Amplification of Nucleic Acids

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Analysis of DNA

Polymerase Chain Reaction

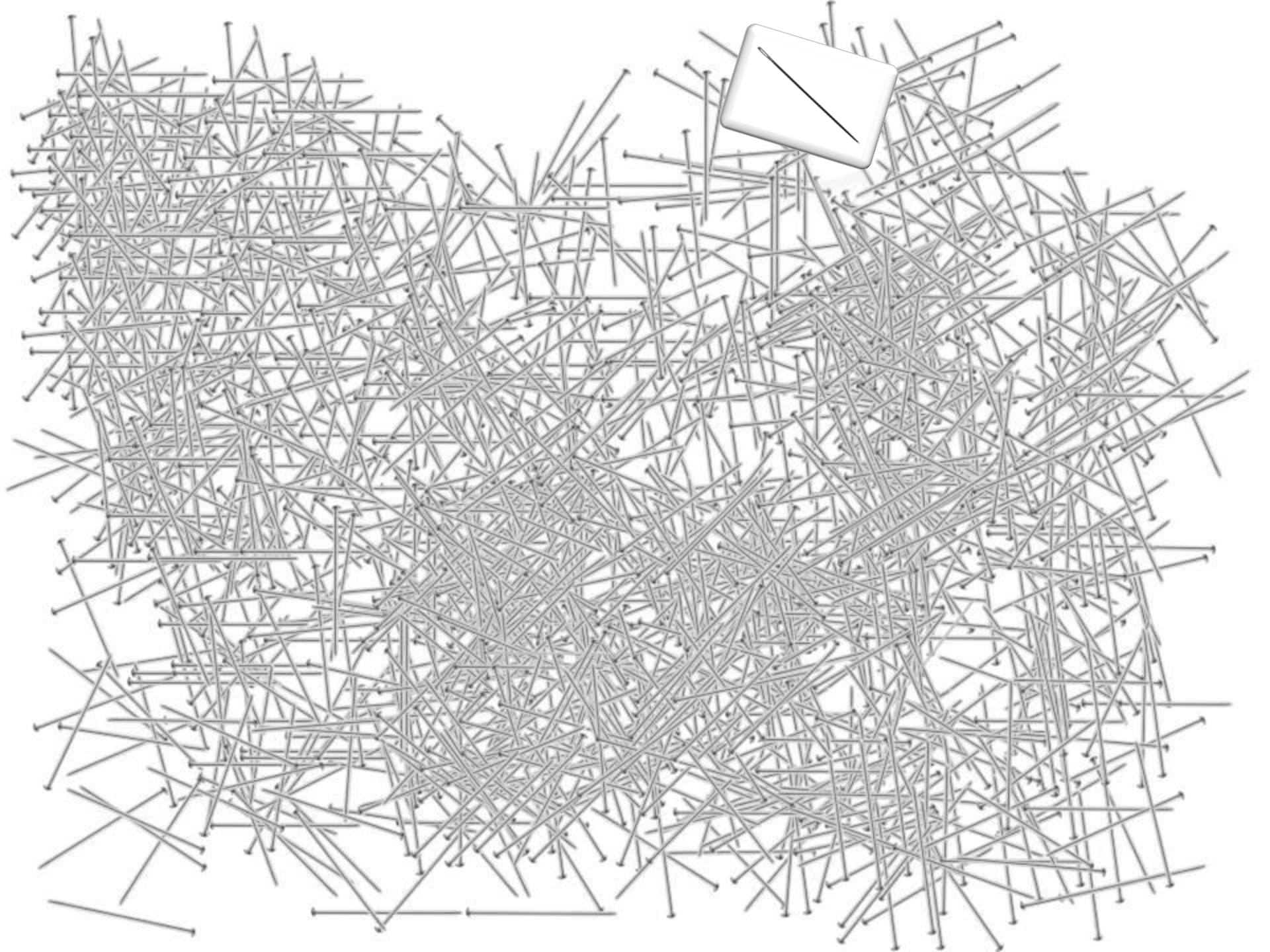
Polymerase Chain Reaction

- Discovered in 1983 (Kary Mullis, Cetus)
- Patented technique
- Patent sold to Roche in 1991 for $300 Million

Polymerase Chain Reaction

- Problems before PCR
  - Quantity of DNA was small
  - The target was a tiny fraction of all DNA


