane 5; a 760 base pair PCR product amplified from DNA of the rat treated with normal saline solution, Lane M; 100 bp DNA ladder.

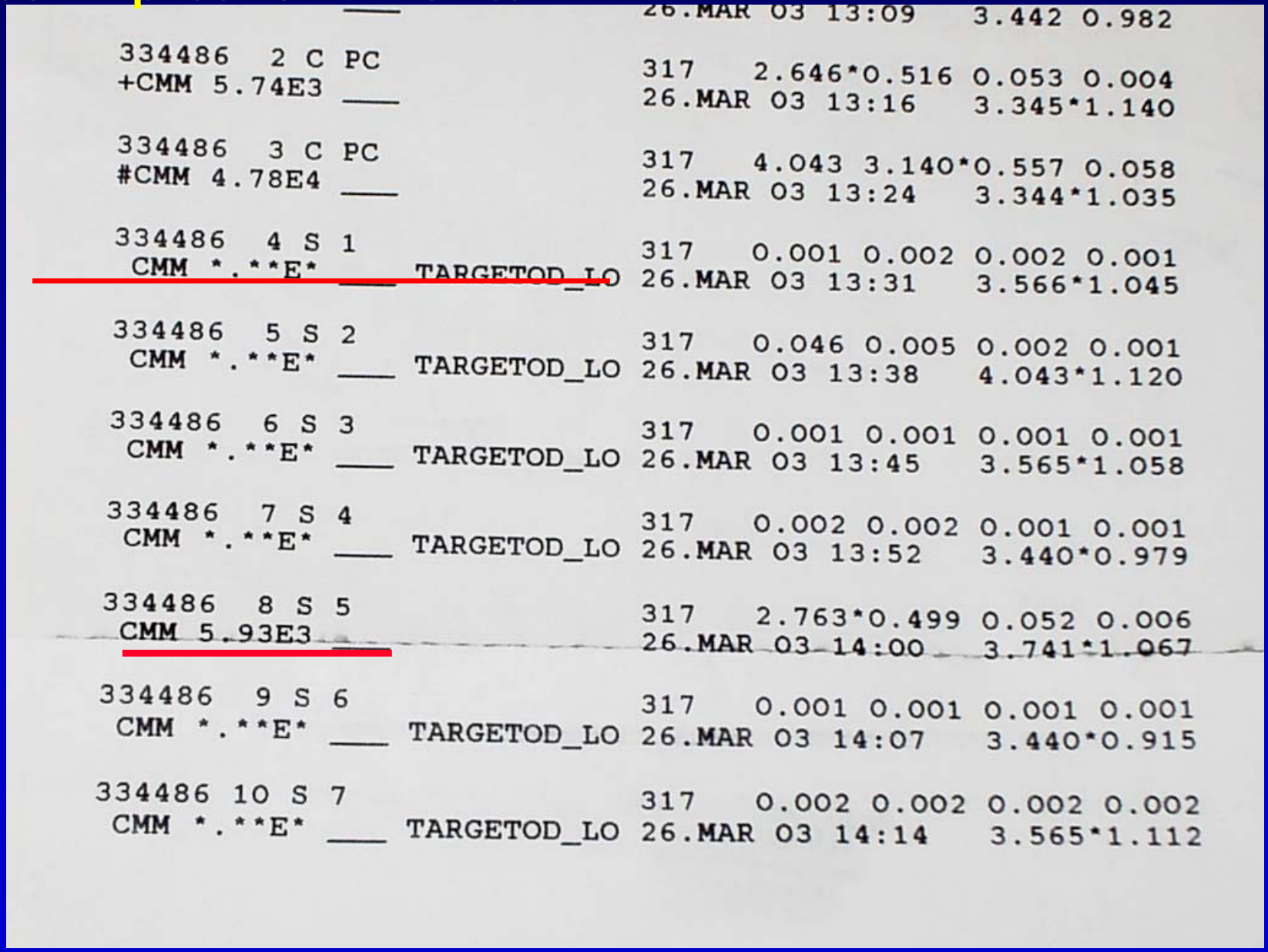
Polimeraz Zincir Reaksiyonu

Çoğaltma sonrası saptama

Cobas Amplicor CMV Monitor

Negatif

Pozitif

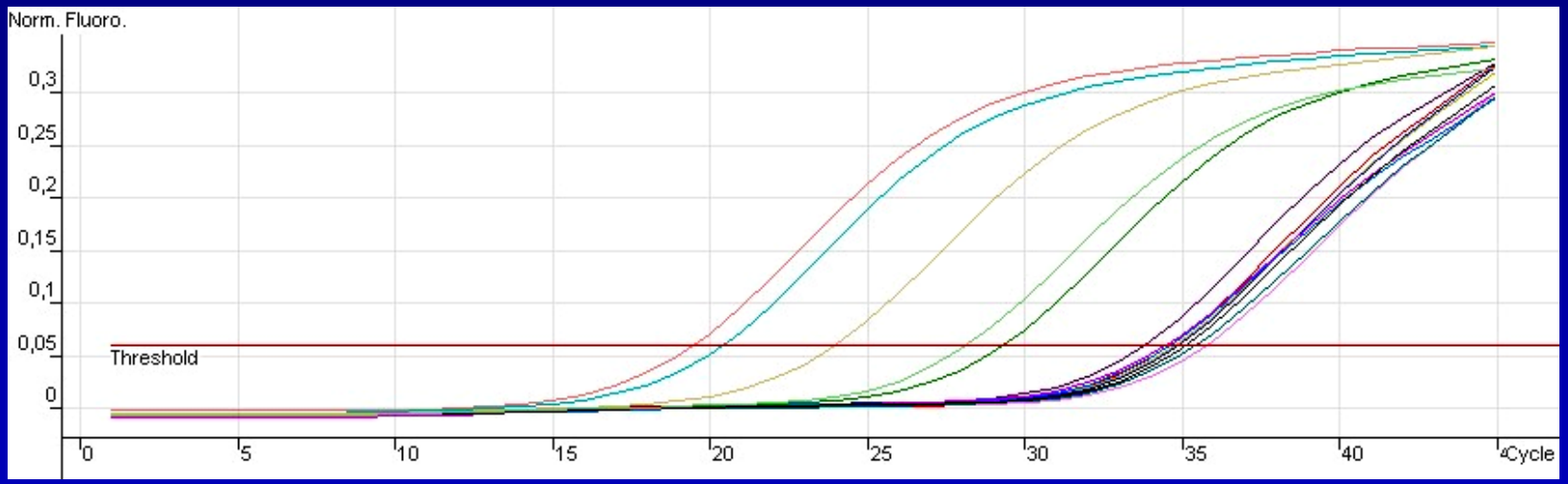


```
                                                  26.MAR 03 13:09    3.442 0.982

334486   2 C PC
+CMM 5.74E3 ____                                  317    2.646*0.516 0.053 0.004
                                                  26.MAR 03 13:16    3.345*1.140

334486   3 C PC
#CMM 4.78E4 ____                                  317    4.043 3.140*0.557 0.058
                                                  26.MAR 03 13:24    3.344*1.035

334486   4 S 1                                    317    0.001 0.002 0.002 0.001
 CMM *.**E* ____ TARGETOD_LO                      26.MAR 03 13:31    3.566*1.045

334486   5 S 2                                    317    0.046 0.005 0.002 0.001
 CMM *.**E* ____ TARGETOD_LO                      26.MAR 03 13:38    4.043*1.120

334486   6 S 3                                    317    0.001 0.001 0.001 0.001
 CMM *.**E* ____ TARGETOD_LO                      26.MAR 03 13:45    3.565*1.058

334486   7 S 4                                    317    0.002 0.002 0.001 0.001
 CMM *.**E* ____ TARGETOD_LO                      26.MAR 03 13:52    3.440*0.979

334486   8 S 5                                    317    2.763*0.499 0.052 0.006
 CMM 5.93E3 ____                                  26.MAR 03 14:00    3.741*1.067

334486   9 S 6                                    317    0.001 0.001 0.001 0.001
 CMM *.**E* ____ TARGETOD_LO                      26.MAR 03 14:07    3.440*0.915

334486 10 S 7                                     317    0.002 0.002 0.002 0.002
 CMM *.**E* ____ TARGETOD_LO                      26.MAR 03 14:14    3.565*1.112
```

Polimeraz Zincir Reaksiyonu:

- Multiplex PCR
- Nested PCR
- RT-PCR (RNA için cDNA sentezi)
  - Riboz şeker
  - A=U
  - G=C
- Real-Time PCR

