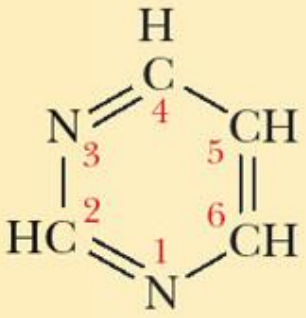
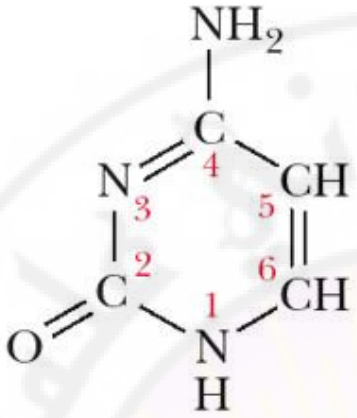
Molecular Biology

البيولوجيا الجزيئية

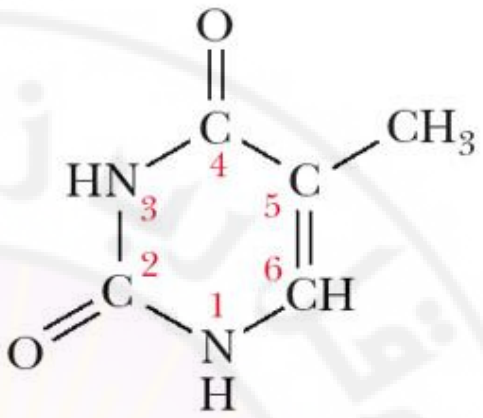
Dr.Shaden HADDAD



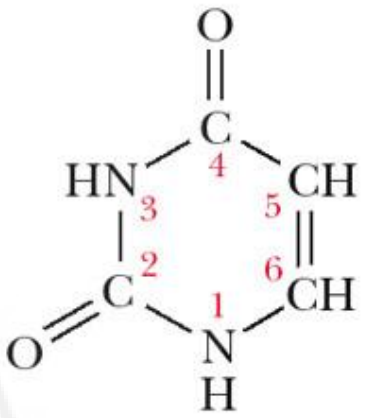
Pyrimidine



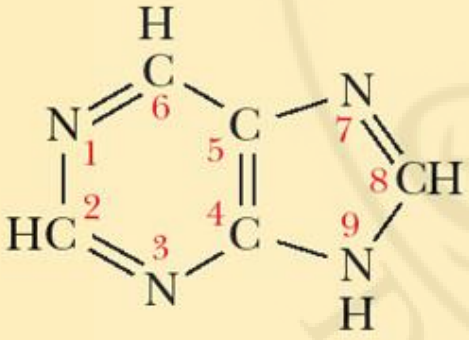
Cytosine
(in DNA & RNA)



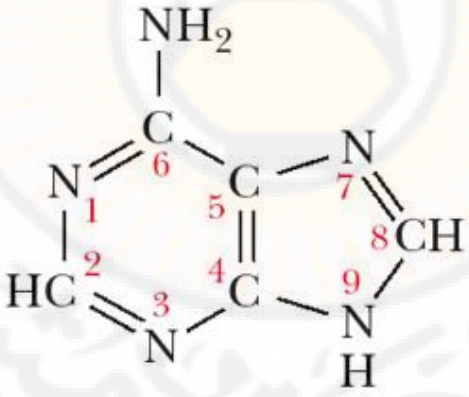
Thymine
(in DNA & some RNA)



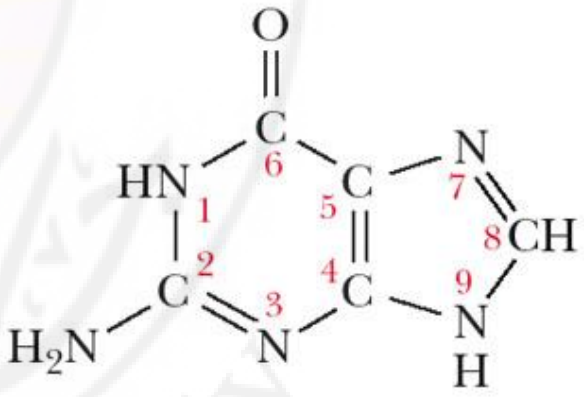
Uracil
(in RNA)