Quiz



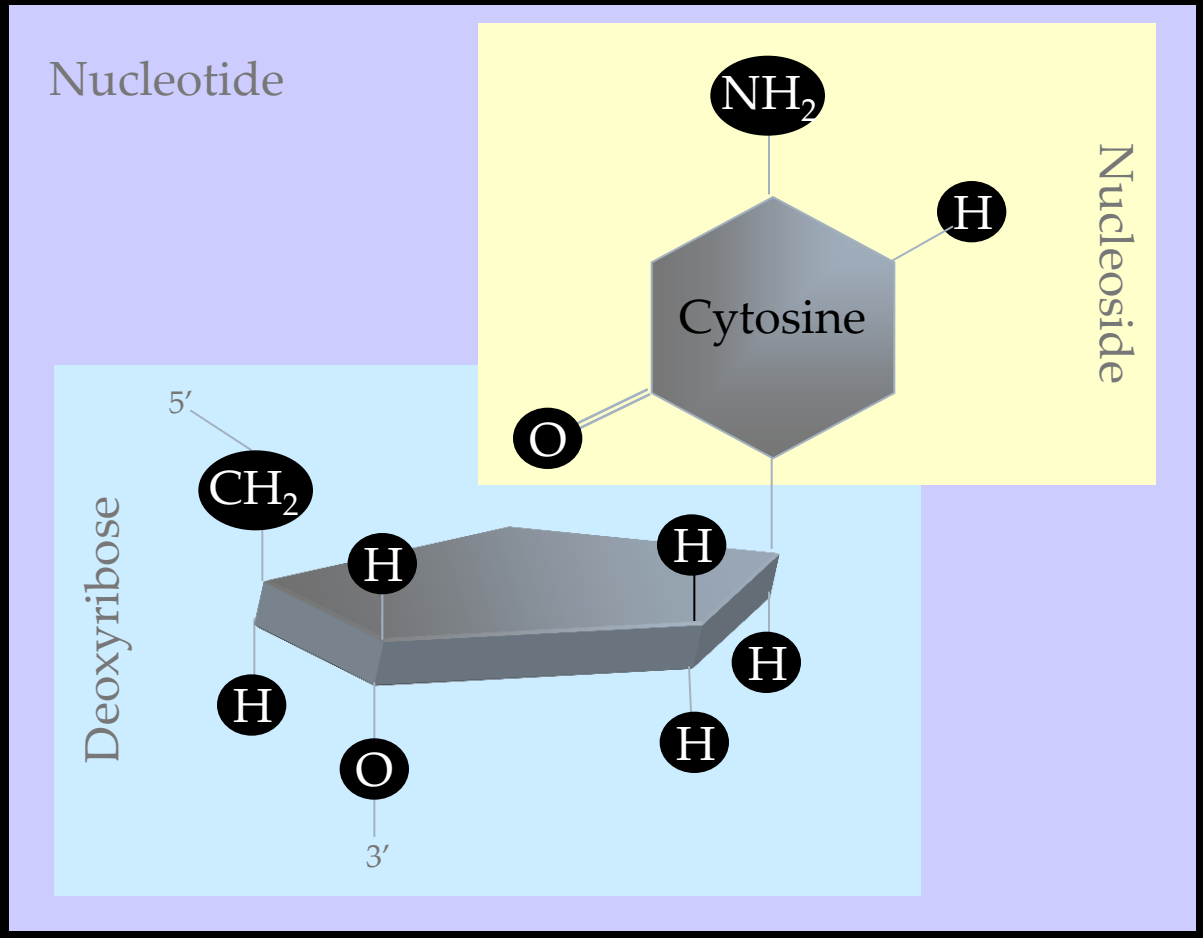
Polymerase Chain Reaction

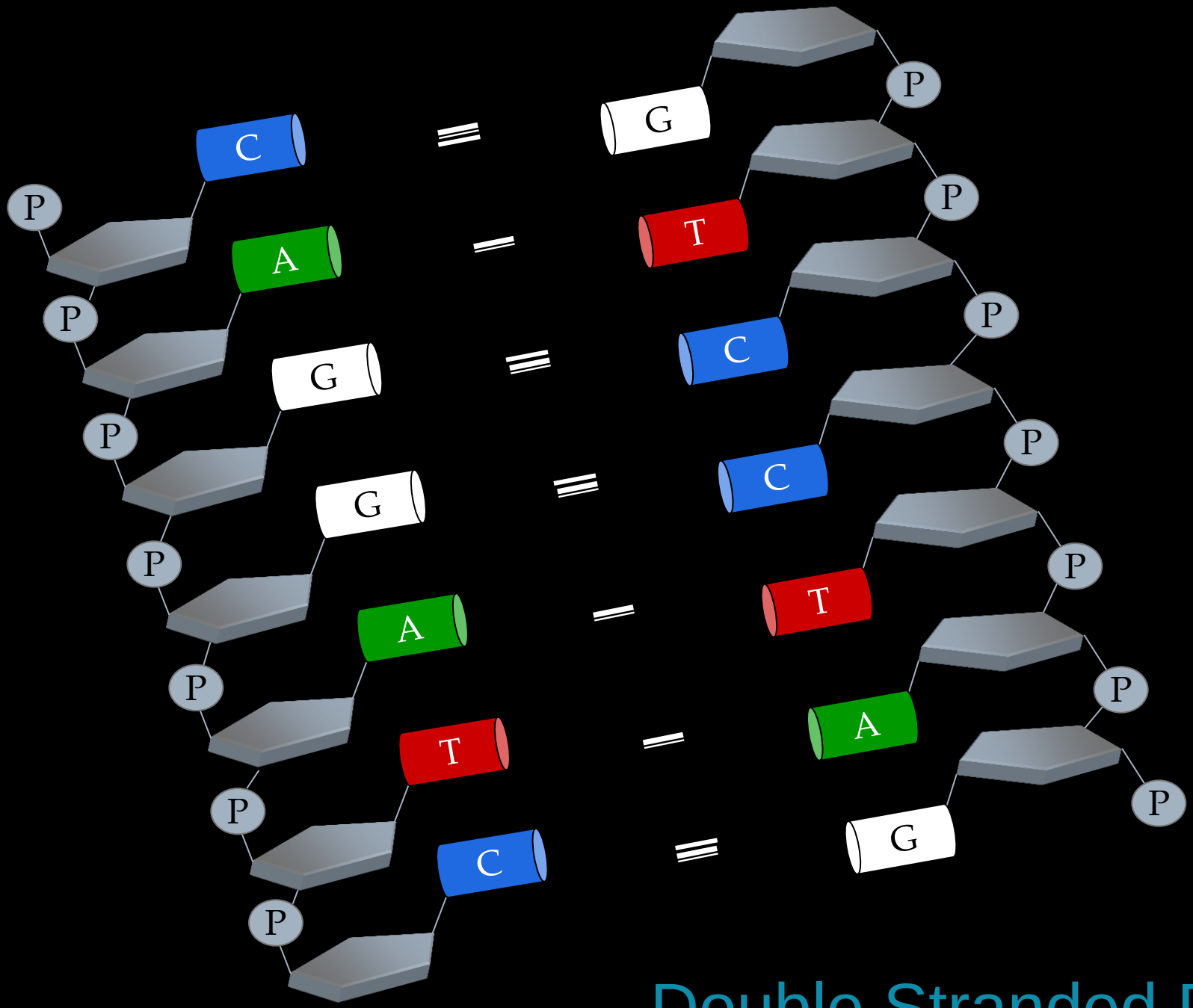
- PCR enabled
  - Amplification for quantity
  - Target specific enrichment

Polymerase Chain Reaction

- Template DNA
- Primers
- Taq enzyme
- Nucleotide building blocks

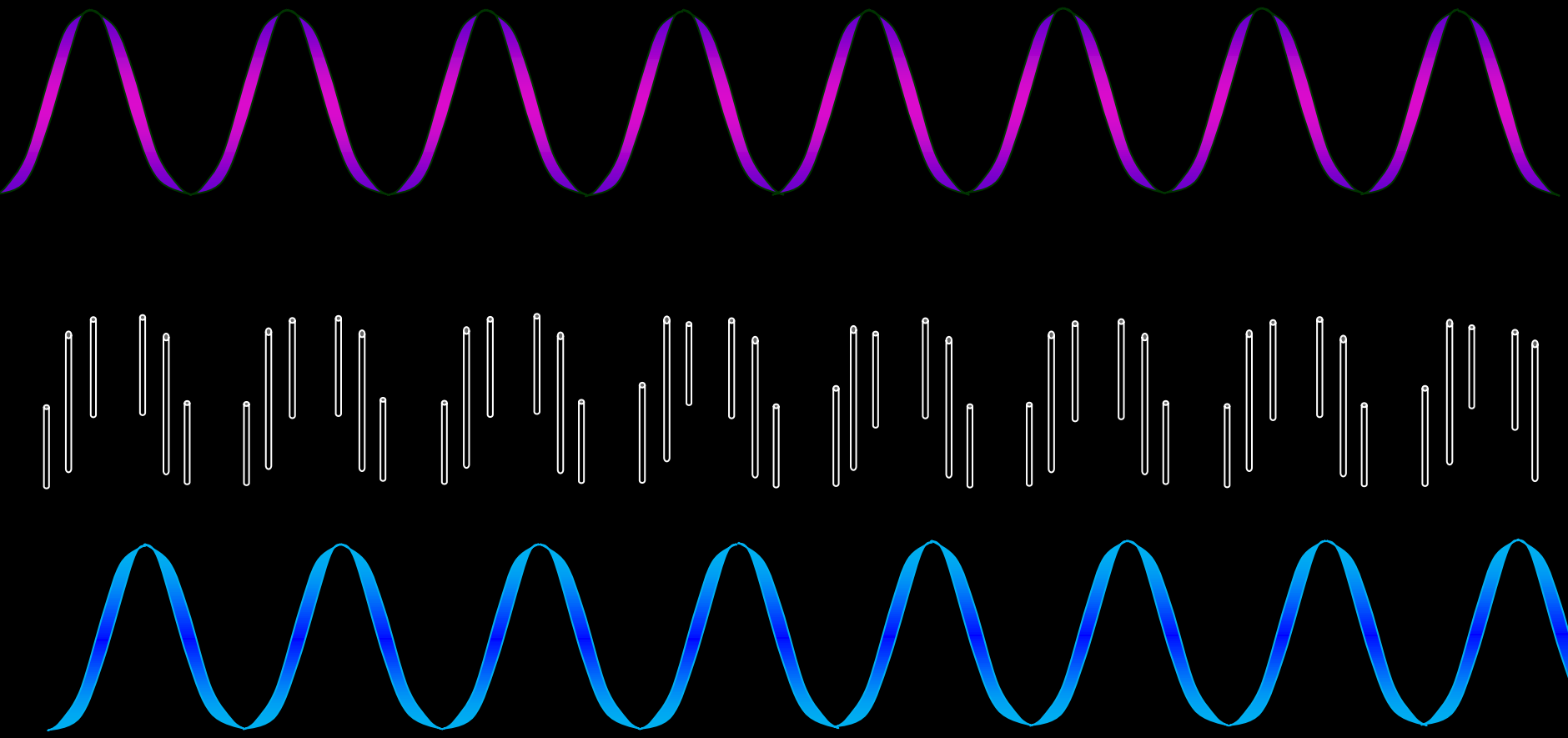
Template DNA: Bases





Double Stranded DNA

Double Stranded DNA



Primer Design

- Primers
  - Short DNA sequence (18-24 nucleotides)
  - Forward and Reverse
    - “Sense & Antisense”