Polimeraz Zincir Reaksiyonu: Real-Time PCR



LCR

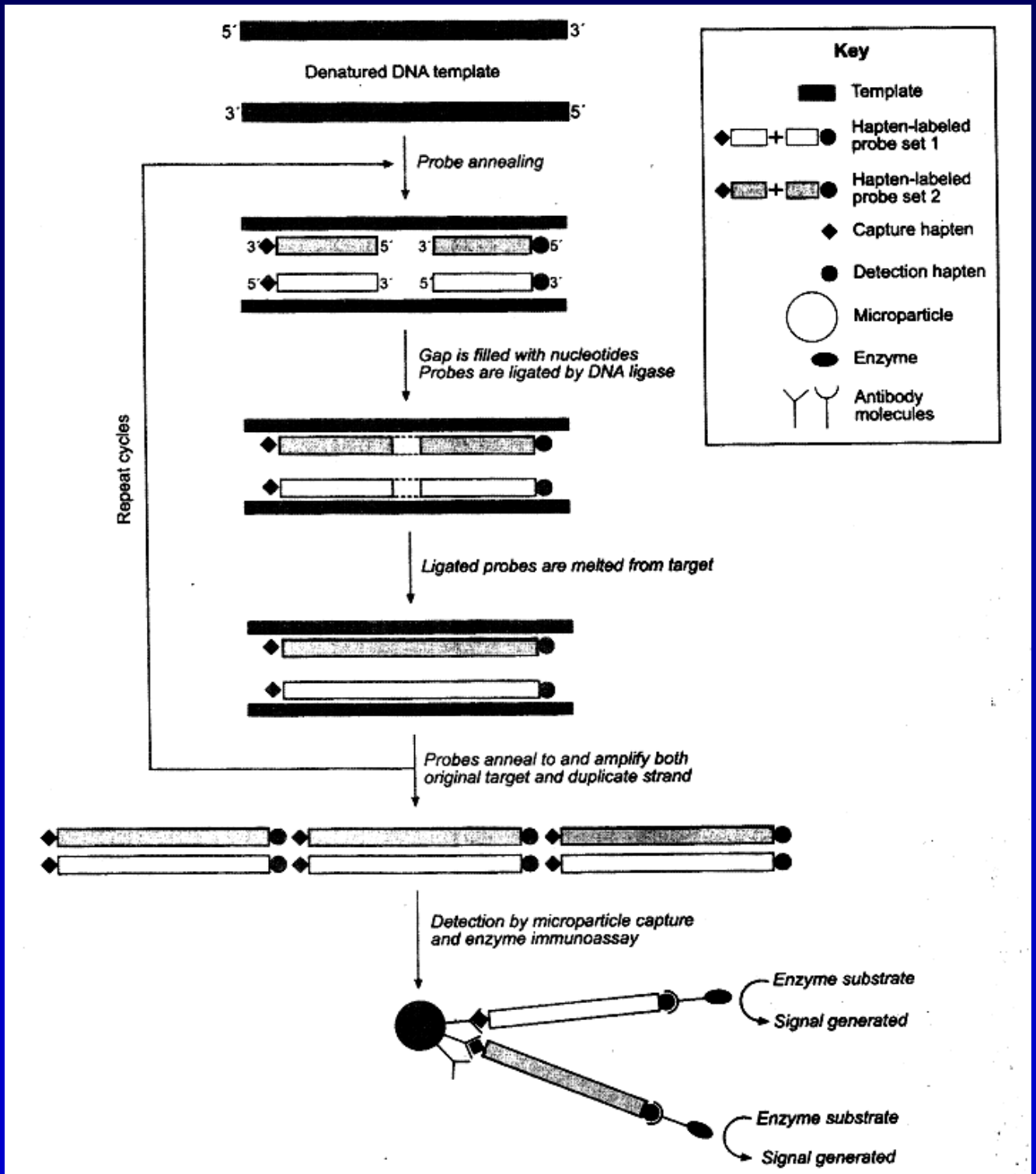


Figure 5. Scheme for LCR. Reprinted with permission (181).

