

**PCR**

# PCR

- = Polymerase Chain Reaction (Reactia in lant a polimerazei)
- Mullis & Faloona – *Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction*, 1987
- Mullis: Premiul Nobel, Chimie, 1993
- Amplificarea cu ajutorul unei enzime (ADN-polimeraza) a numărului de copii ale unei regiuni specifice dintr-un lanț ADN

➤ **Amplificarea selectivă a unui fragment particular al ADN-ului (de exemplu un exon al unei gene umane)**

➤ **Permite obținerea în 2 ore a unei cantități de ADN detectabilă pe gel de agaroză prin colorare cu bromură de etidiu (teoretic metoda PCR poate amplifica un segment provenit de la o singură moleculă de ADN; după 30 de cicluri se vor obține aproximativ 1 miliard de copii alături de alte câteva zeci de fragmente ADN diferite)**

➤ **Principiile de bază au fost expuse de Gobid Khorana în 1971, însă dificultățile în sinteza primerilor și purificarea polimerazelor au întârziat impunerea metodei**

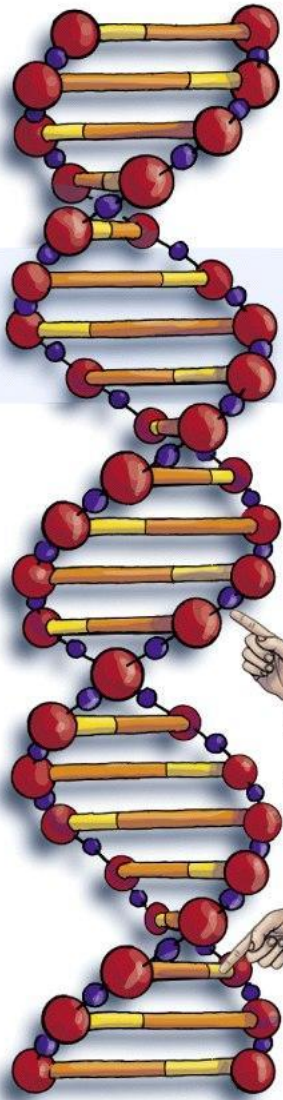
# ADN

- Acid nucleic (dezoxiribonucleic)
- Lant dublu catenar:
  - 5' (fosfatat) → 3' (hidroxilat)
  - 3' → 5'
- Nucleotid:
  - Nucleozid:
    - Baza azotata
    - Pentoza
  - Grupare fosfat

# BAZELE AZOTATE

- Baze purinice:
  - Adenina (A)
  - Guanina (G)
- Baze pirimidinice:
  - Citozina (C)
  - Timina (T)
- Legături de hidrogen:
  - A:T
  - C:G