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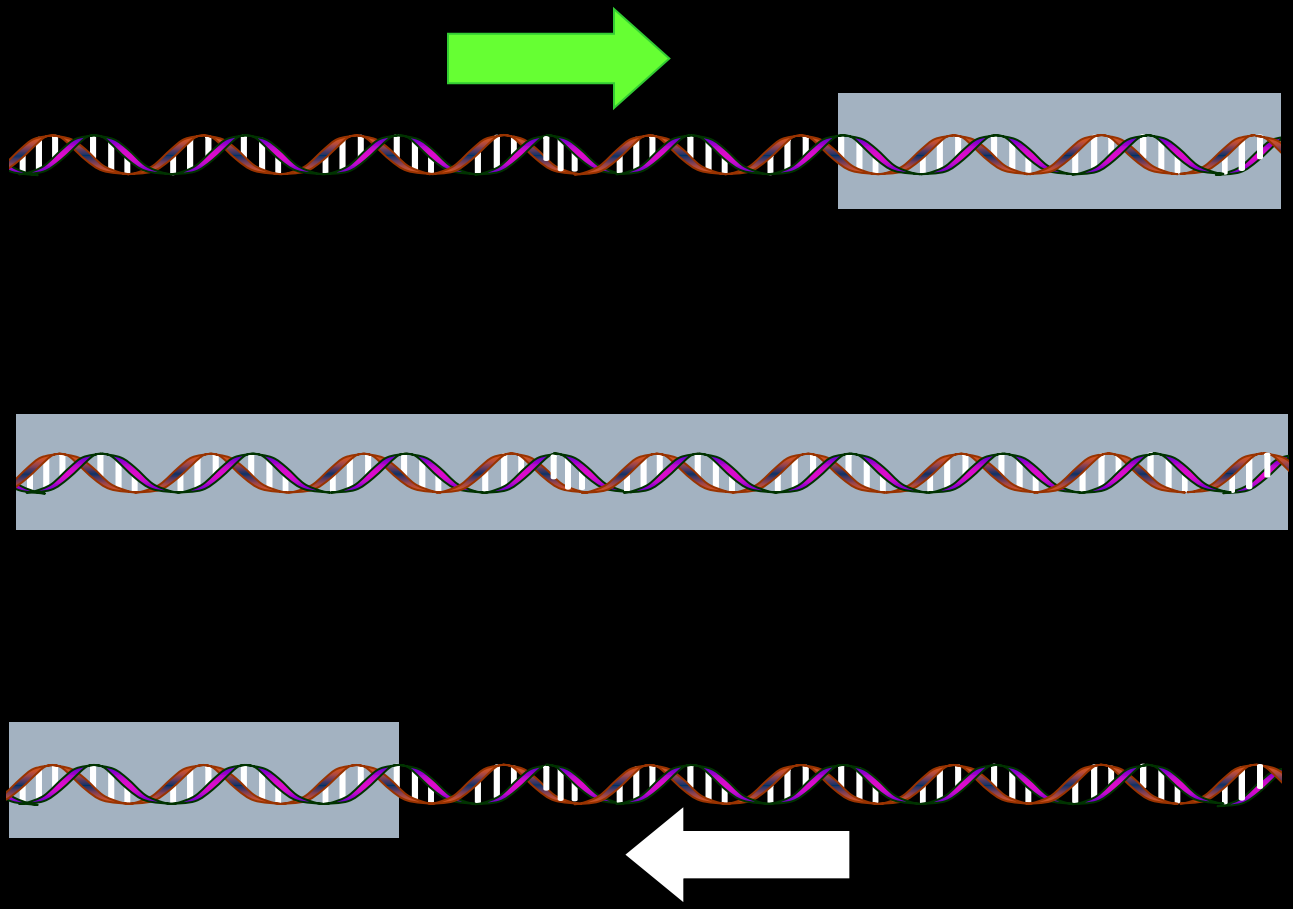
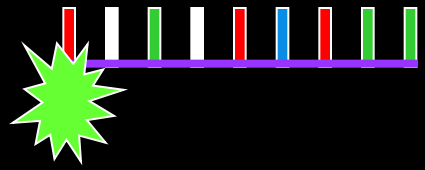
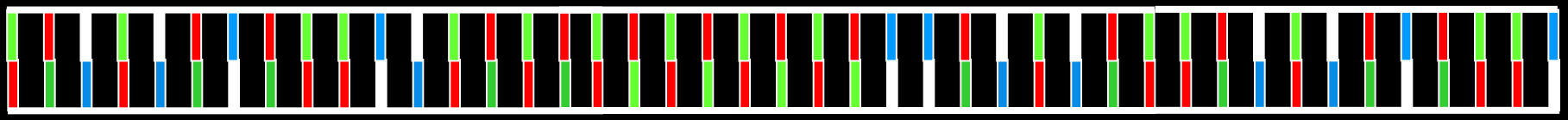
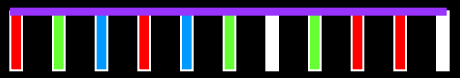
Primer Design



Primer Binding







Polymerase

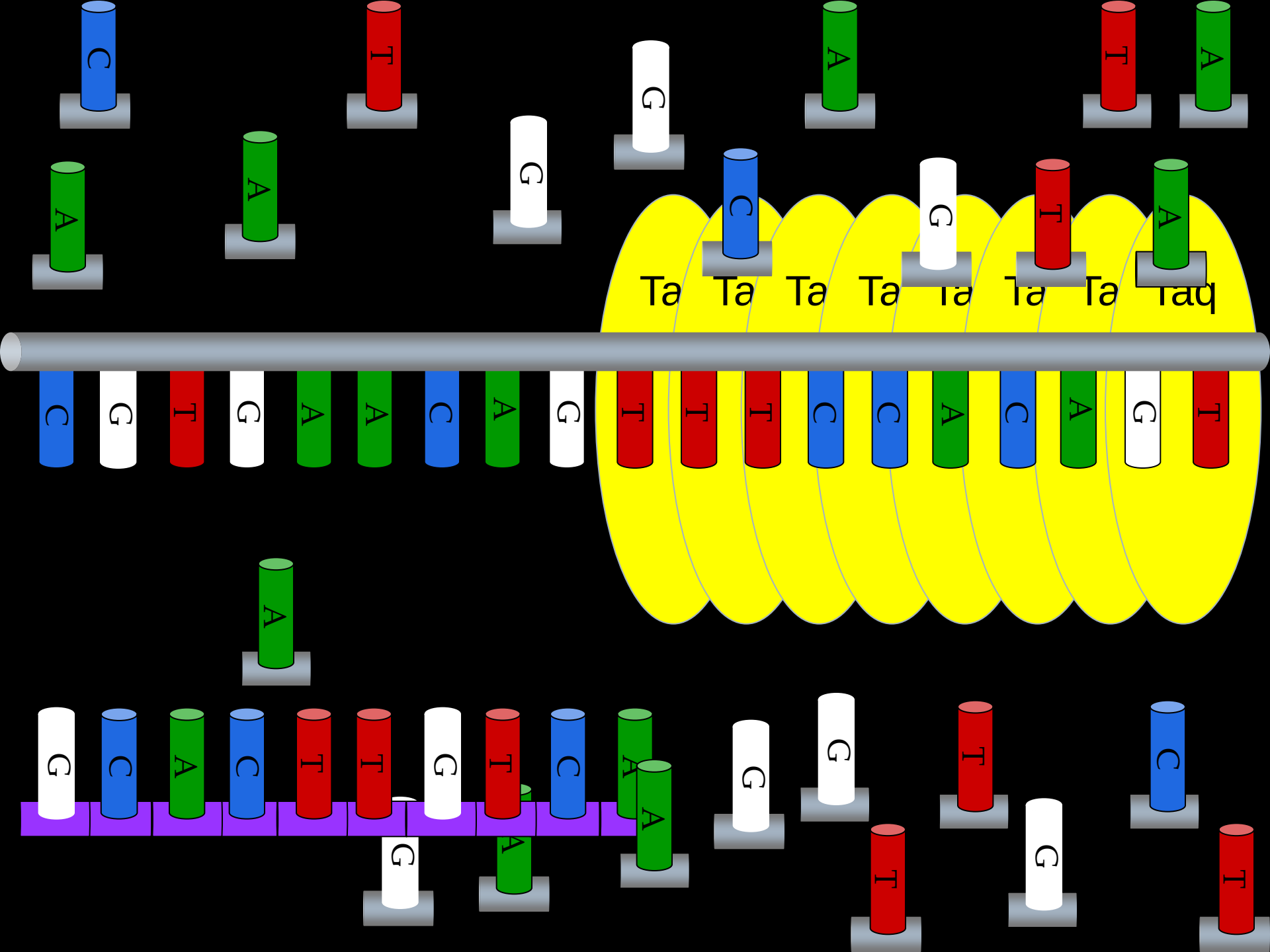
- Taq Enzyme
  - *Thermus Aquaticus*
  - DNA polymerase
  - Heat activated