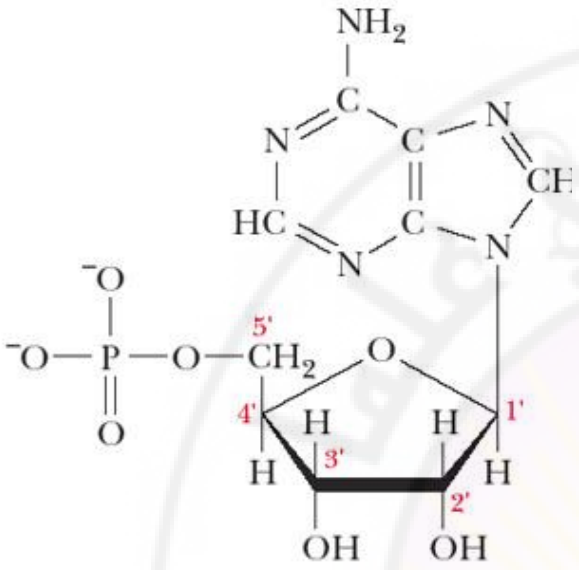
Purine



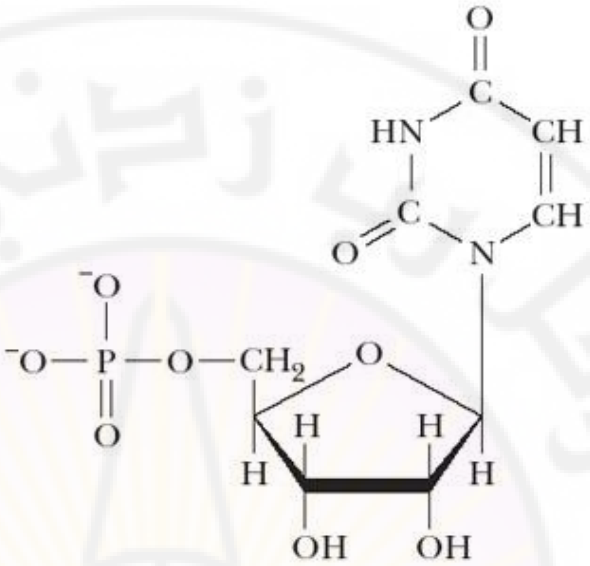
Adenine
(in DNA & RNA)



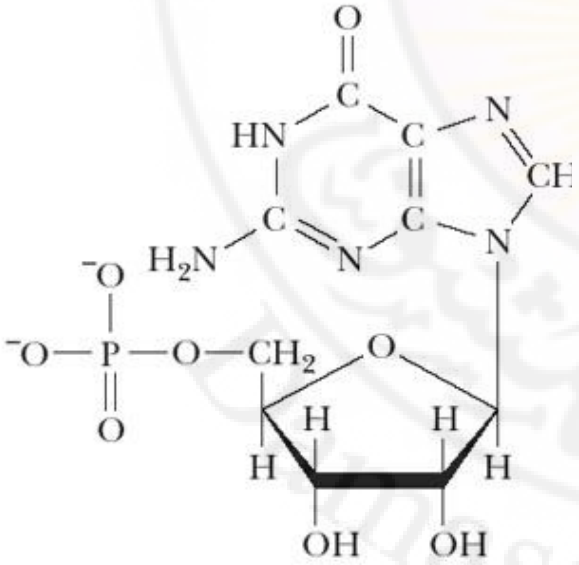
Guanine
(in DNA & RNA)