SDA

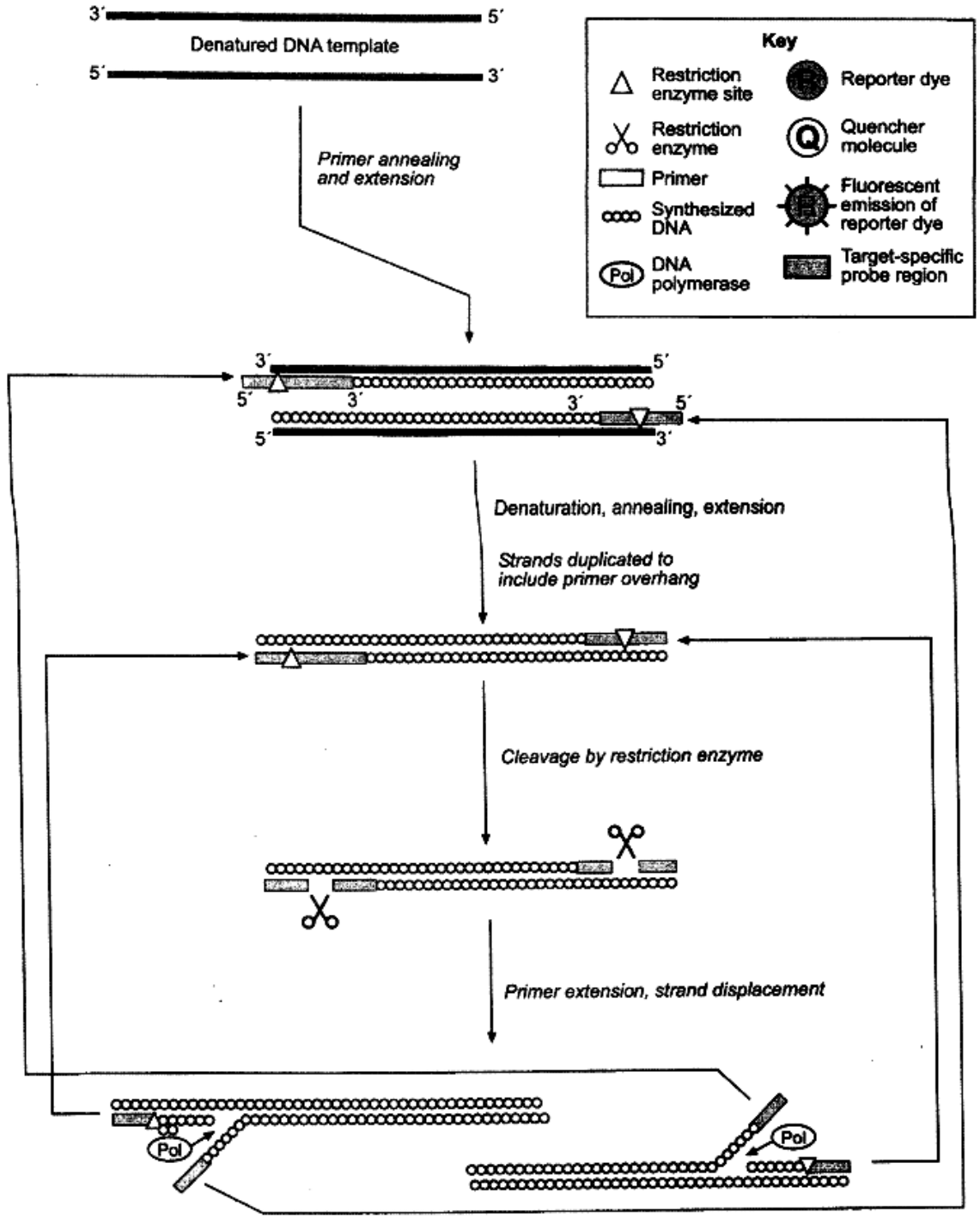


Figure 6. Scheme for SDA. Reprinted with permission (181).