**DNA double helix is made of two strands.**



**"Handrails" made of sugars and phosphates**

**"Rungs" made of nitrogenous bases**

**Each strand is a chain of of antiparallel nucleotides.**

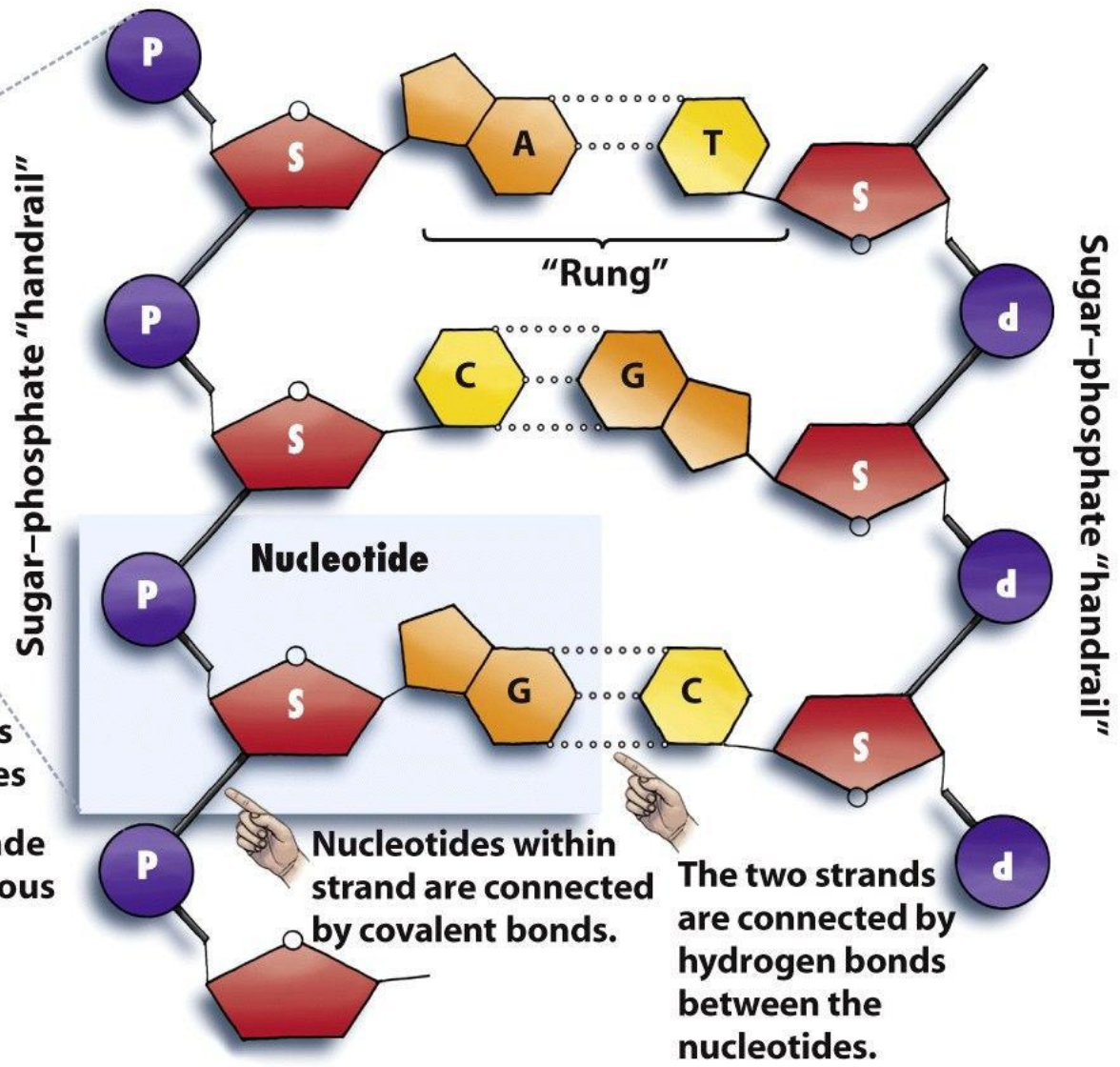
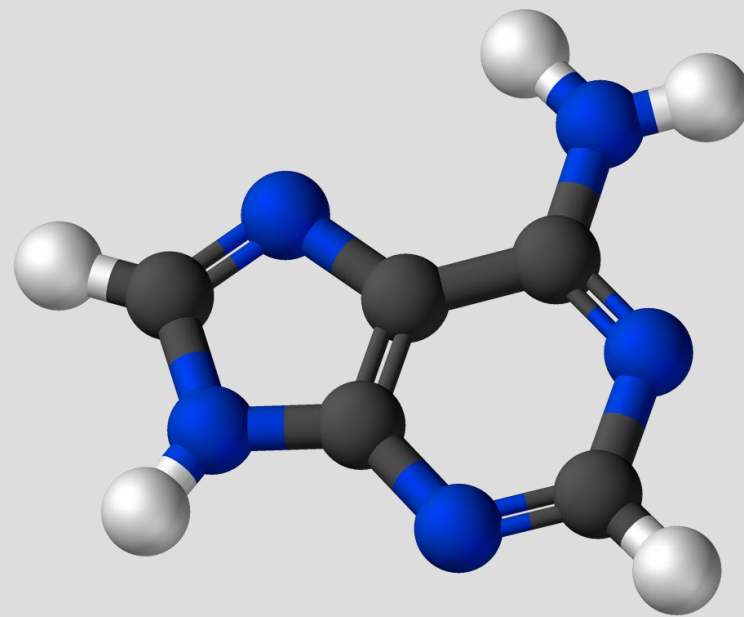
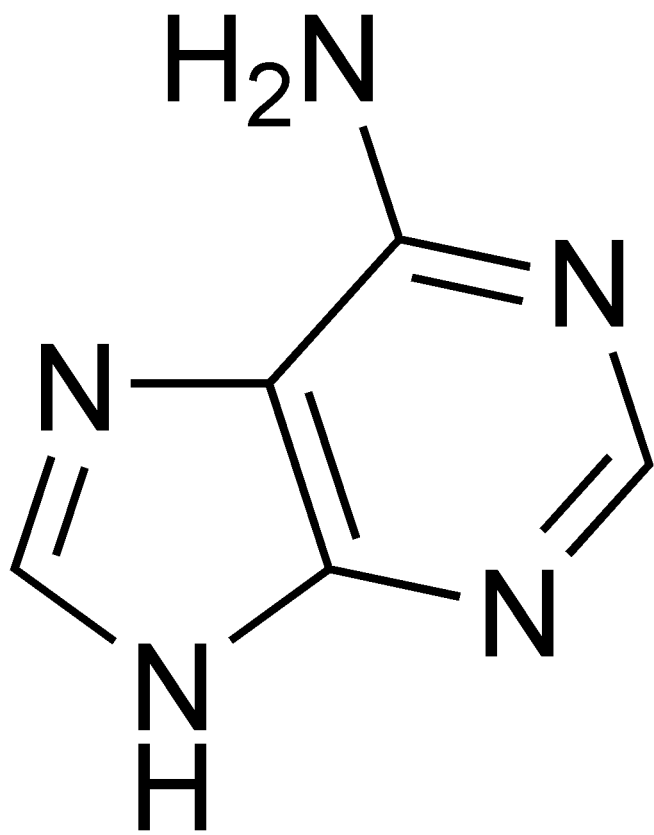
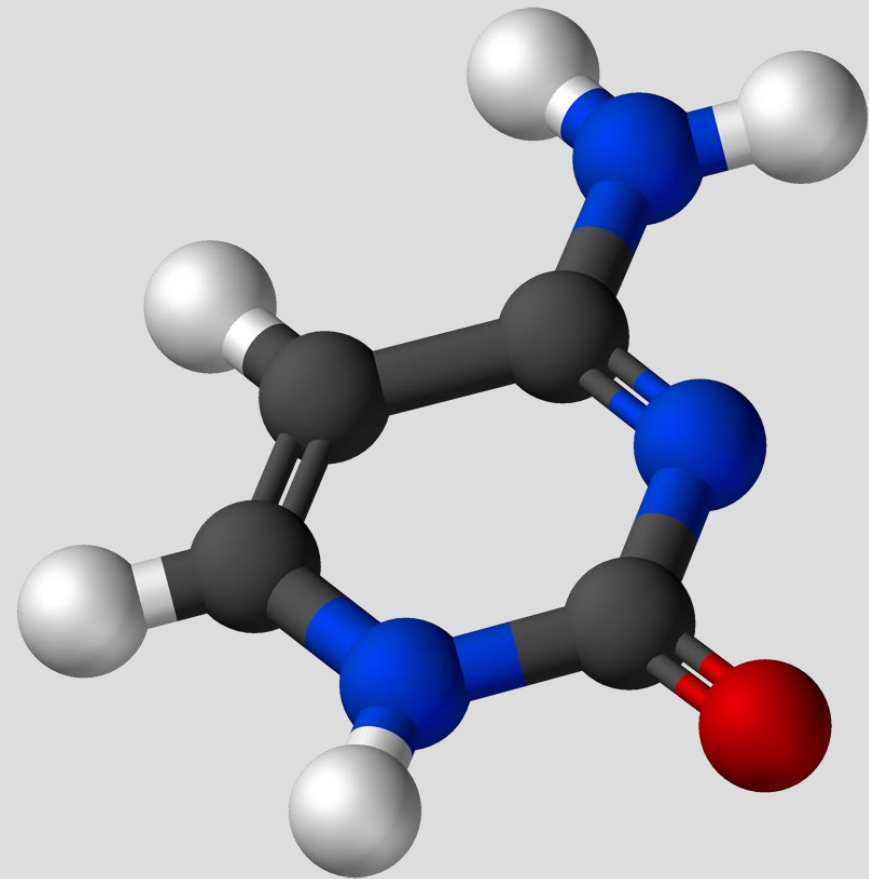
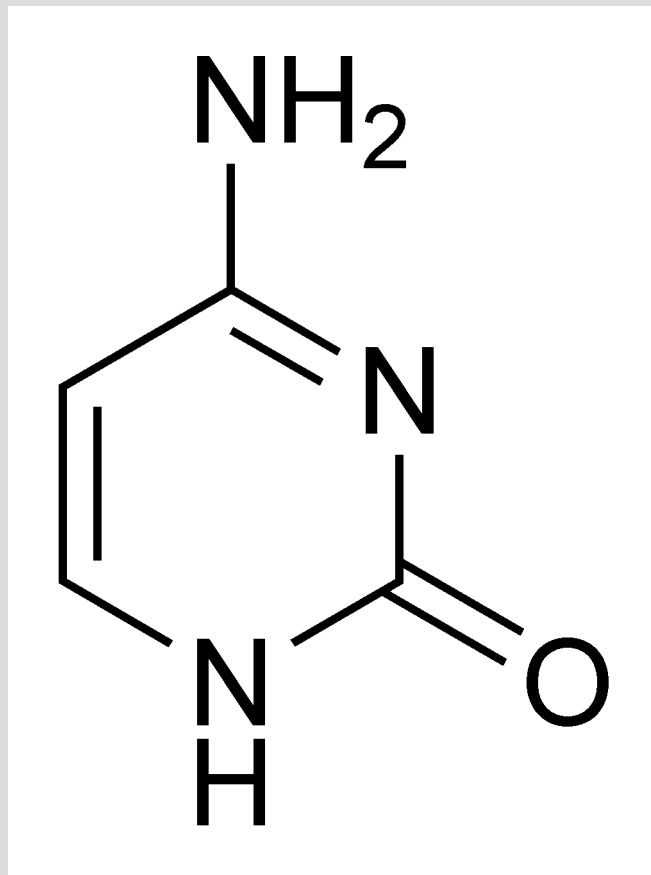


Figure 2-13ab Biology: Science for Life, 2/e  
© 2007 Pearson Prentice Hall, Inc.

# ADENINA

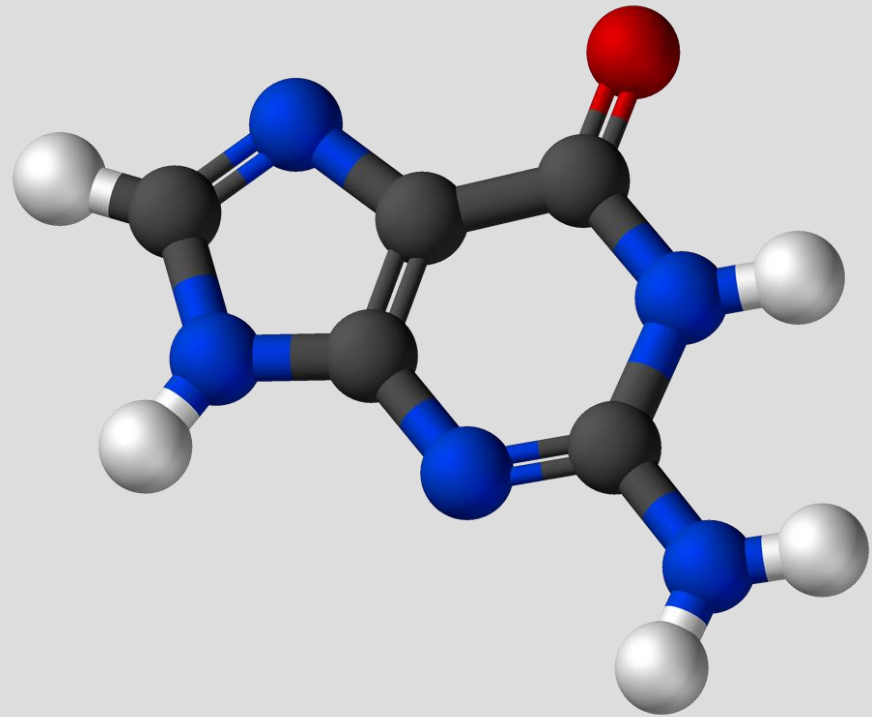
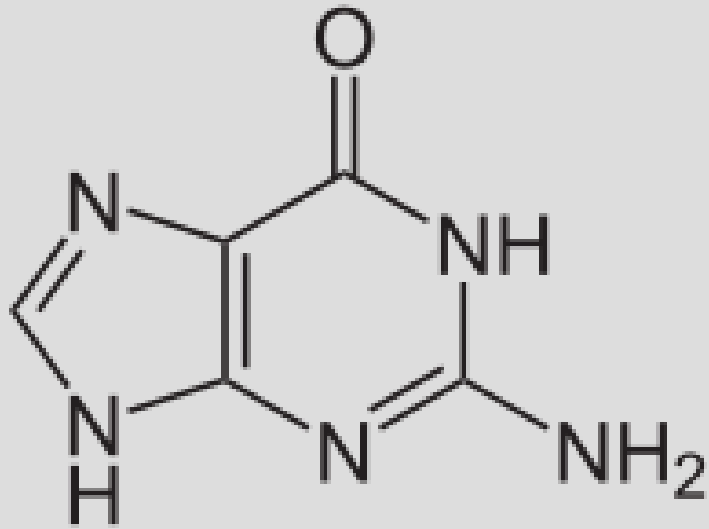