Nucleotides

	Base name	Nucleotide name
A	Adenine	Deoxyadenosine
G	Guanine	Deoxyguanosine
C	Cytosine	Deoxycytidine
T	Thymine	Deoxythymidine

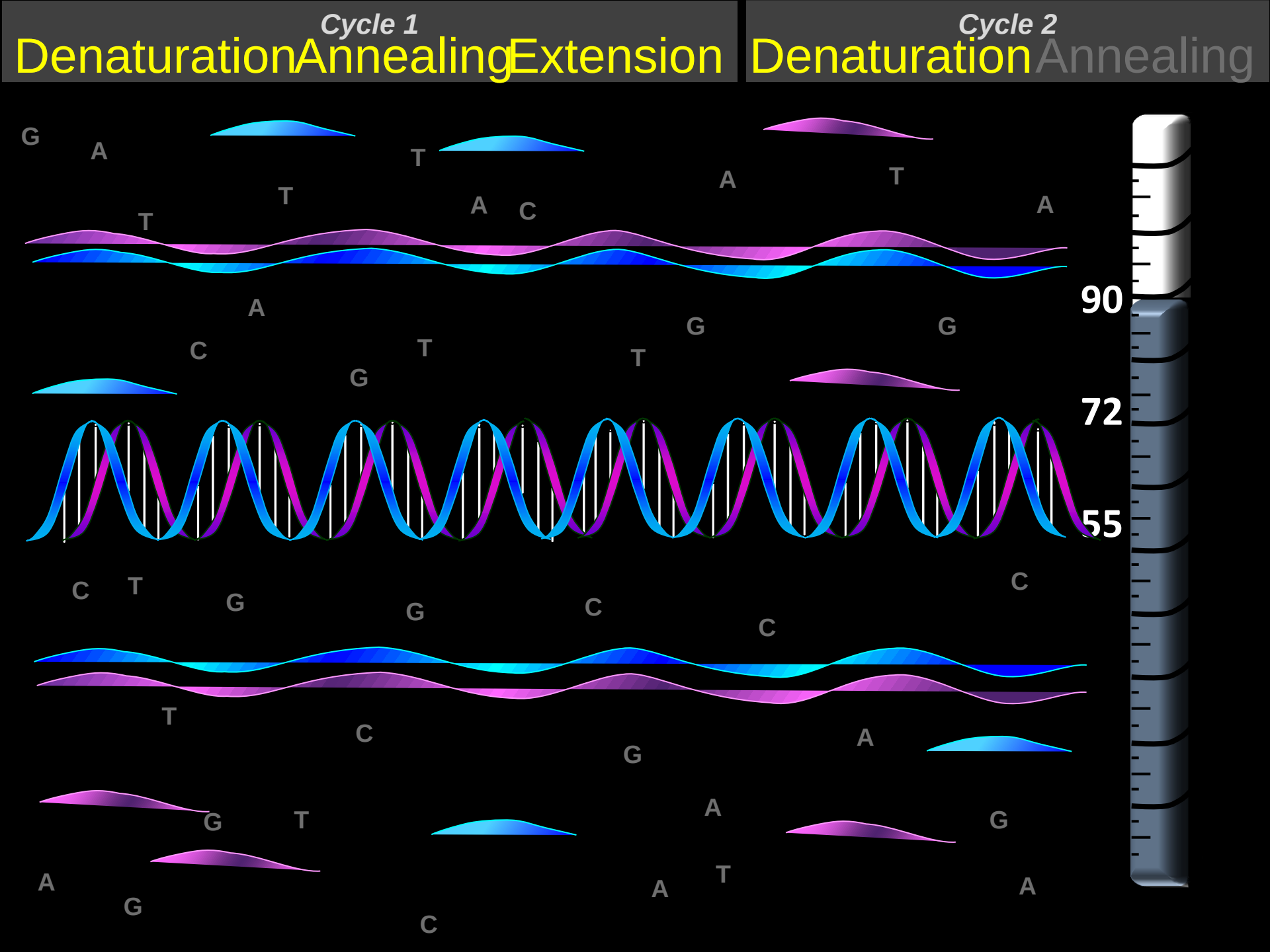
Purines

Pyrimidines

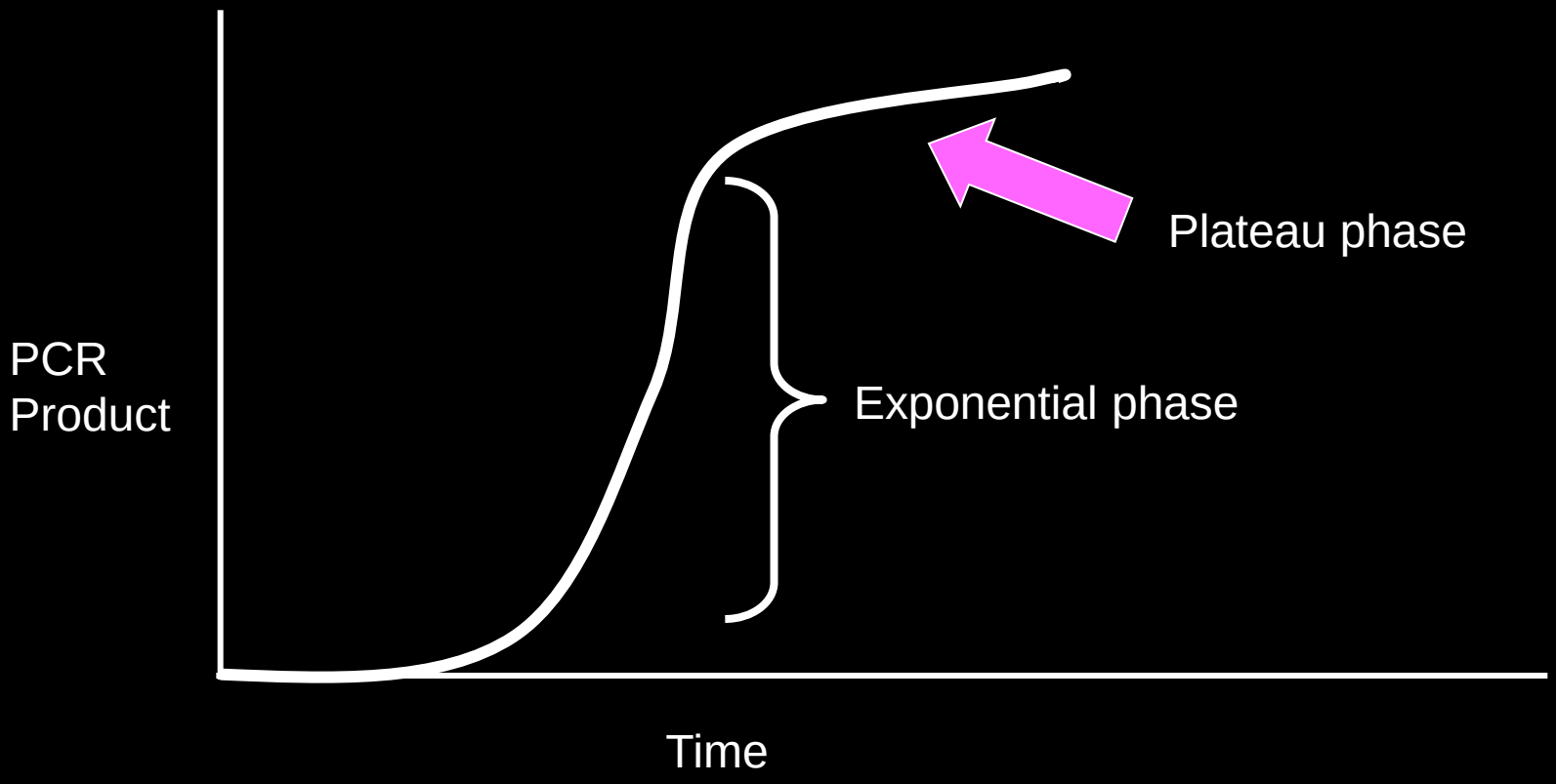

Taq

PCR Amplification

- Occurs in a machine: Thermocycler (thermal cylcer)
- Series of 3 different temperatures = 1 Cycle