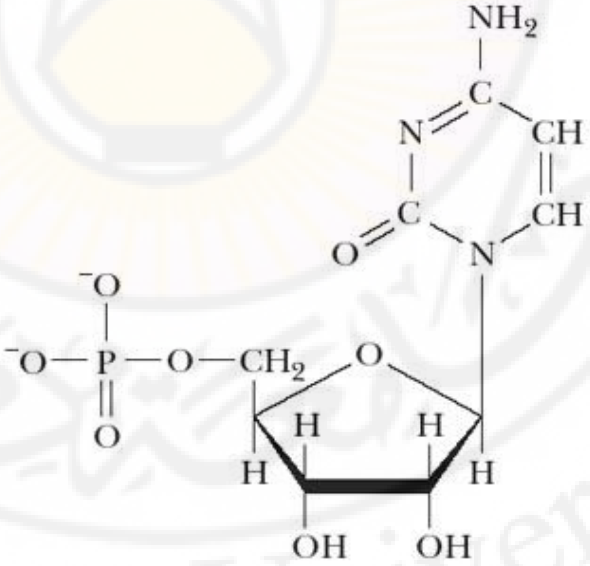
Adenosine 5'-monophosphate



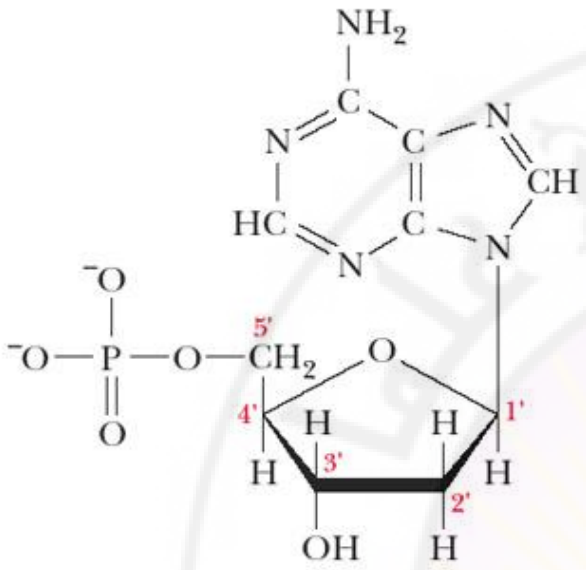
Uridine 5'-monophosphate



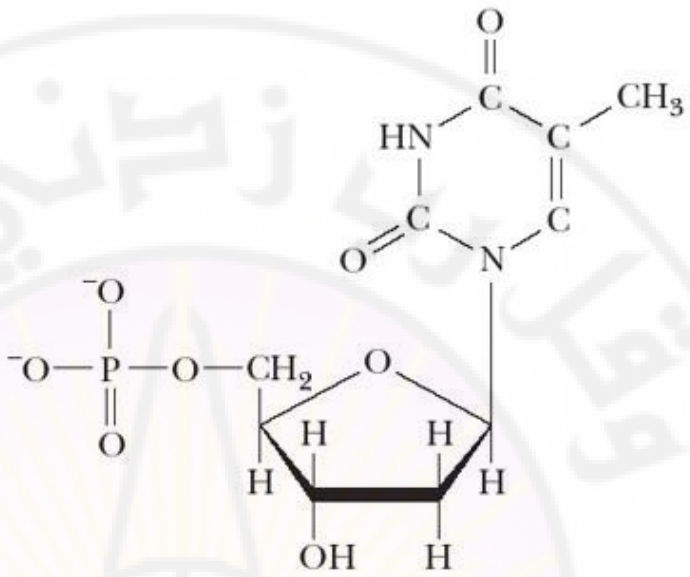
Guanosine 5'-monophosphate