# CITUZINA

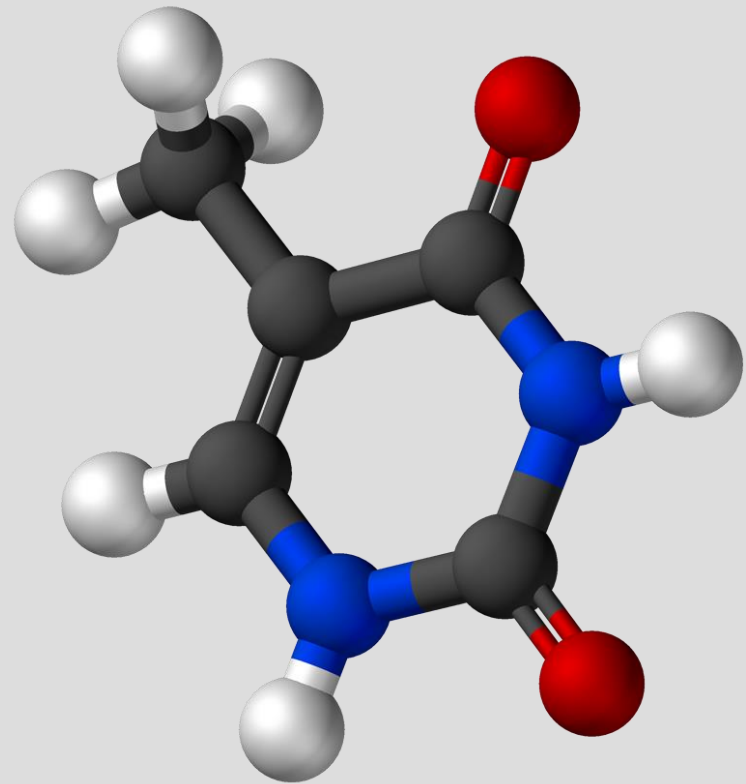
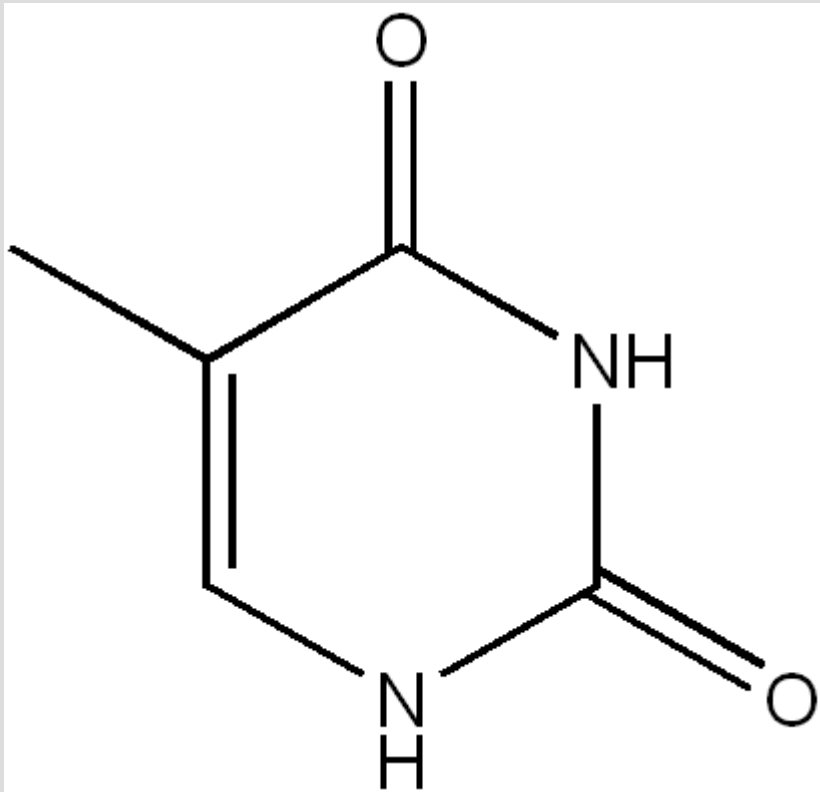




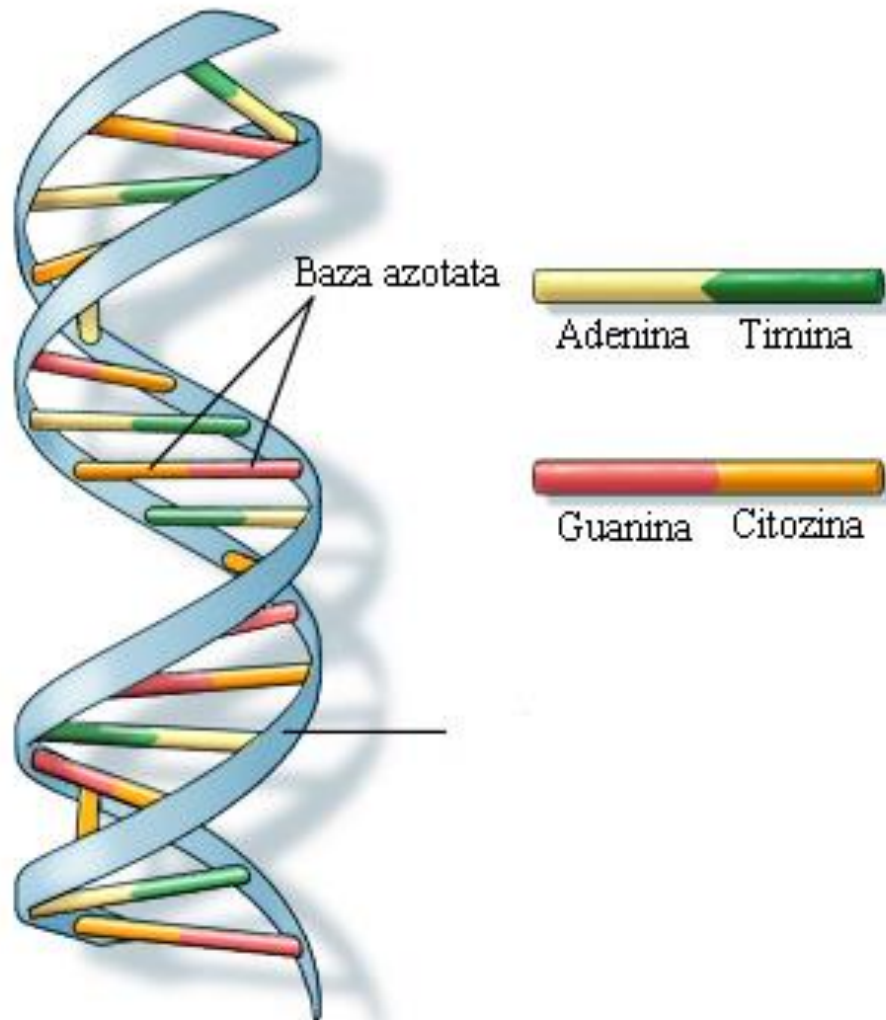
# GUANINA



# TIMINA



# ADN



5' → 3'