Cycle 1
Denaturation
Annealing
Extension
Cycle 2
Denaturation
Annealing
90
72
55

PCR Amplification



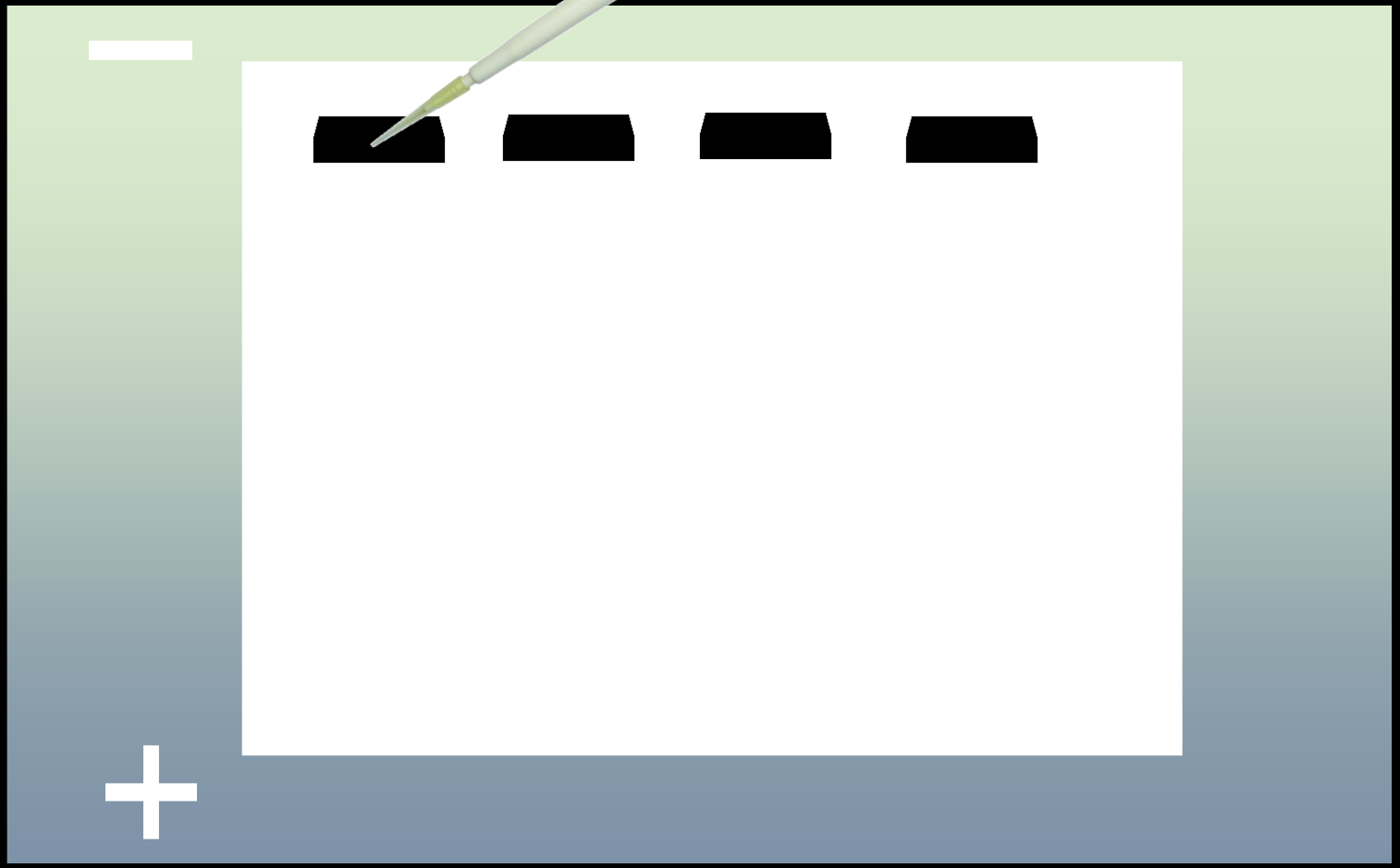
PCR Product Analysis

- What do we do after PCR?
  - Detect the sequence in the product
    - The actual sequence (*Sequencing*)
    - Differences that cause altered migration (*MSI*)
  - Detect the amount of product
    - Quantitative (*Q-PCR*)
    - Semi-quantitative (*LOH*)

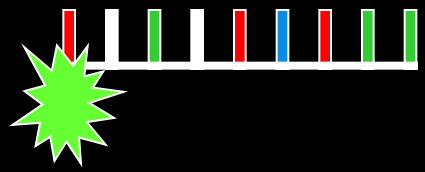
PCR product detection

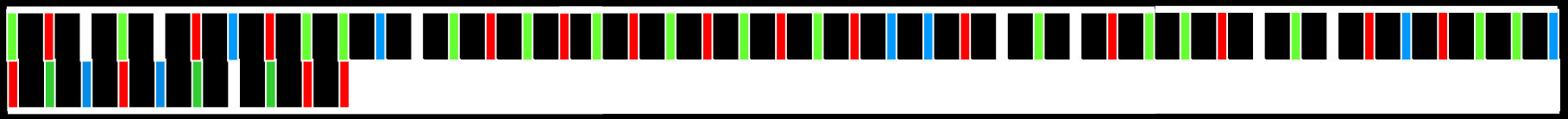
- Gel: Agarose or polyacrylamide
- Capillary electrophoresis
- Quantitative PCR