TMA

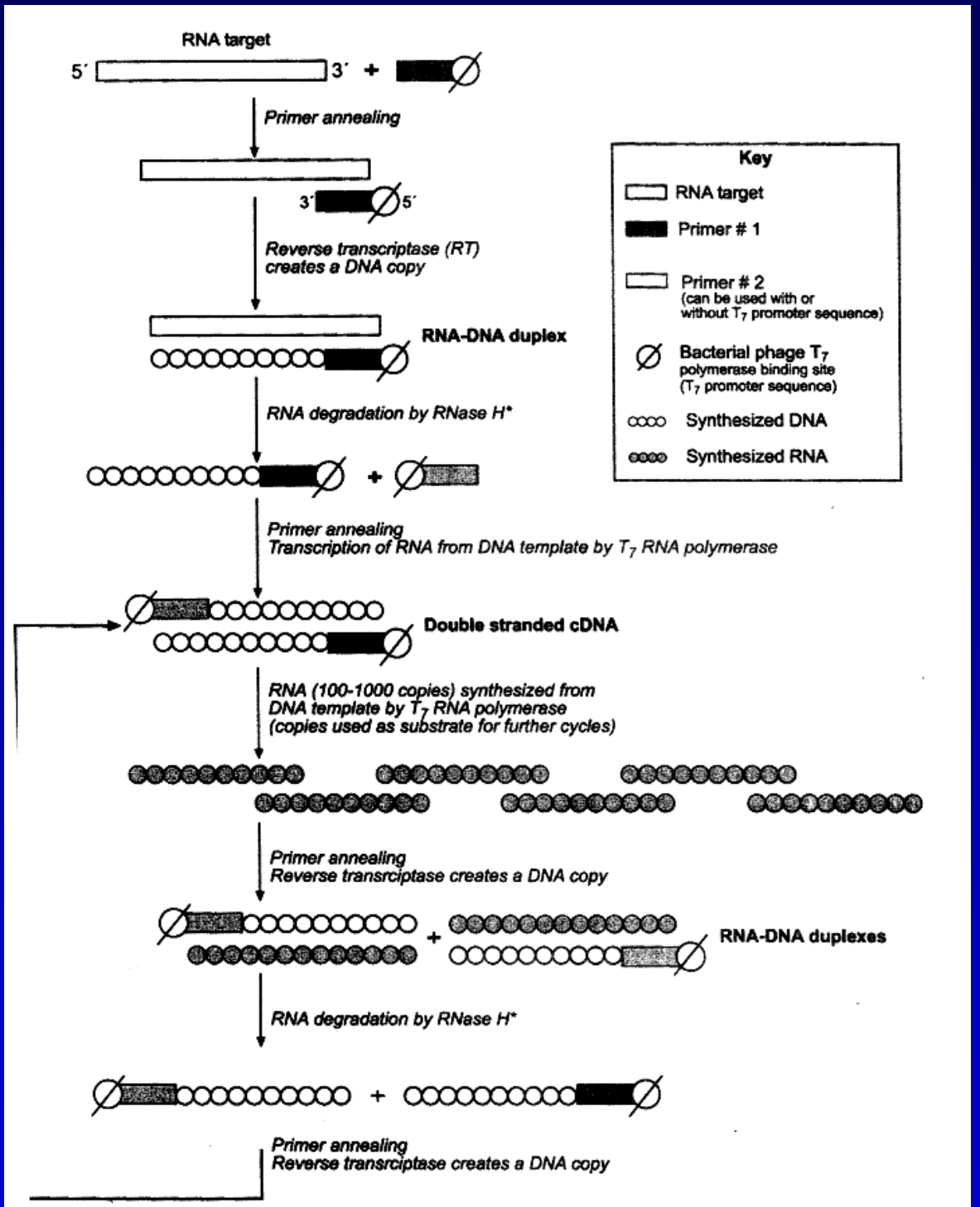


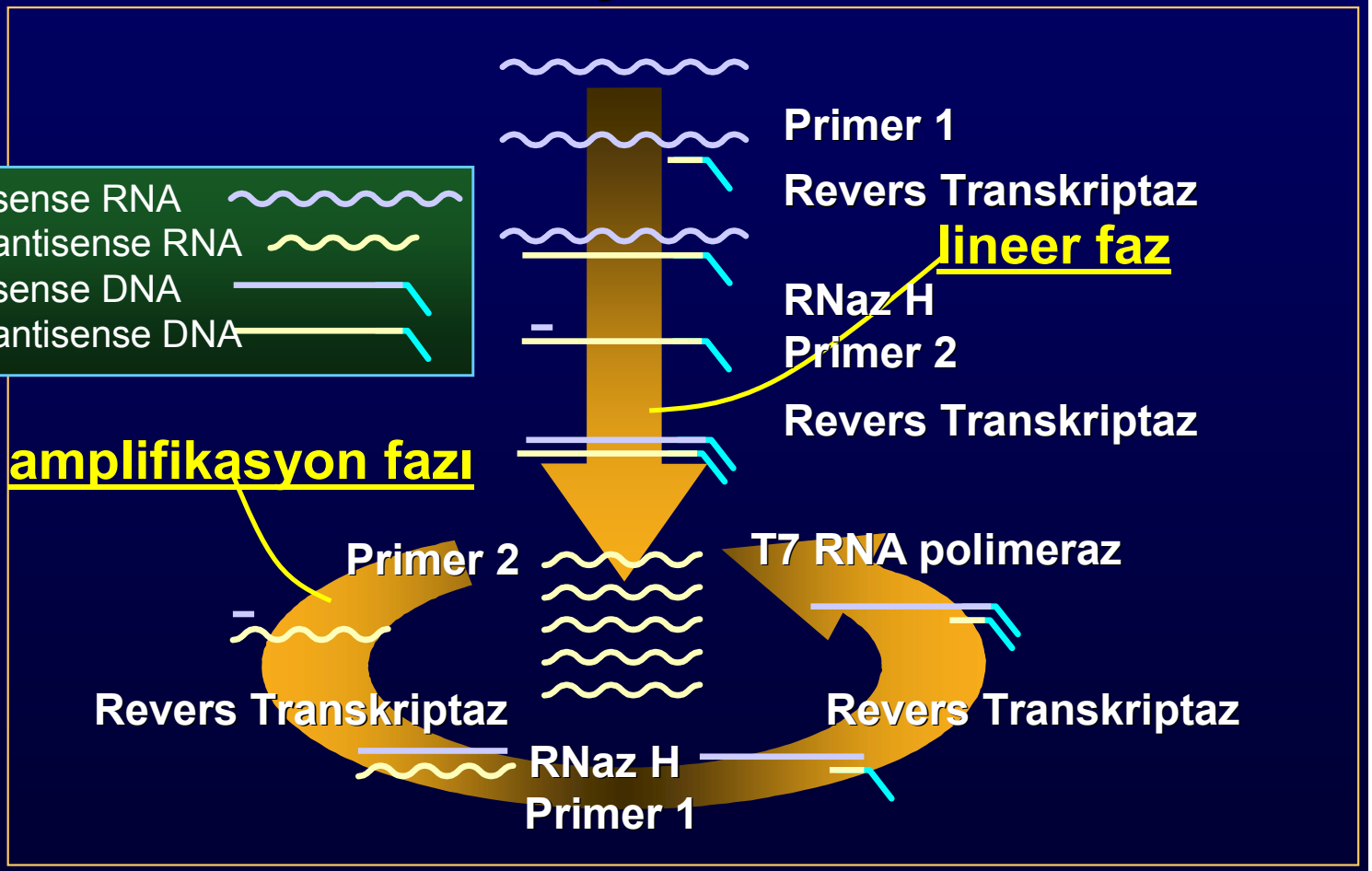
Figure 7. Scheme for transcription-based amplification systems. Reprinted with permission (181).

Nuclisens pp67 mRNA NASBA

1- Tam kandan nükleik asit izolasyonu

2- İzotermal amplifikasyon (41°)

3- Hibridizasyon kemilumin. saptama (CMV, IC)