TCACACTGAGCGTGCTG

AGTGTGACTCGCACGAC

3' → 5'

# TEHNICA PCR

- ADN de amplificat
- Primeri (oligonucleotide, amplimeri)
- ADN-polimeraza
- dNTP (dezoxiribonucleotid trifosfati)
- Solutie tampon (**Mg<sup>2+</sup>** sau Mn<sup>2+</sup>, K<sup>+</sup>)

# ADN-UL DE AMPLIFICAT

- Cantitati mici (1 molecula)
- Poate fi impur, vechi, degradat
- Surse:
  - Sange
  - Sperma
  - Par
  - Periuta de dinti
  - Insecte (in chilhlimbar)
  - Mumii
  - Amprente
  - Urina

# PRIMERII

- 20-30 de dezoxiribonucleotide complementare extremitatilor 5' sau 3' ale fragmentului de amplificat
- Sintetizati in laborator
- Lungimea ~ specificitatea
- In exces in mediul de reactie

# ADN-POLIMERAZA

- = enzima care catalizeaza polimerizarea dTNP in catene ADN (elongarea primerilor)
- Taq polimeraza:
  - *Thermus aquaticus* (bacterie termofilica)
  - Ape termale
  - Activitate optima la 75-80 °C
- Alte polimeraze:
  - E. Coli
  - Pfu (*Pyrococcus furiosus*)

# TEHNICA PCR

- Etapa de menținere a unei temperaturi înalte (90 °C, timp de 1-9 minute)
- 20-40 cicluri (serii de modificări de temperatura repetate)
- Fiecare ciclu are 2-3 trepte de temperatura
- Temperaturile și durata aplicării variază în funcție de:
  - ADN-polimeraza
  - Concentrația ionilor bivalenți
  - Concentrația dNTP-urilor
  - Temperatura de legare a primerilor

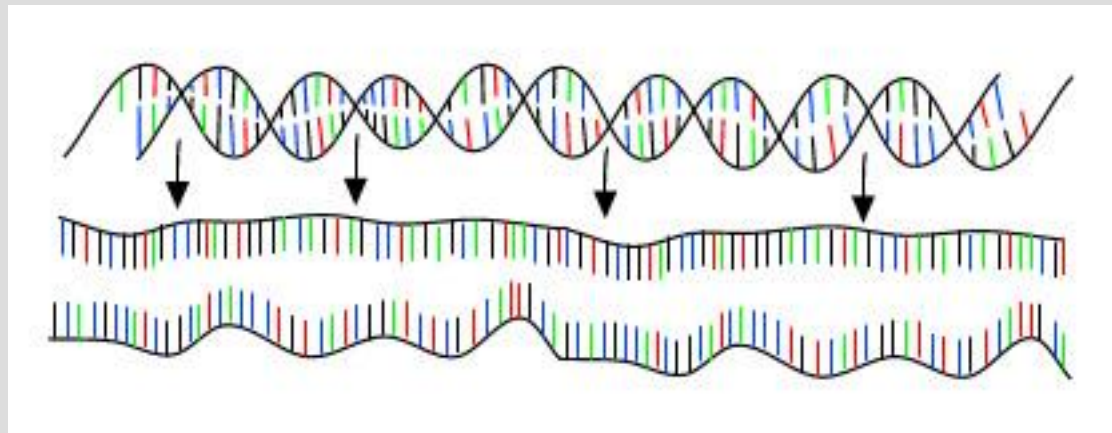


# ETAPELE PCR

1. Denaturarea ADN-ului dublu catenar
2. Cuplarea primerilor de ADN-ul simplu catenar
3. Extensia primerilor

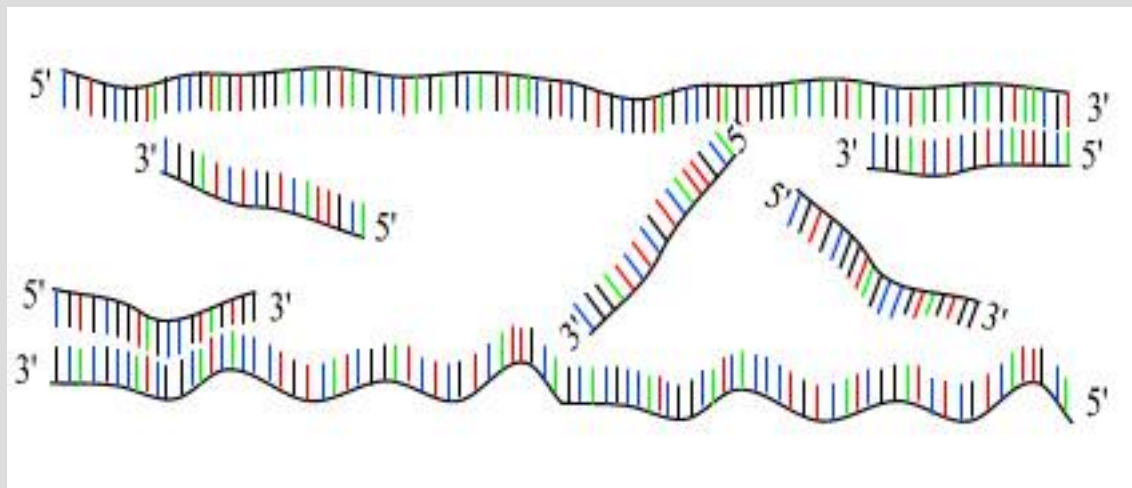
# DENATURAREA ADN-ULUI

- 94-98 °C, timp de 20-30 de secunde
- ADN-ul dublu catenar este desfasurat in doua lanturi complementare, de care se vor lega apoi primerii
- La sfarsitul acestei etape in mediul de reactie vor fi prezente doua lanturi ADN simplucatenare



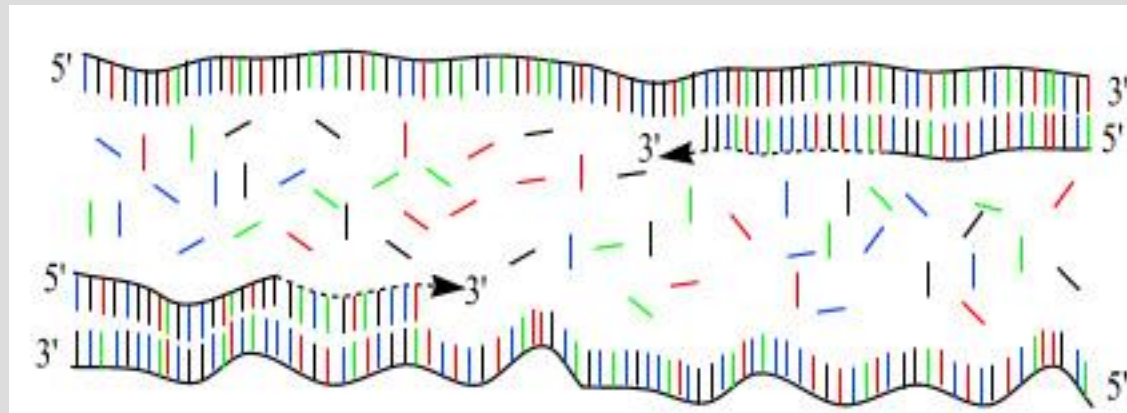
# CUPLAREA PRIMERILOR

- Se scade temperatura la 50-65 °C, timp de 20-40 de secunde
- Primerii sunt in exces in mediul de reactie
- Lant dublu-catenar hibrid – primeri si ADN de amplificat