Agarose Gel Electrophoresis



Capillary Electrophoresis





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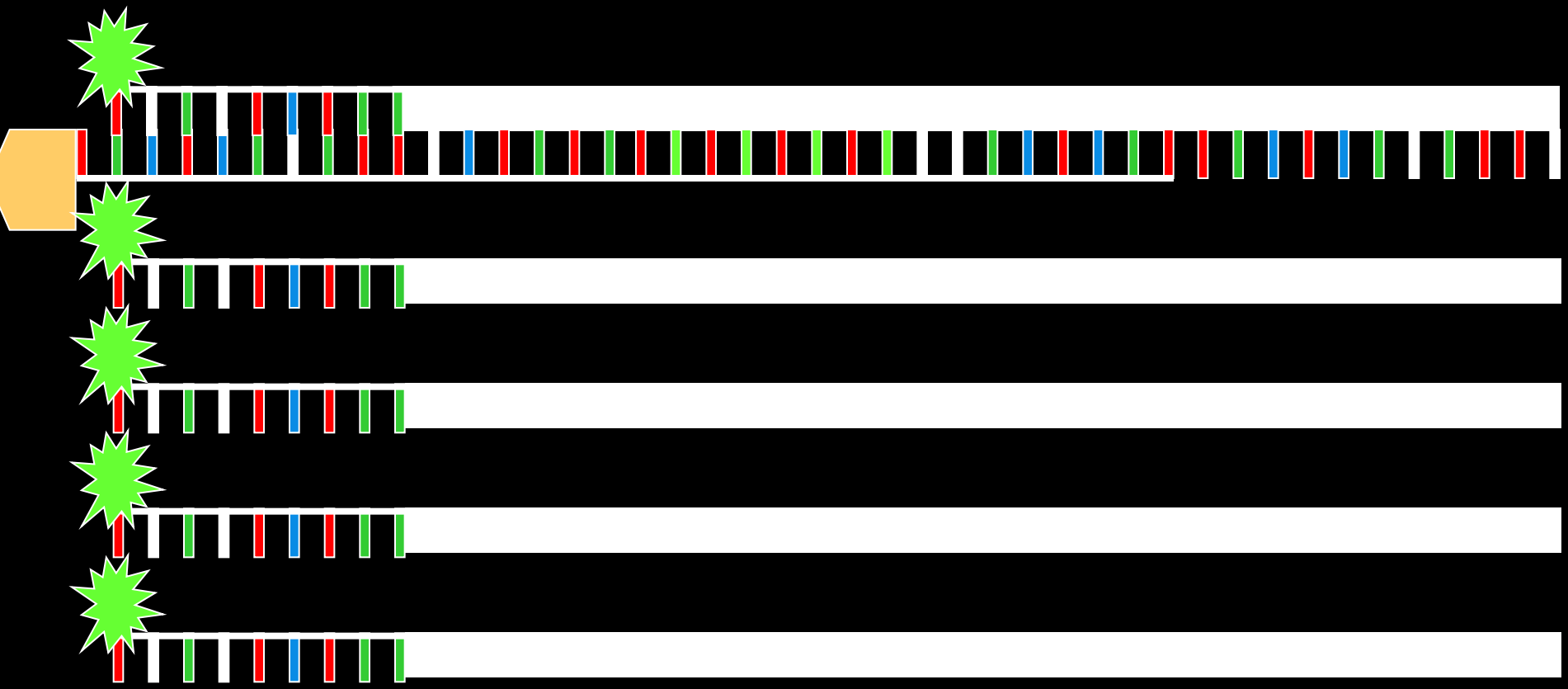
Capillary Electrophoresis



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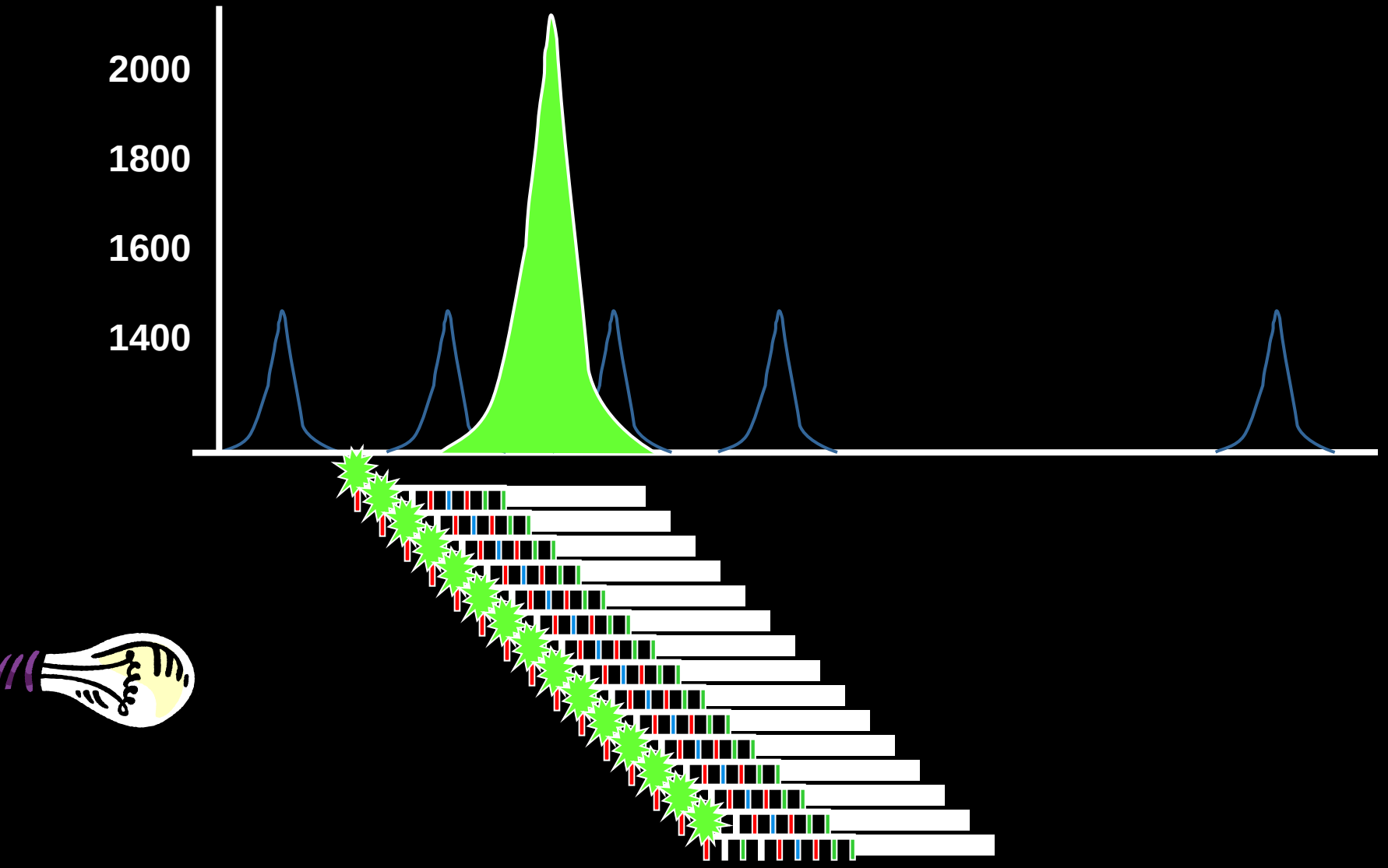
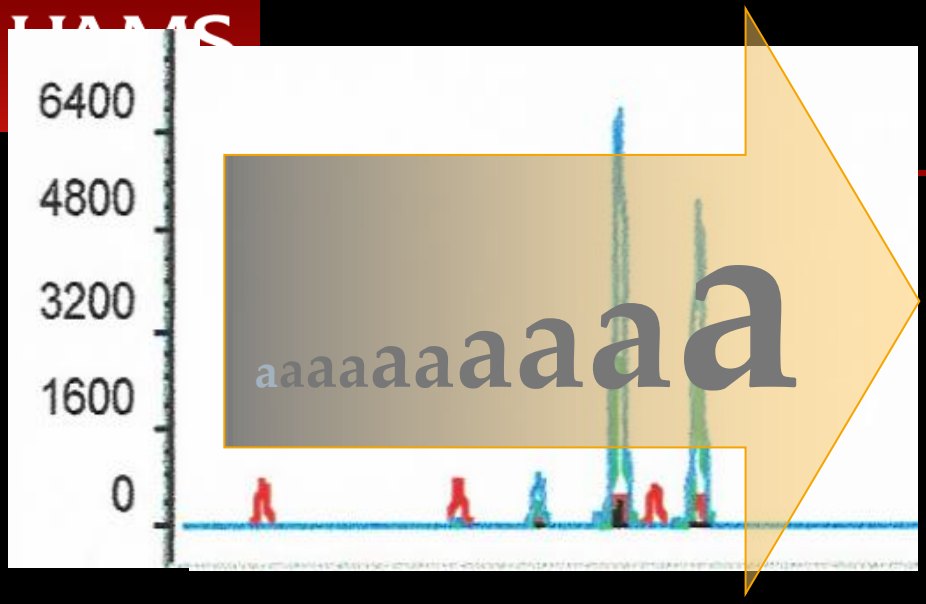
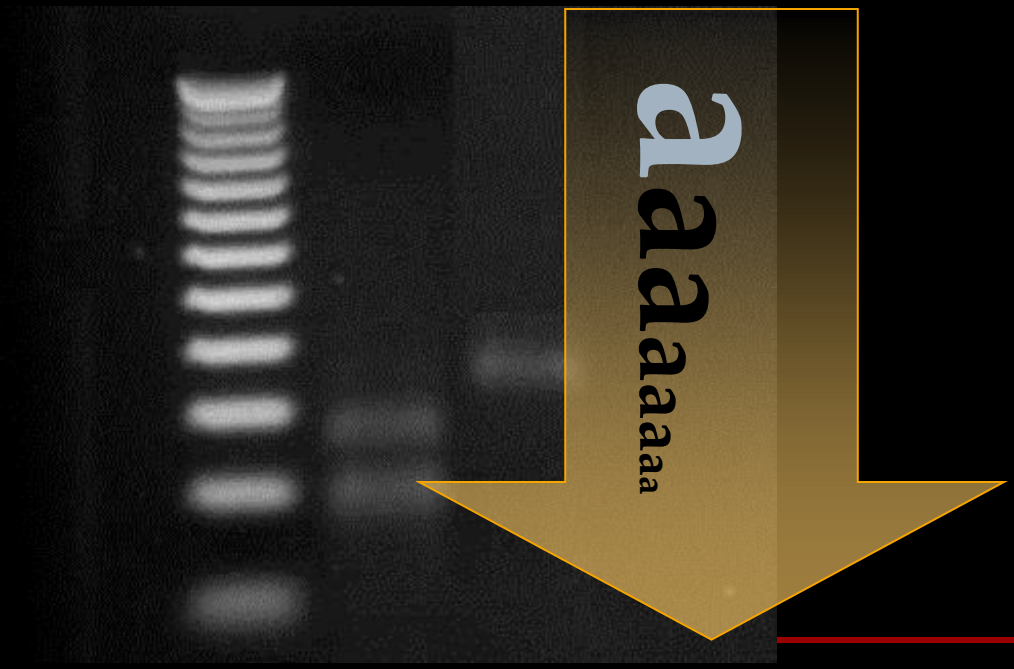
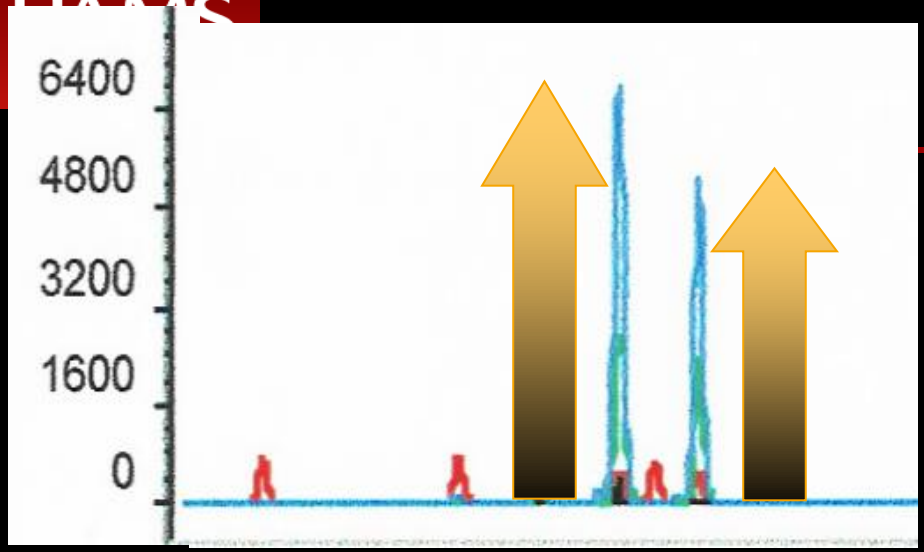
Capillary Gel Electrophoresis