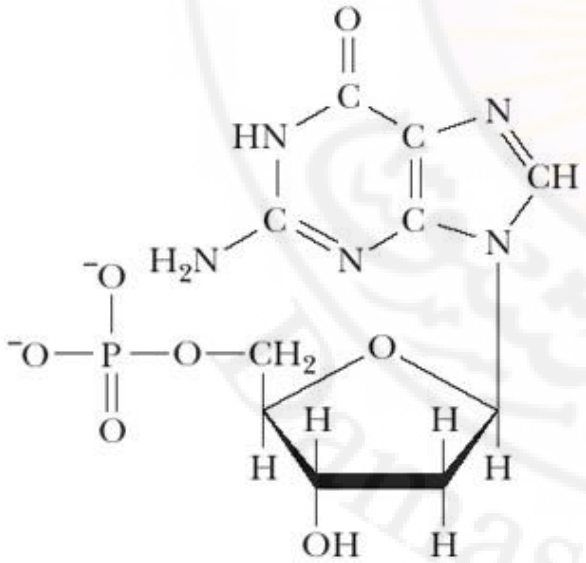
Cytidine 5'-monophosphate



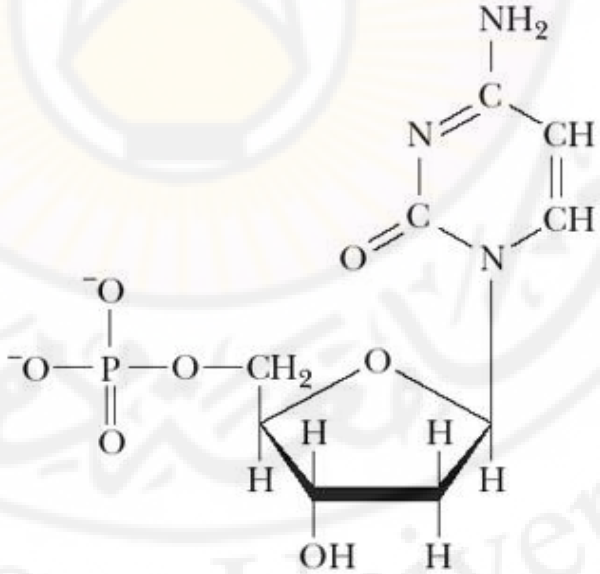
Deoxyadenosine 5'-monophosphate



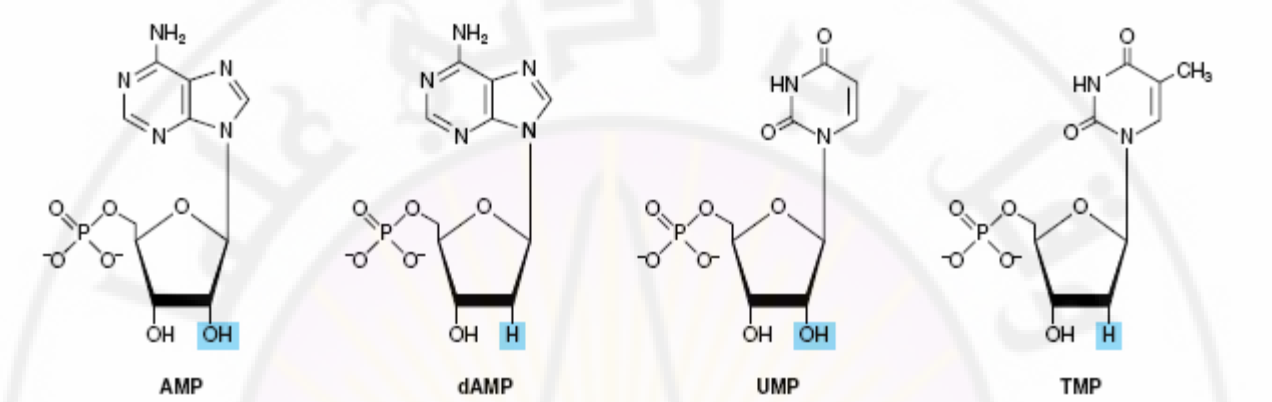