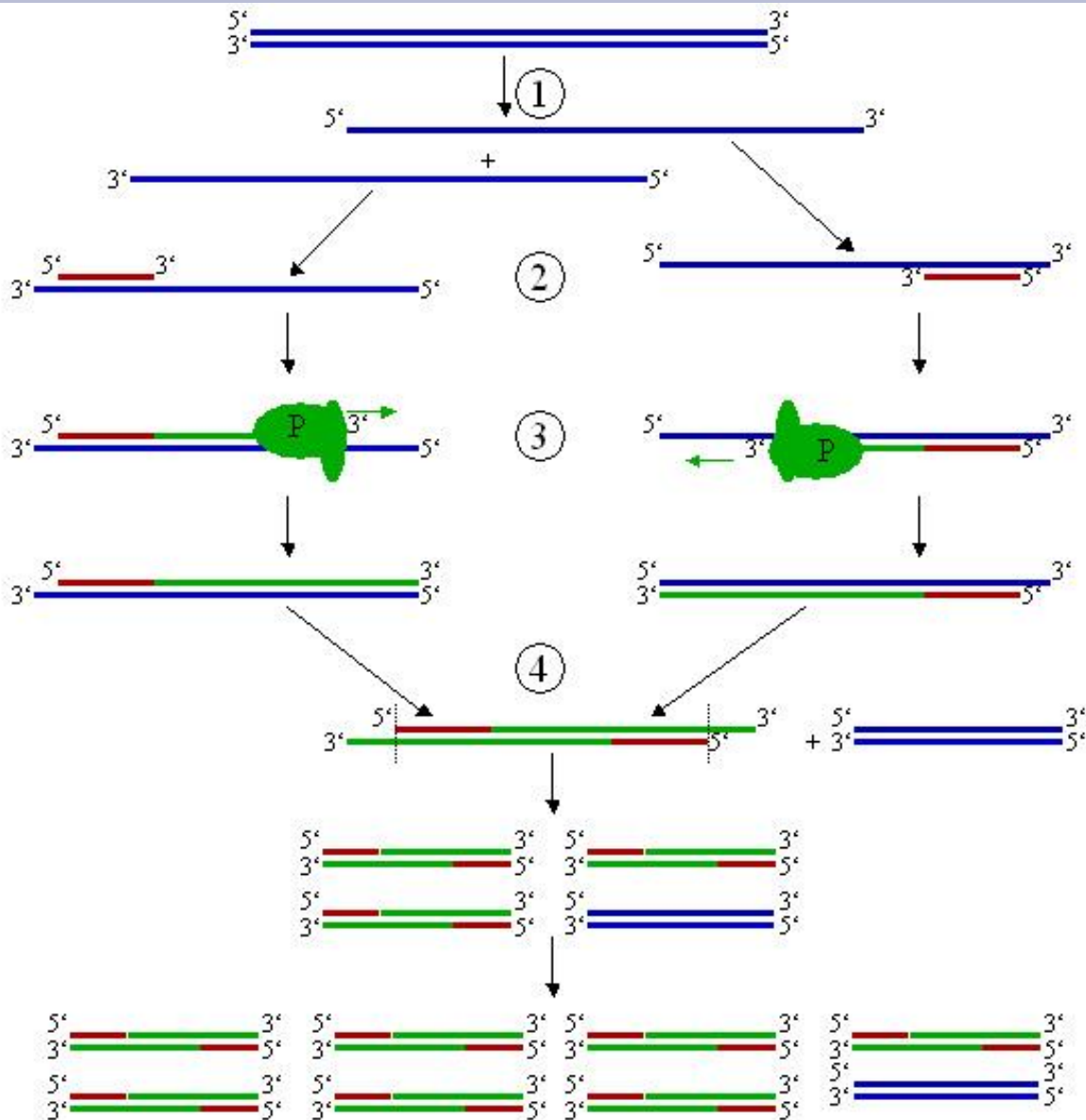


# EXTENSIA PRIMERILOR

- Temperatura depinde de ADN-polimeraza (de obicei 72°C)
- ADN-polimeraza:
  - Se leaga de lantul hibrid
  - „Citeste” secvența lantului ADN-tinta
  - Alungeste primerii prin adaugarea de dNTP, pe baza regulii complementaritatii

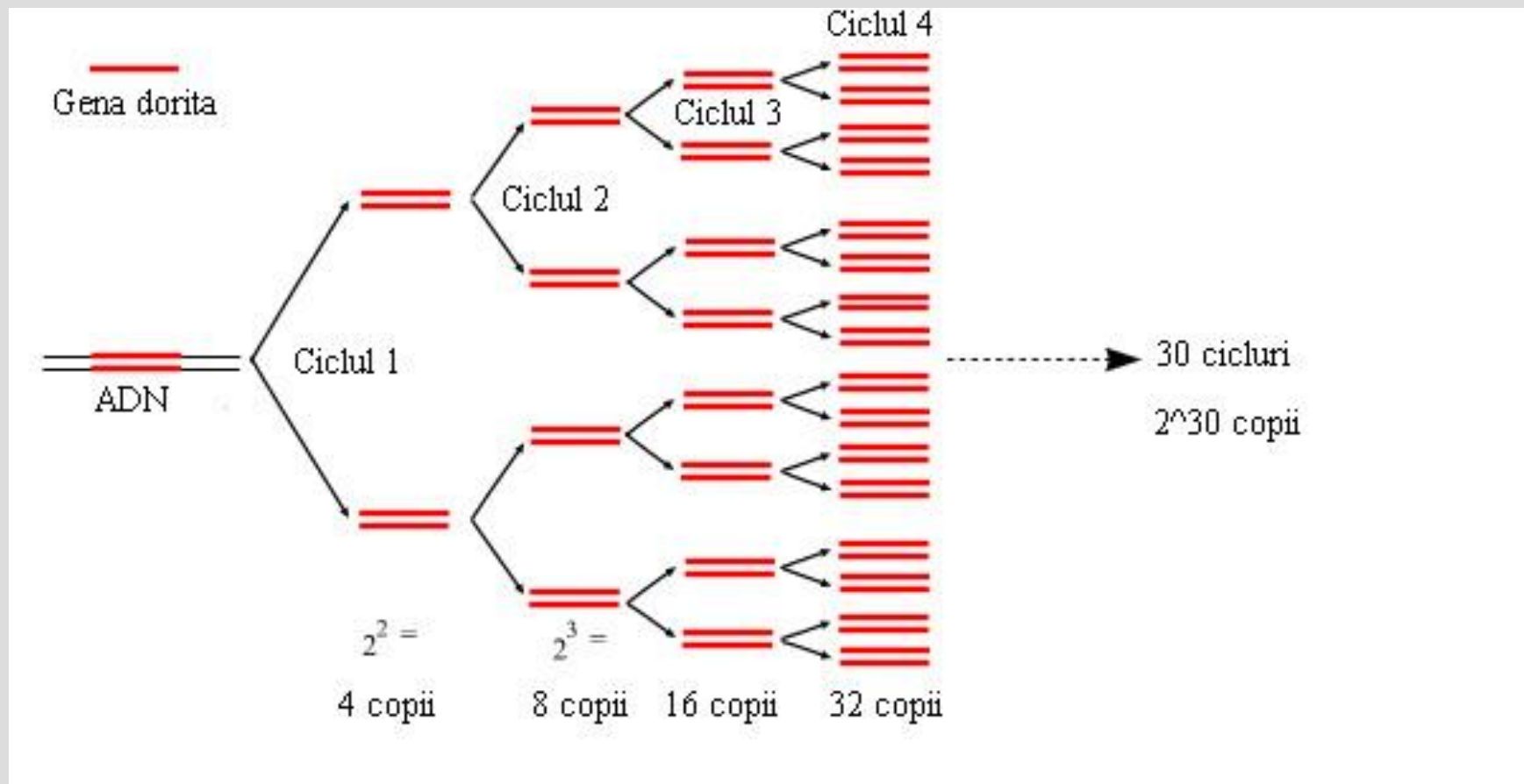


# TEHNICA PCR



1. Denaturarea la 94-98 °C
2. Cuplarea primerilor la 50-65 °C
3. Extensia la 72 °C
4. Terminarea primului ciclu si dublarea cantitații de ADN