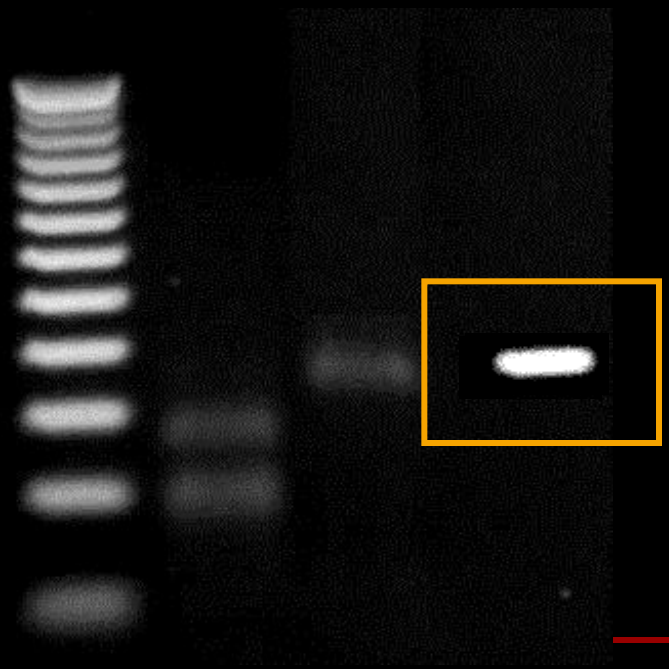




Size of PCR product (basepairs)