Deoxythymidine 5'-monophosphate



Deoxyguanosine 5'-monophosphate



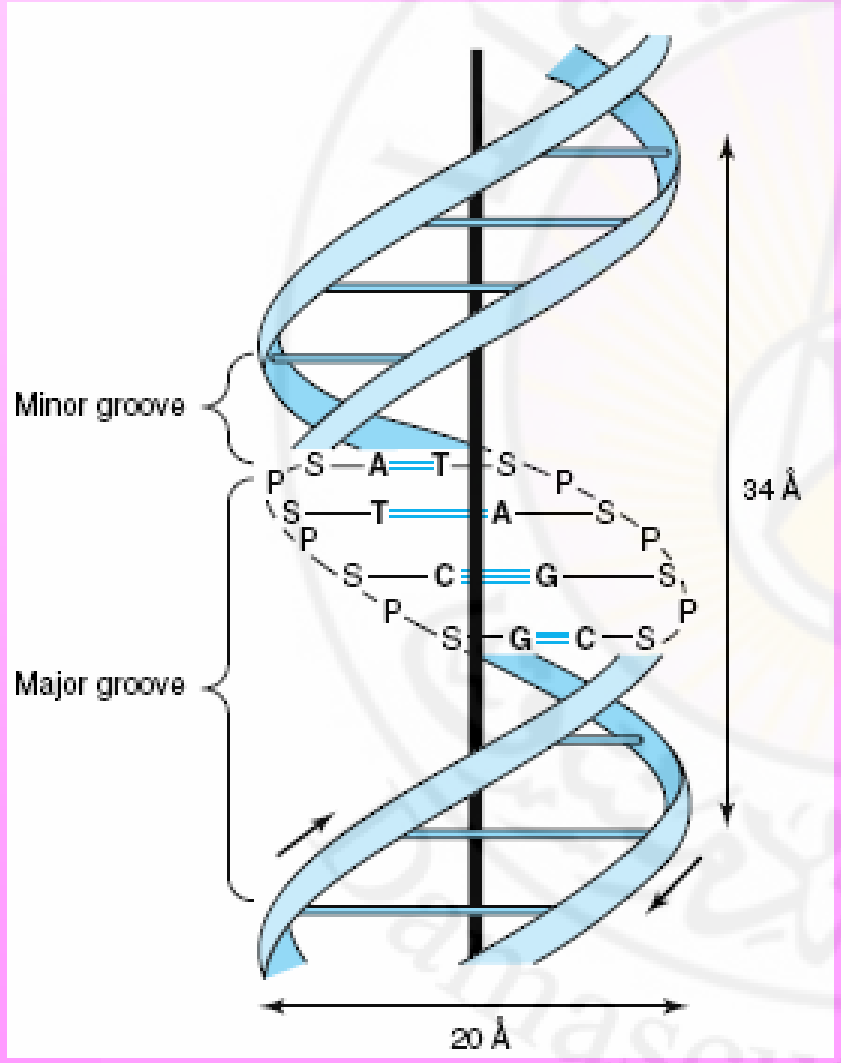
Deoxycytidine 5'-monophosphate

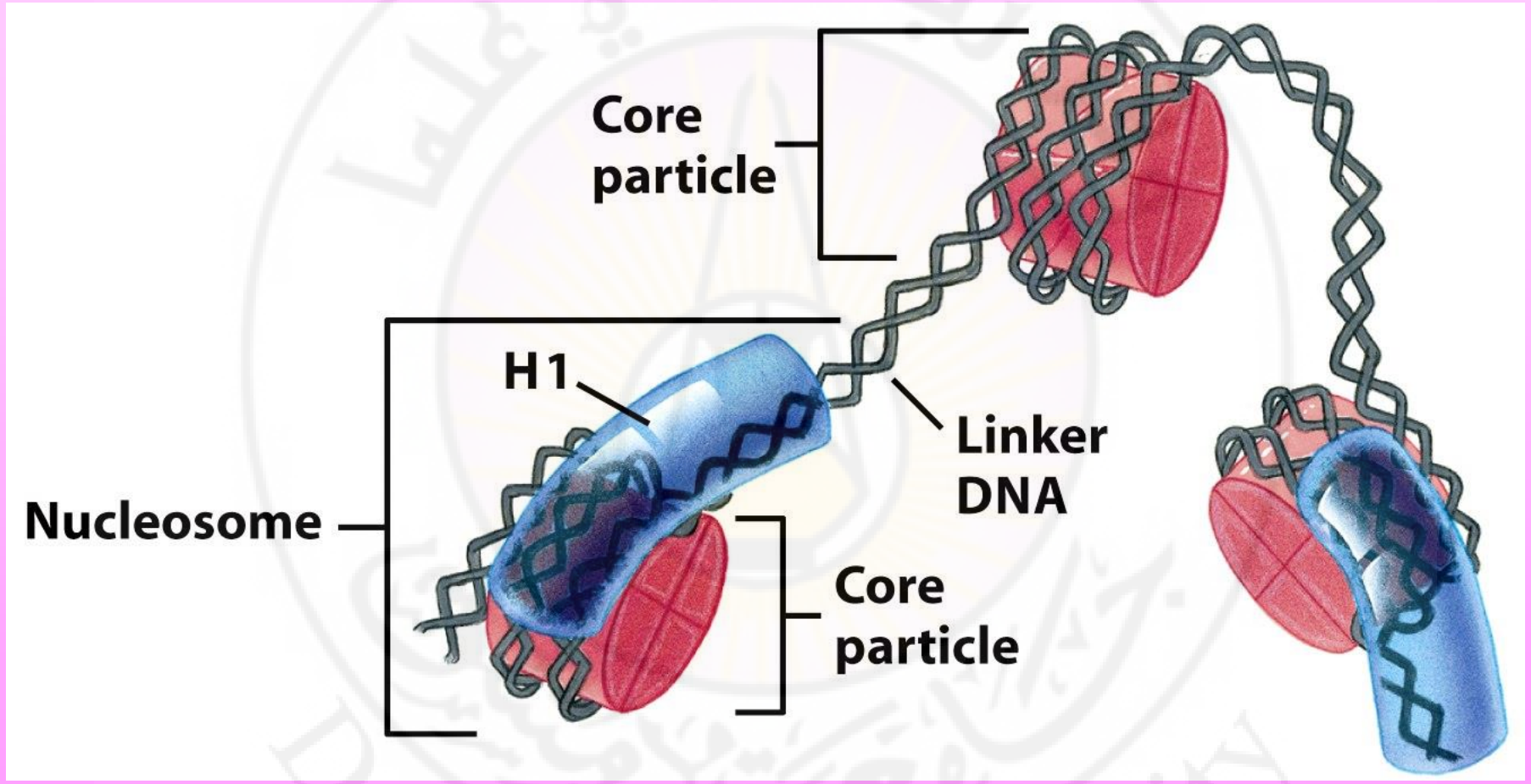


AMP, dAMP, UMP, and TMP.