# TEHNICA PCR



# TERMOCICLU

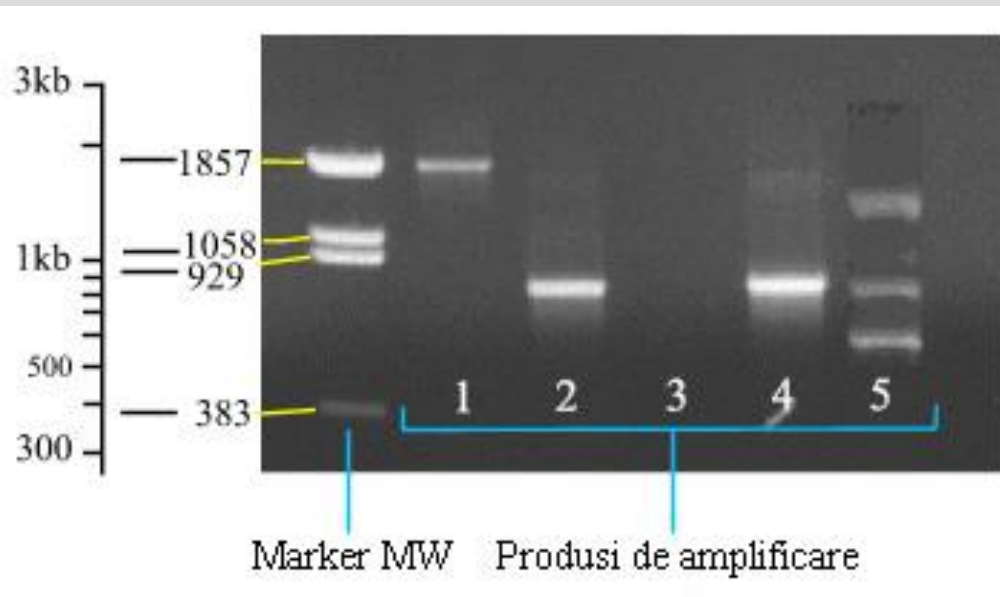


# FAZELE AMPLIFICARII

- **Amplificare exponentiala:** la fiecare etapa cantitatea de ADN se dubleaza
- **Levelling off:** se consuma reactivii, reactiile se defasoara mai lent
- **Platou:** epuizarea reactivilor (primeri, dNTP)



# VIZUALIZAREA PRODUSILOR DE AMPLIFICARE



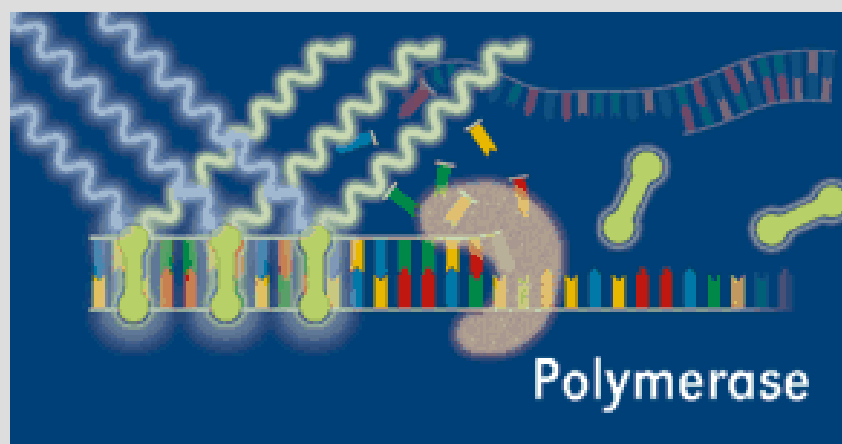
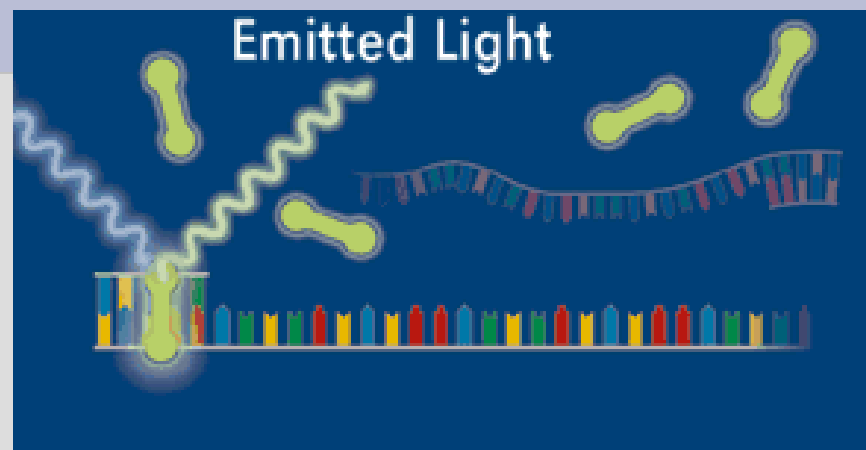
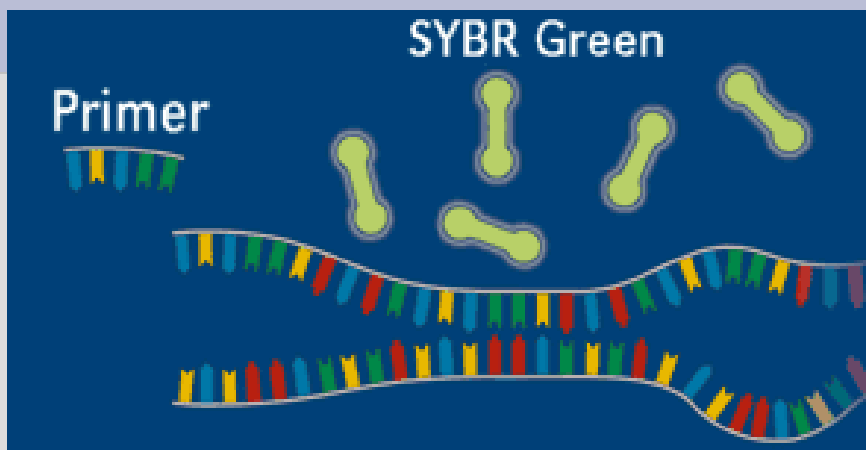
- MW: amestec de fragmente cu dimensiuni cunoscute
- 1: ~1850 bp
- 2: ~800 bp
- 3: nu s-a amplificat
- 4: ~800 bp
- 5: benzi multiple