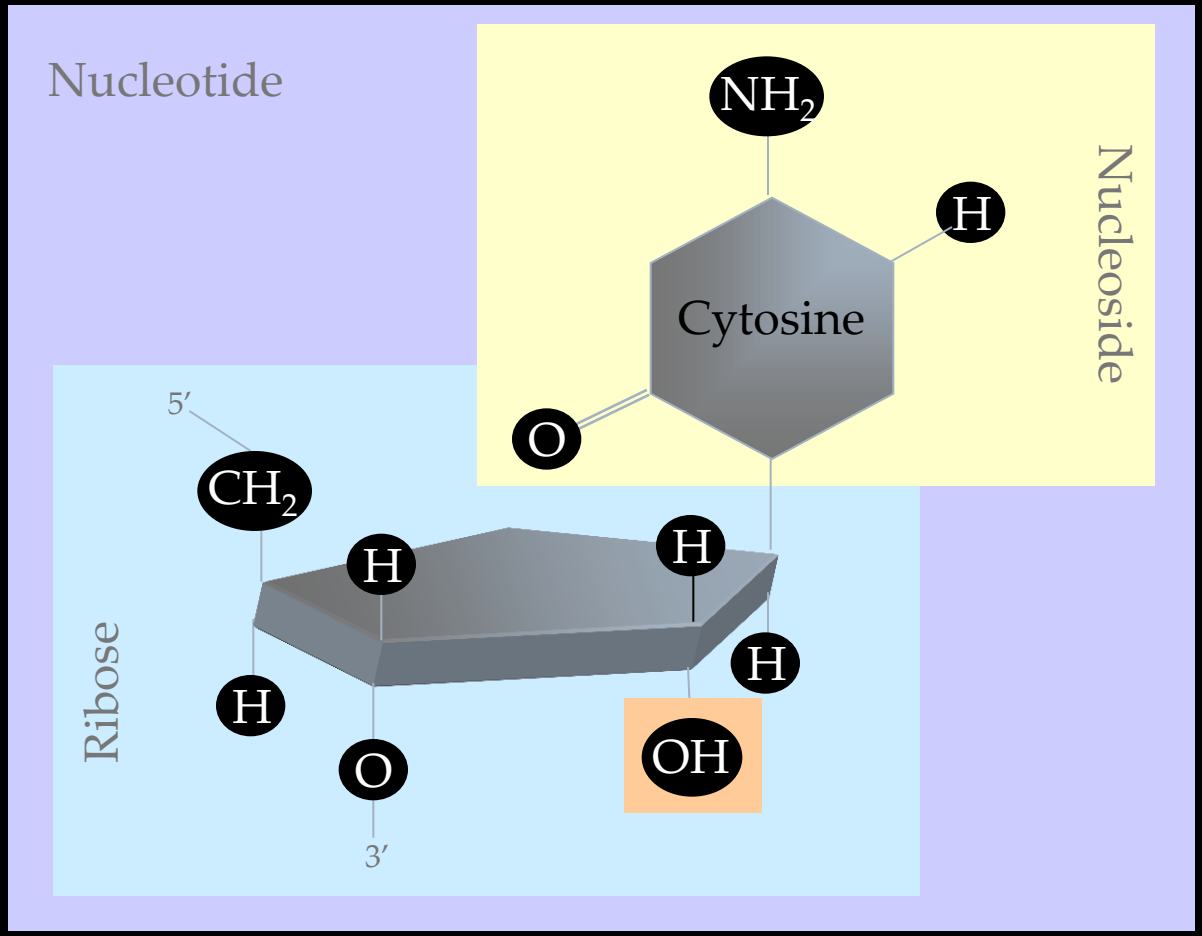






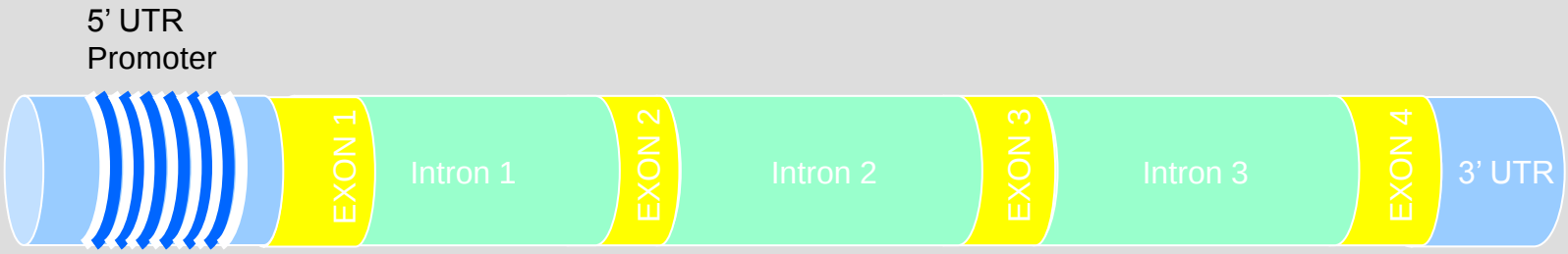
Relative amount of PCR product

RNA: Bases (A, C, G, U)



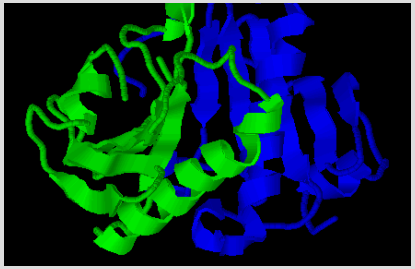
Gene Structure and Translation

DNA

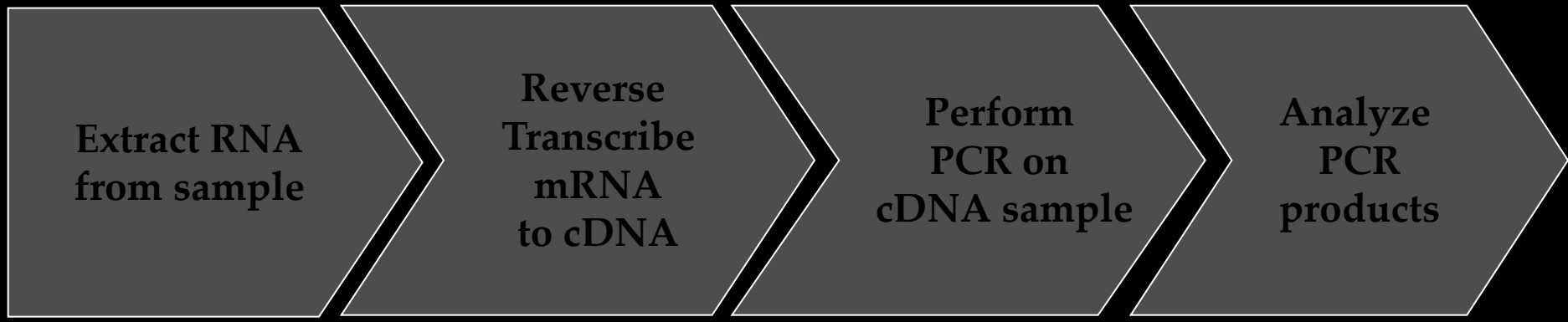


mRNA

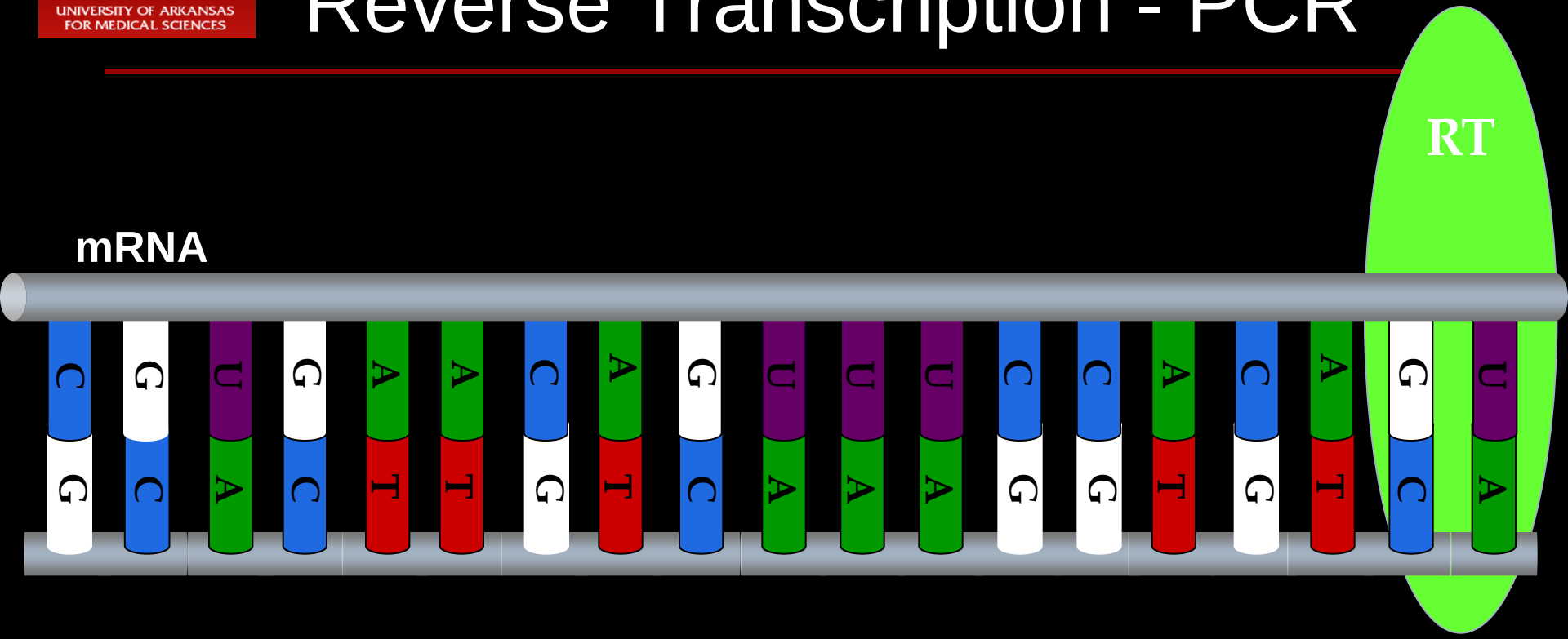
Protein



Reverse Transcription – PCR



Reverse Transcription - PCR



cDNA

RT-PCR Product Analysis

- What do we do after RT-PCR?
  - Detect the presence or absence of product
    - Qualitative
  - Detect the sequence in the product, which represents the sequence of the DNA
    - Translocations
  - Detect the amount of product, which represents the amount of expression of a gene
    - Quantitative (*Q-RT-PCR*)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GGCTTTCGGAGATGTTTTGATAGCGACGGGAATTTTAACTTTCTCACCTTCTGGGATC