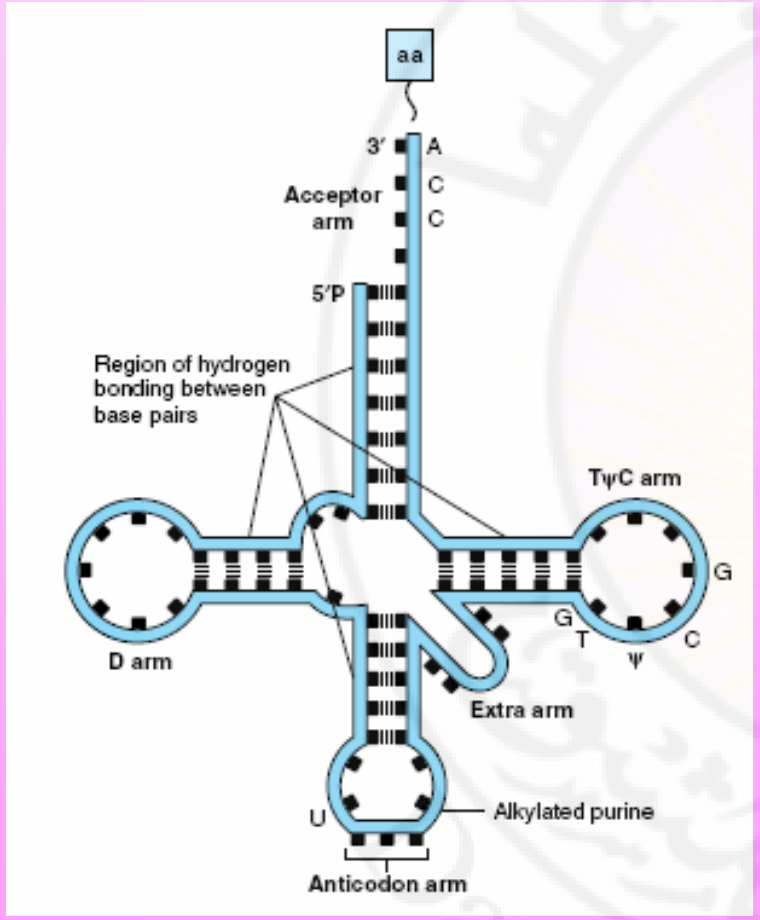
Base	Ribonucleoside	Ribonucleotide (5′-monophosphate)
Adenine (A)	Adenosine	Adenosine 5′-monophosphate (AMP); adenylate[a]
Guanine (G)	Guanosine	Guanosine 5′-monophosphate (GMP); guanylate[a]
Cytosine (C)	Cytidine	Cytidine 5′-monophosphate (CMP); cytidylate[a]
Uracil (U)	Uridine	Uridine 5′-monophosphate (UMP); uridylate[a]
Base	**Deoxyribonucleoside**	**Deoxyribonucleotide (5′-monophosphate)**
Adenine (A)	Deoxyadenosine	Deoxyadenosine 5′-monophosphate (dAMP); deoxyadenylate[a]
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5′-monophosphate (dGMP); deoxyguanylate[a]
Cytosine (C)	Deoxycytidine	Deoxycytidine 5′-monophosphate (dCMP); deoxycytidylate[a]
Thymine (T)	Deoxythymidine or thymidine	Deoxythymidine 5′-monophosphate (dTMP); deoxythymidylate[a] or thymidylate[a]

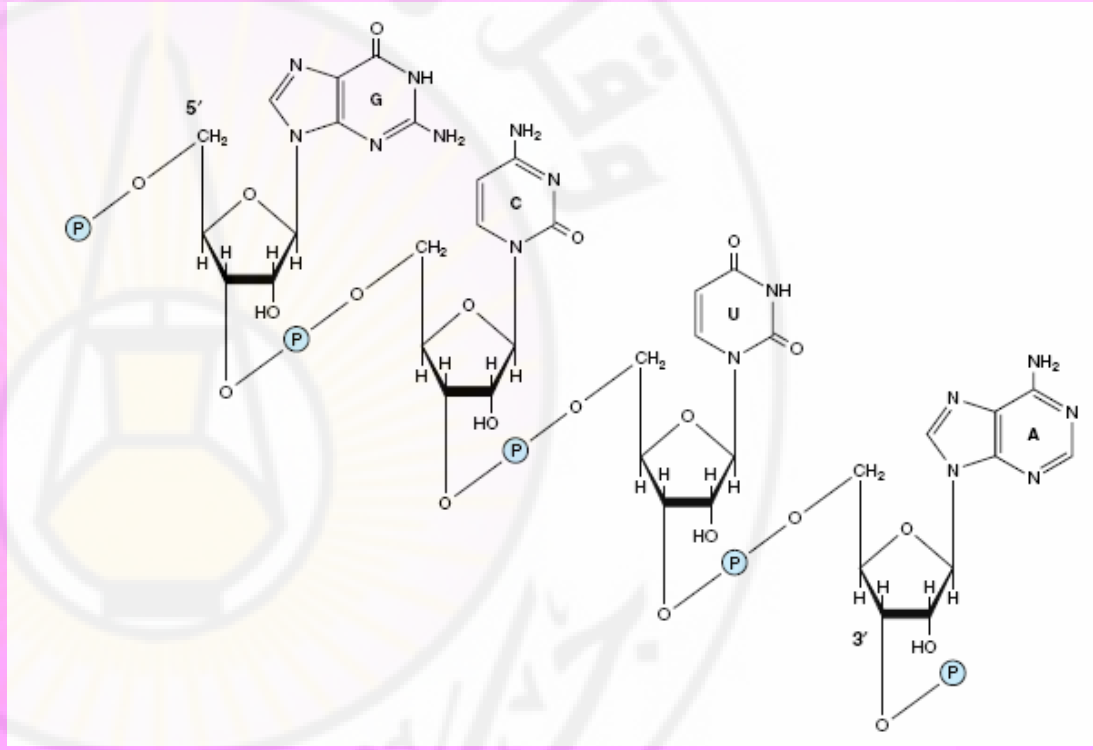
DNA Provides a Template for Replication & Transcription