## ***PCR – Real time***

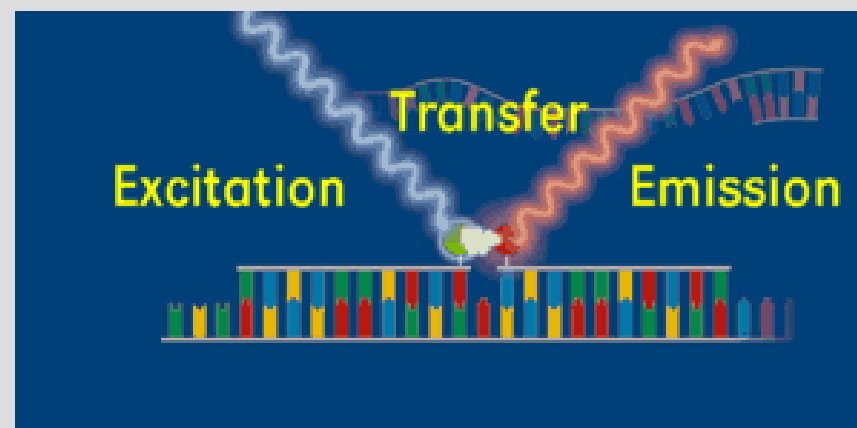
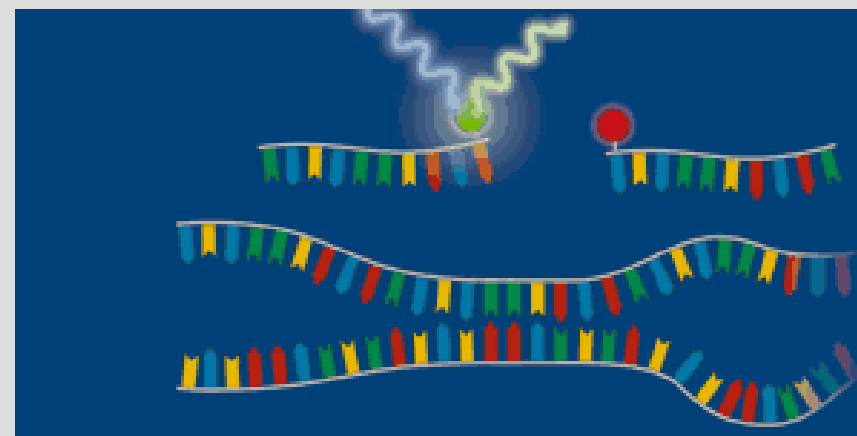
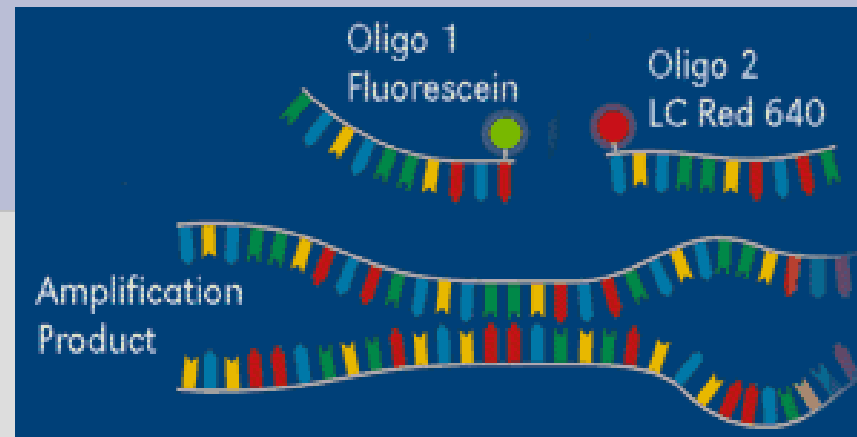
### **PRINCIPIUL:**

În metoda ***PCR- real time*** fragmentul ADN amplificat (ampliconul) este vizualizat pe măsură ce procesul de amplificare înaintează. Această urmărire în “timp real” a procesului de amplificare este posibilă prin marcarea cu molecule fluorogenice a primerilor, probelor sau ampliconului.

# Detectarea directă a ampliconului prin agenți fluorescenți de legare ADN



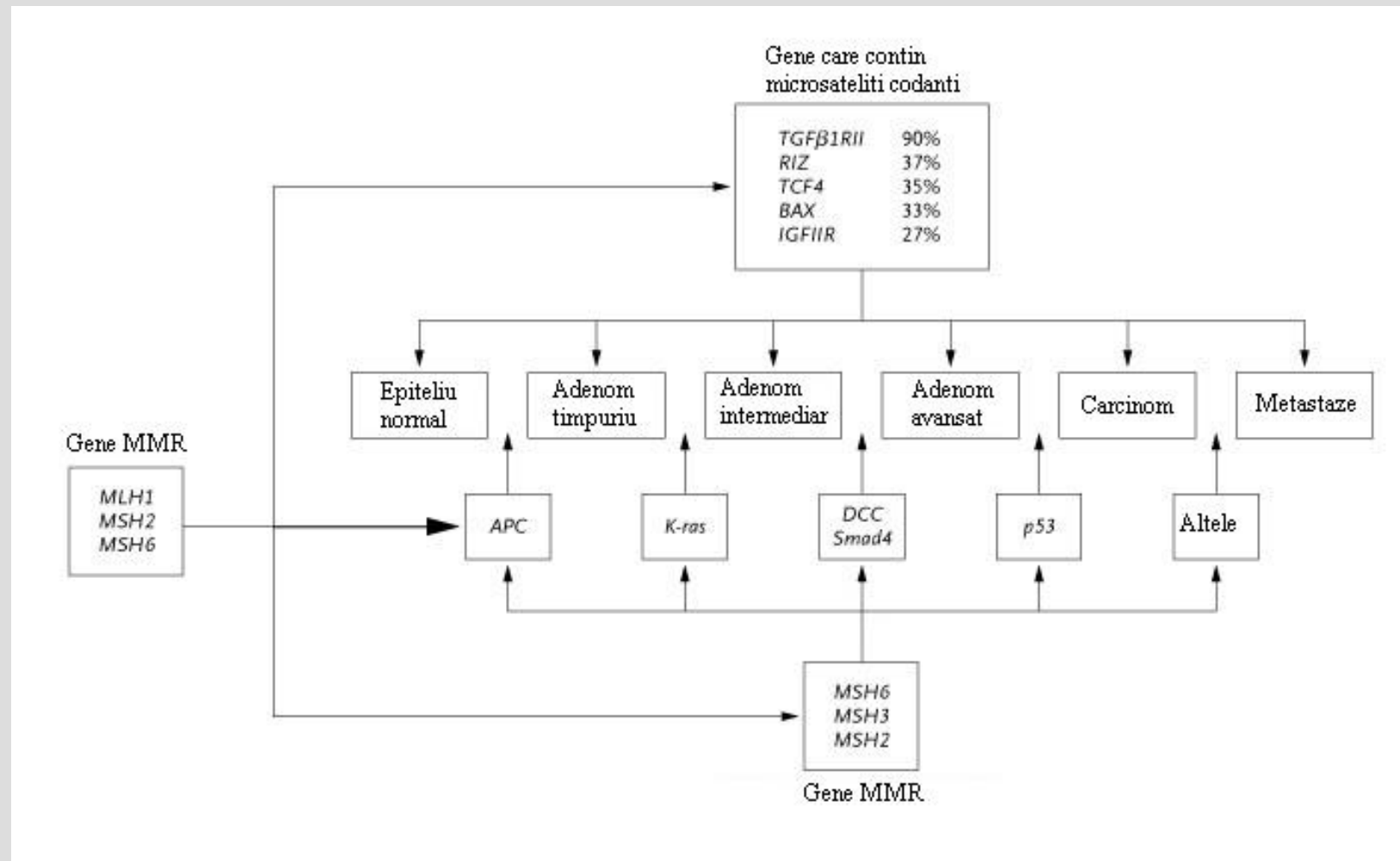
# Detectarea directă a ampliconului prin oligoprobe liniare



# APLICATII

- Testarea unor mutatii (ex.: fibroza chistica, gene tumorale)
- Testare prenatala (si screening preimplantare)
- Infectii virale (HIV), bacteriene (BK)
- Teste de histocompatibilitate
- Medicina legala:
  - Teste de paternitate
  - Identificarea de persoane
- Cladistica = metodă de clasificare a speciilor de organisme în clade, membrii cărora au un strămoș filogenetic comun de la care au evoluat.