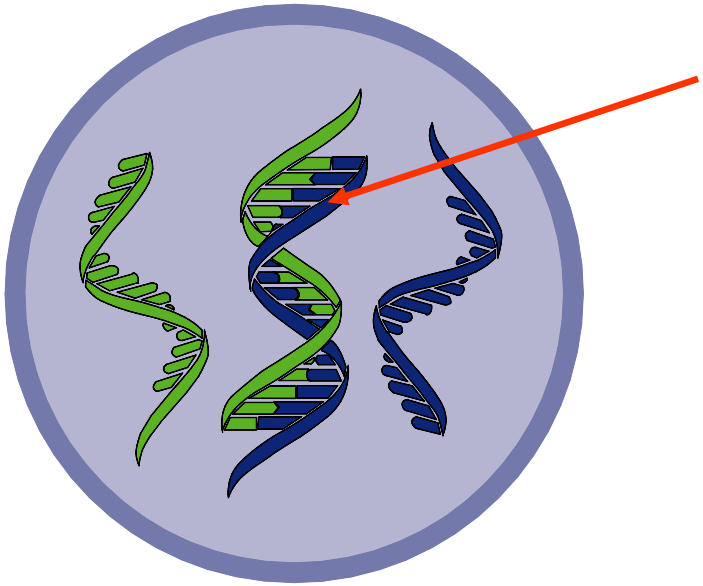


4. Duyarlılık: 700 RNA molekülü

Sinyal Çoğaltma

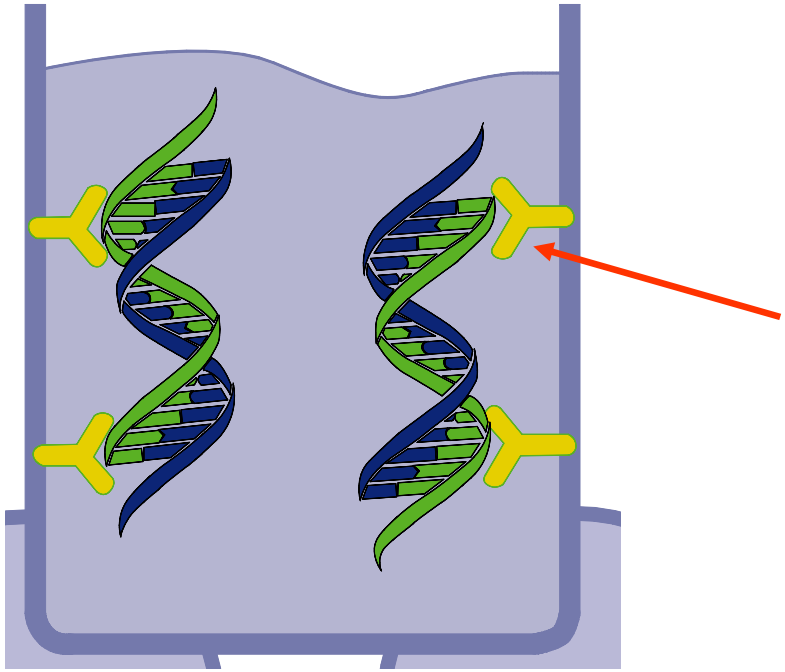
Hibridizasyon-1

DNA ekstraksiyonu ve denatürasyon



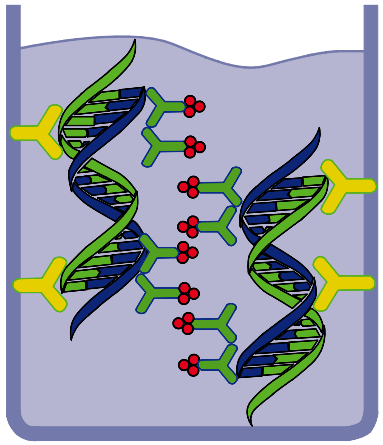
Spesifik prob ile hibridizasyon

Hibridizasyon-2

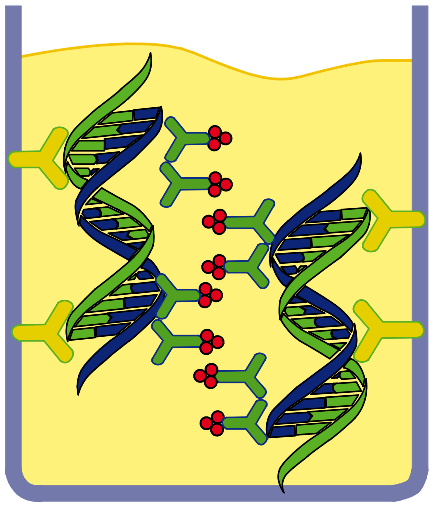


Oluşan hibridin antikorla kaplı tüplerde yakalanması

Hibridizasyon-3

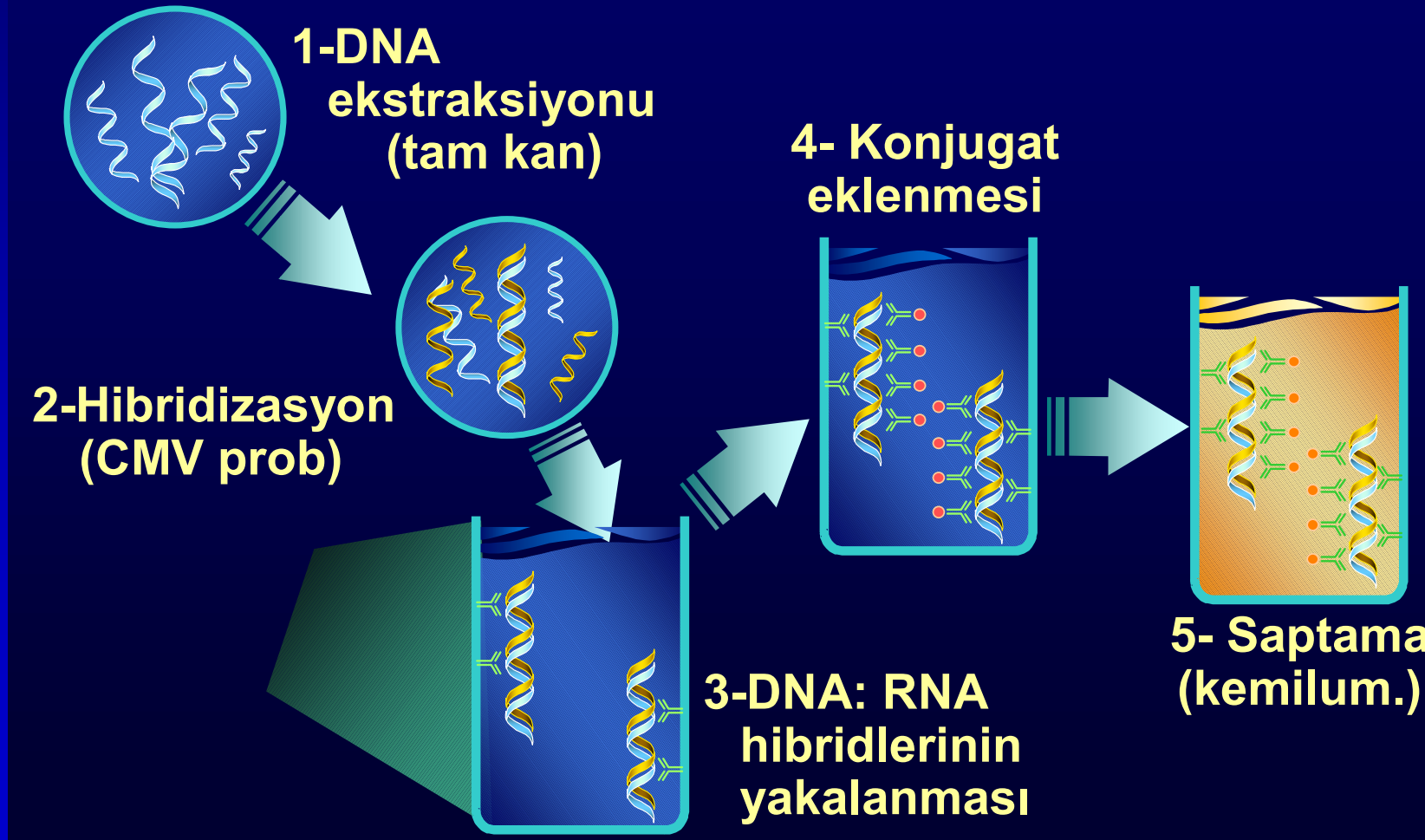


Ortama enzimle işaretli antikorların eklenmesi



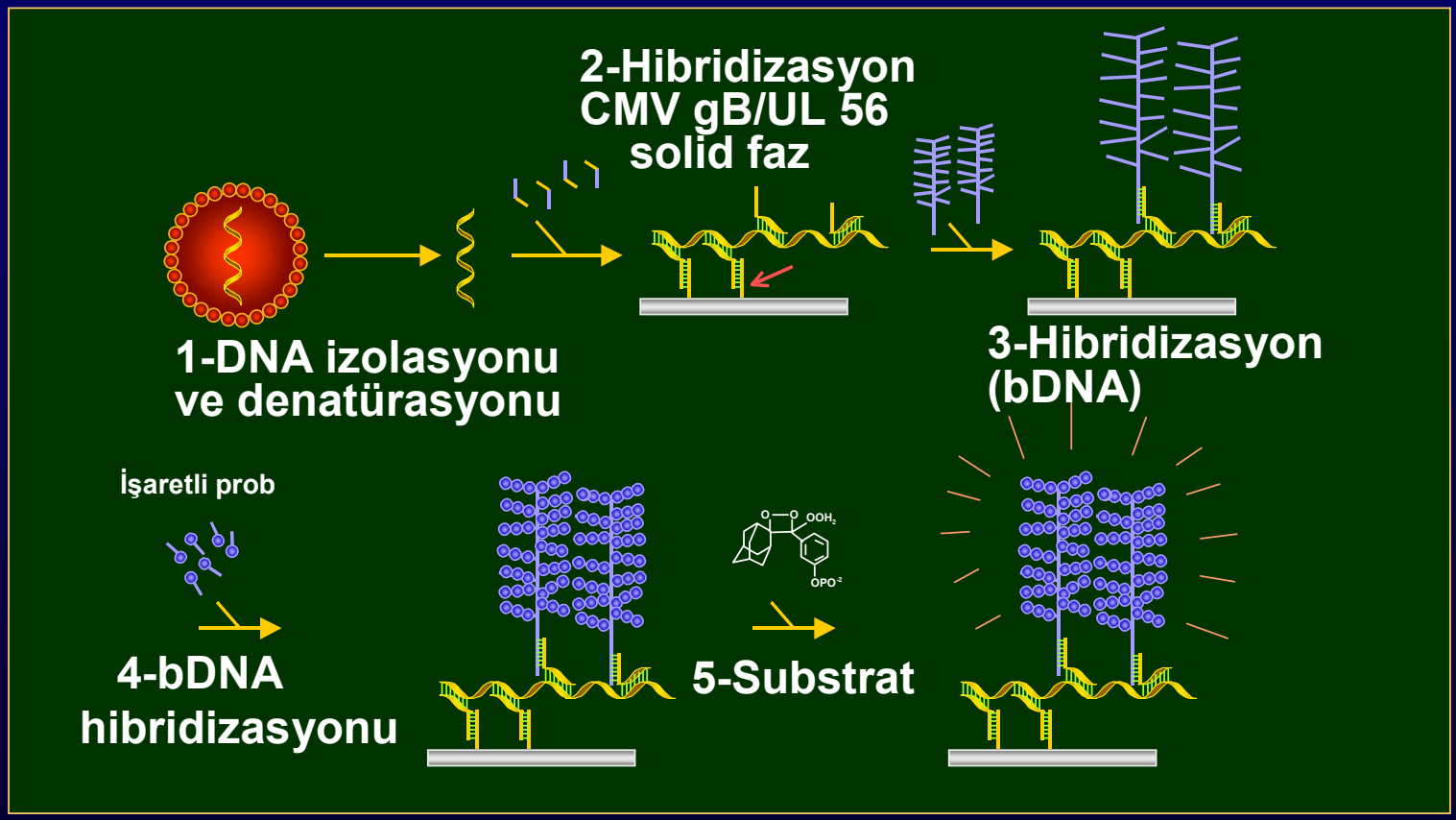
Ortama substrat eklenmesi ve renklenme

Hybrid capture CMV DNA testi



Duyarlılık: 2.1-830 pg DNA

Quantiplex (bDNA) CMV testi



Duyarlılık: 900 kopya/10^6 lökosit

bDNA

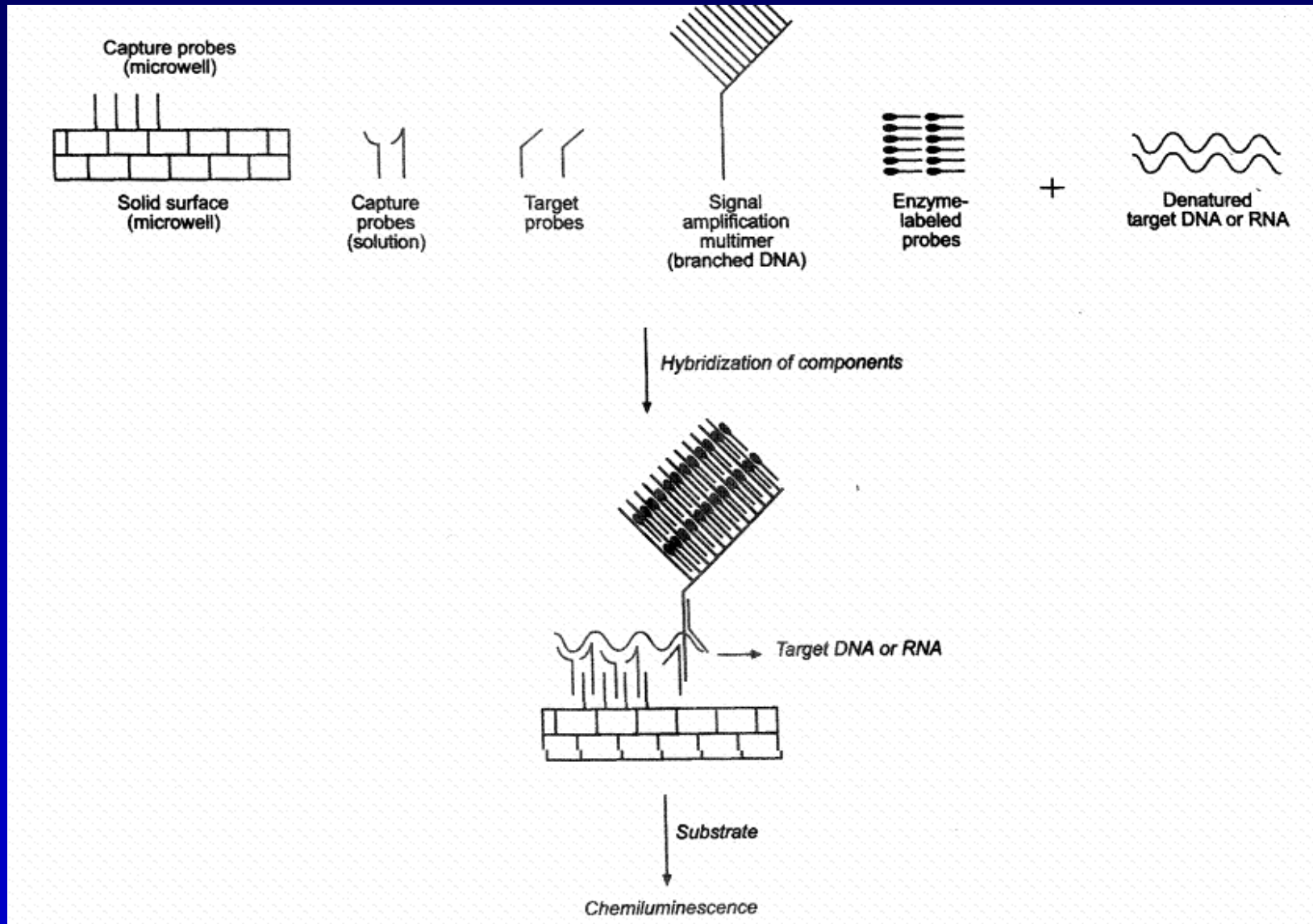


Figure 8. Scheme for bDNA signal amplification. Reprinted with permission (181).

NAAT'ın mikrobiyolojide kullanım alanları:

1. Kültürü yapılamayan veya çok geç üreyen mikroorganizmaların araştırılması
2. Kısa sürede, duyarlılığı ve özgüllüğü yüksek testlerle tanı konulması
3. Antibiyotik direnç genlerinin araştırılması
4. Mutant mikroorganizmaların araştırılması

?

Analitik duyarlılık = Klinik duyarlılık