# GENE TUMORALE



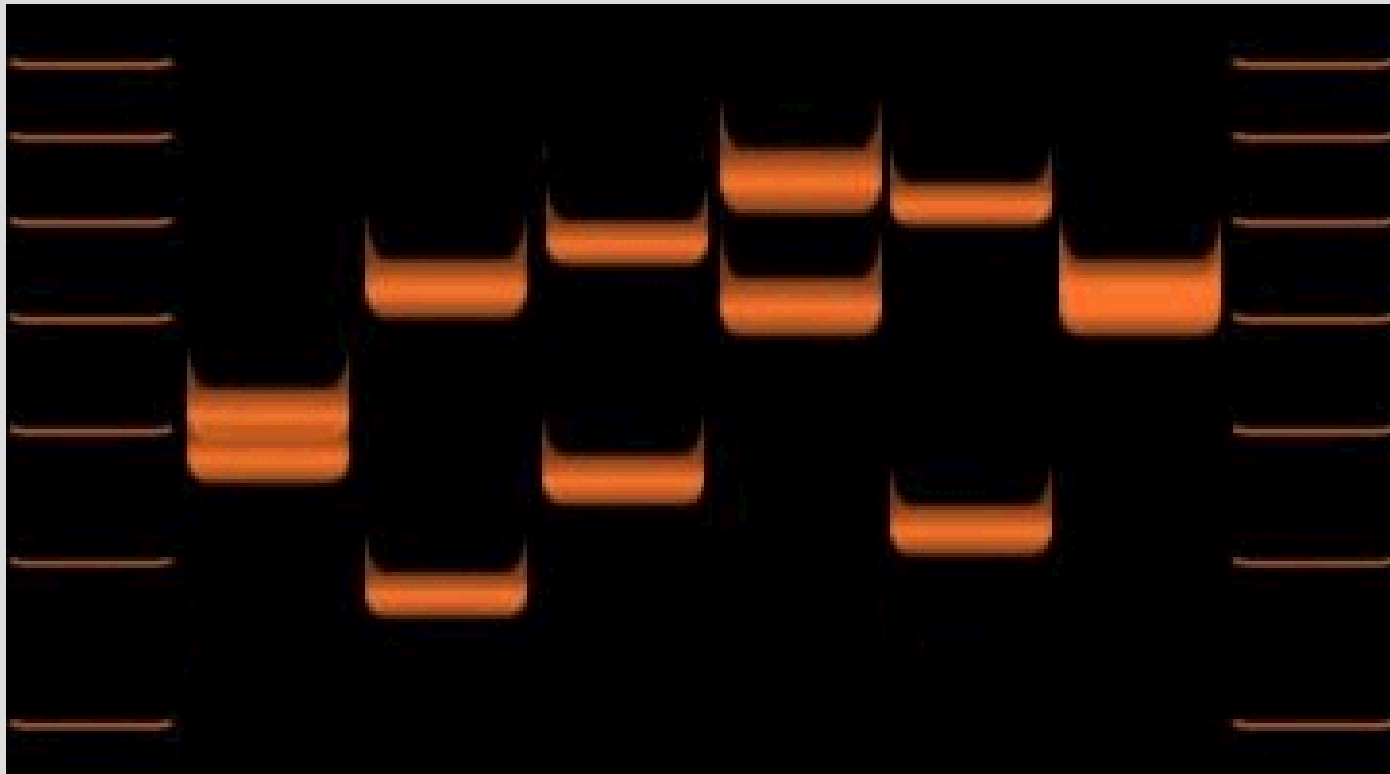
# TESTE DE PATERNITATE

The diagram illustrates the layout of a paternity test kit. It consists of three vertical columns, each containing a series of horizontal lines representing test wells. The columns are labeled at the bottom with circled numbers 1, 2, and 3. Column 1 (labeled '1') contains 10 horizontal lines. Column 2 (labeled '2') contains 10 horizontal lines. Column 3 (labeled '3') contains 10 horizontal lines. Red dotted lines connect the lines across the columns, indicating the alignment of the test wells. The top right corner of the diagram shows a folded corner of a sheet of paper.

- 1 – Tatal
- 2 – Copilul
- 3 – Mama

# IDENTIFICAREA DE PERSOANE

- STR (Short Tandem Repeats)

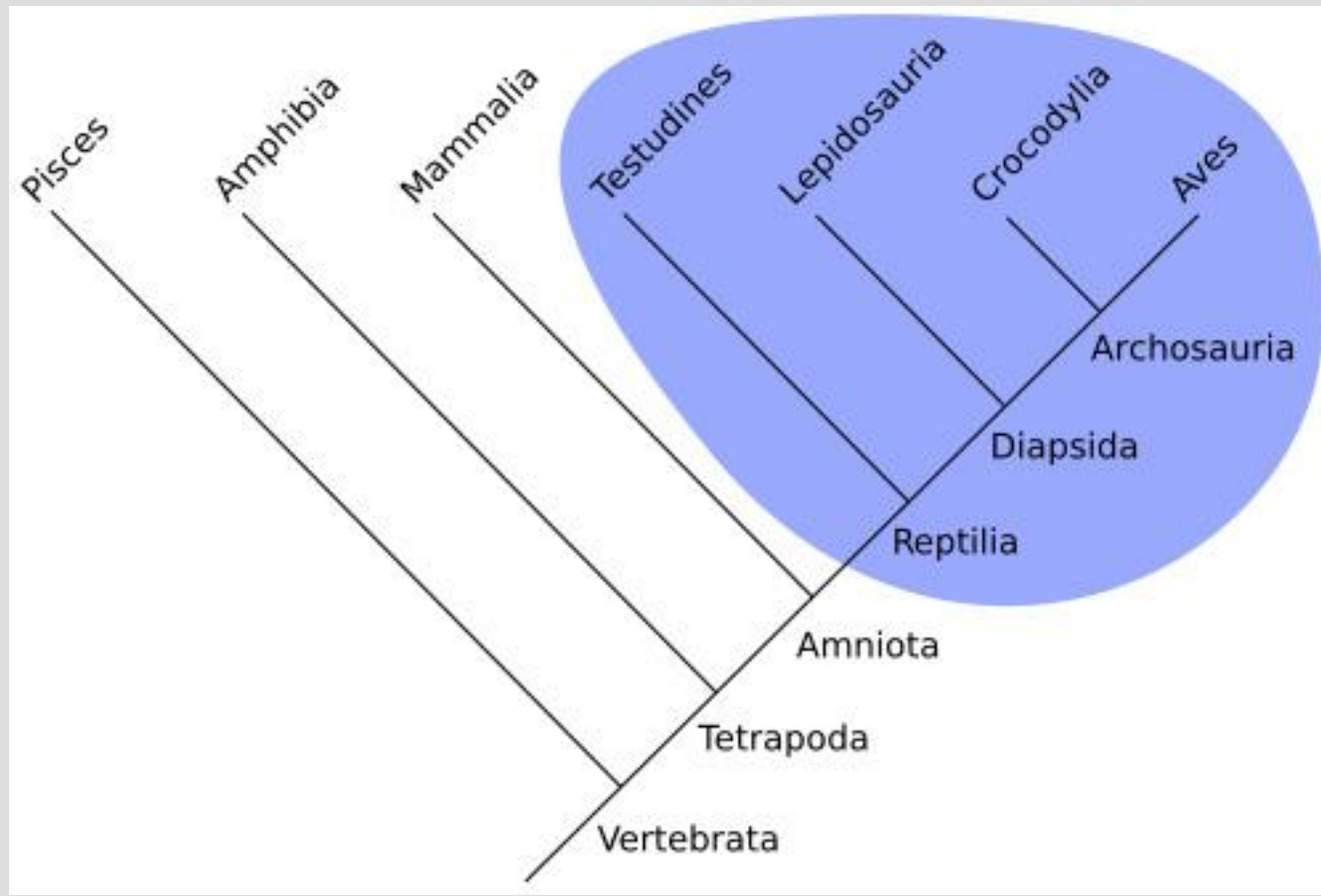




# IDENTIFICAREA DE PERSOANE

Locus	Pacienta	Embrion	Frate	Tata	Bunic matern	Bunic patern
D8S1179	15	15	15	13,15	13,15	10,13
D21S11	28,29	29,30	30	29,30	27,29	28,31.2
D7S820	10	10	10,12	8,10	8	10,12
CSF1PO	11,12	11	10,11	11	11	10,12
D3S1358	15	15	15	15	15,16	16,18
TH01	8,9	8	8,9	9	6,9	6,8
D13S317	11,12	12	12,13	11,13	9,11	12
D16S539	9,11	9,14	9,14	11,14	11	9,12
D2S1338	17,20	17,18	18,20	20	20,24	18,20
D19S433	16,16.2	14,16.2	14,16.2	14,16	12,14	13,15
vWA	14,17	17	17,18	14,18	14,17	17,19
TPOX	8,9	8,11	8,11	8,9	9,10	8,11
D18S51	10,22	10,15	10,15	15,22	14,15	13,14
Amelogenin	X,X	X,X	X,Y	X,Y	X,Y	X,Y
D5S818	13	13	13	13	11,13	13
FGA	20,24	20,21	20,21	20,21	21	21,23

# CLADE



WELL, WE GOT THE  
DNA TEST BACK...  
AND YOU ARE DEFINITELY  
**NOT** OUR DOG.



Vă mulțumesc!

I JUST DID A DNA  
ANALYSIS ON ME AND  
THIS THING



IT TURNS OUT  
WE WERE DRAWN  
WITH THE SAME  
INK!



WHAT ARE YOU DOING?  
EATING POPCORN WITH  
MY BROTHER.