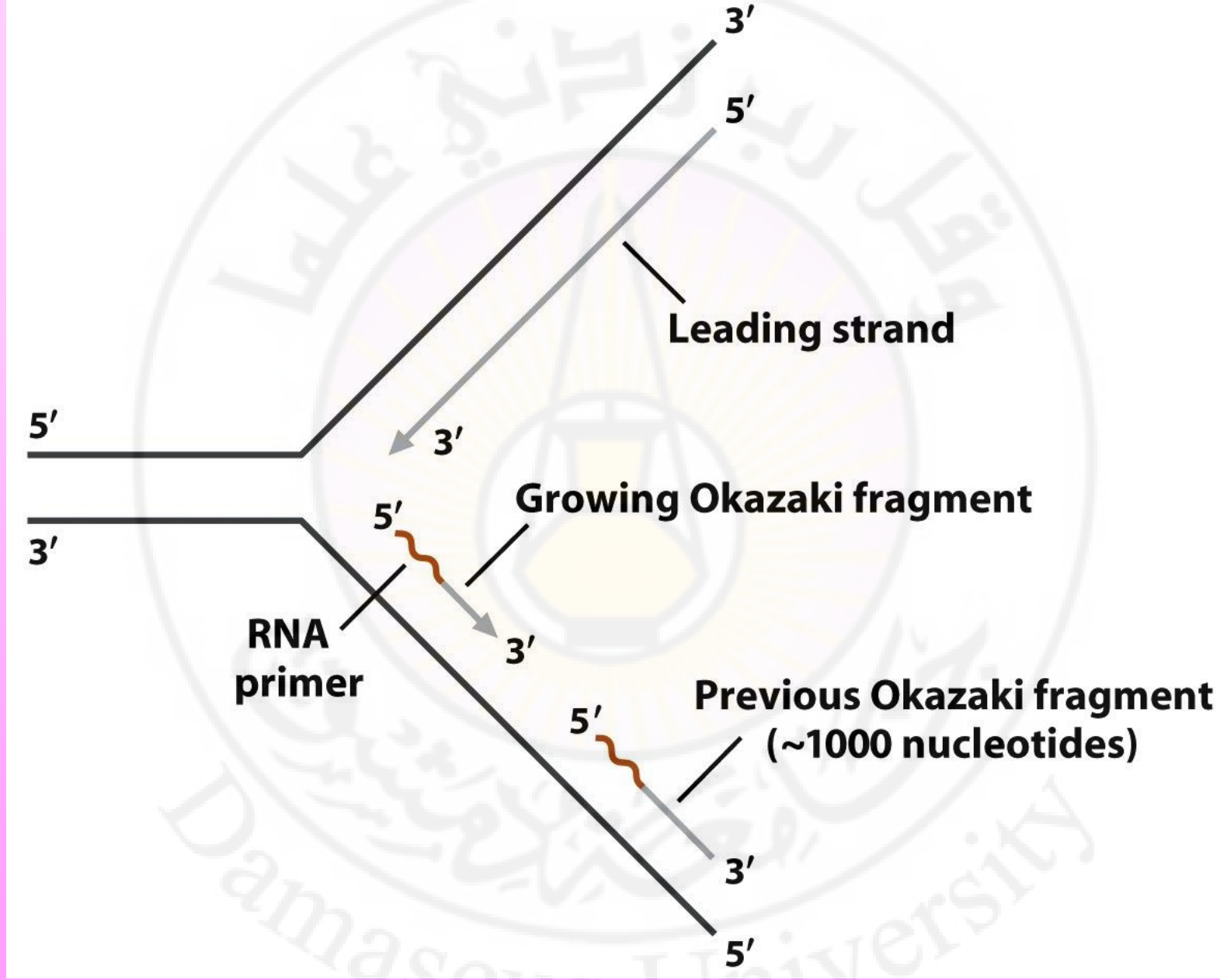

Core particle
H1
Linker DNA
Nucleosome
Core particle

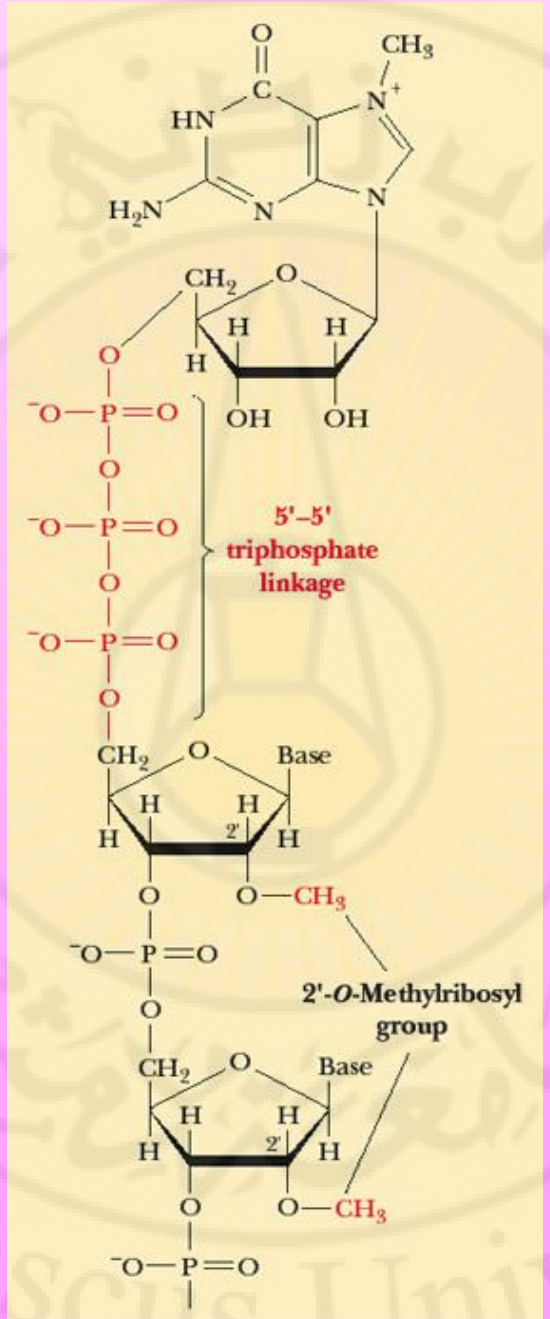
RNA Structure



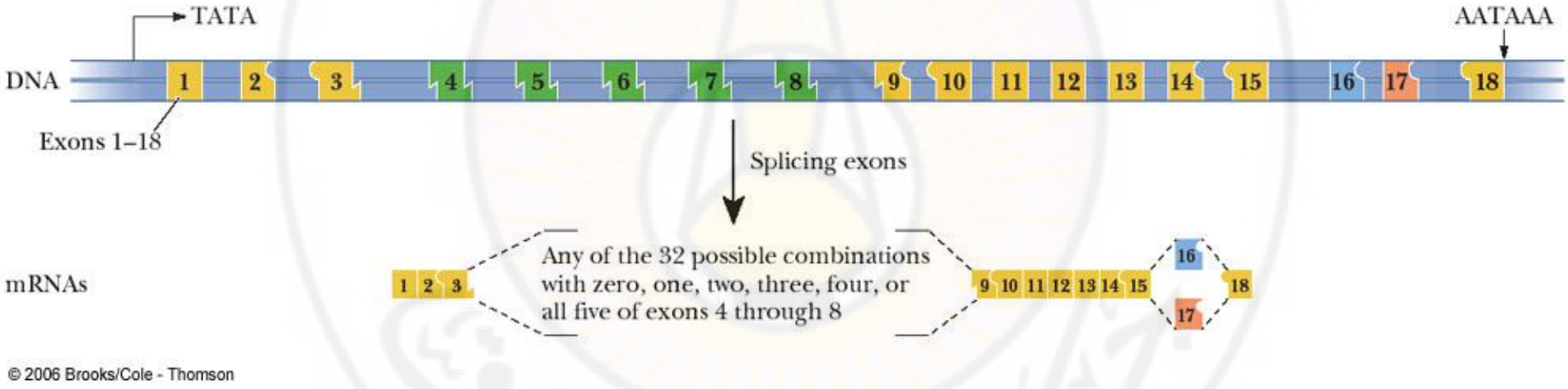


tRNA


3′
5′
Leading strand
5′
3′
5′
Growing Okazaki fragment
3′
RNA
primer
3′
5′
Previous Okazaki fragment
(~1000 nucleotides)
3′
5′



Fast skeletal troponin T gene and spliced mRNAs



Translation

Table 12.2

Base-Pairing Combinations in the Wobble Scheme

Base at 5′ End of Anticodon	Base at 3′End of Codon
I*	A, C, or U
G	C or U
U	A or G
A	U
C	G

I: inosine

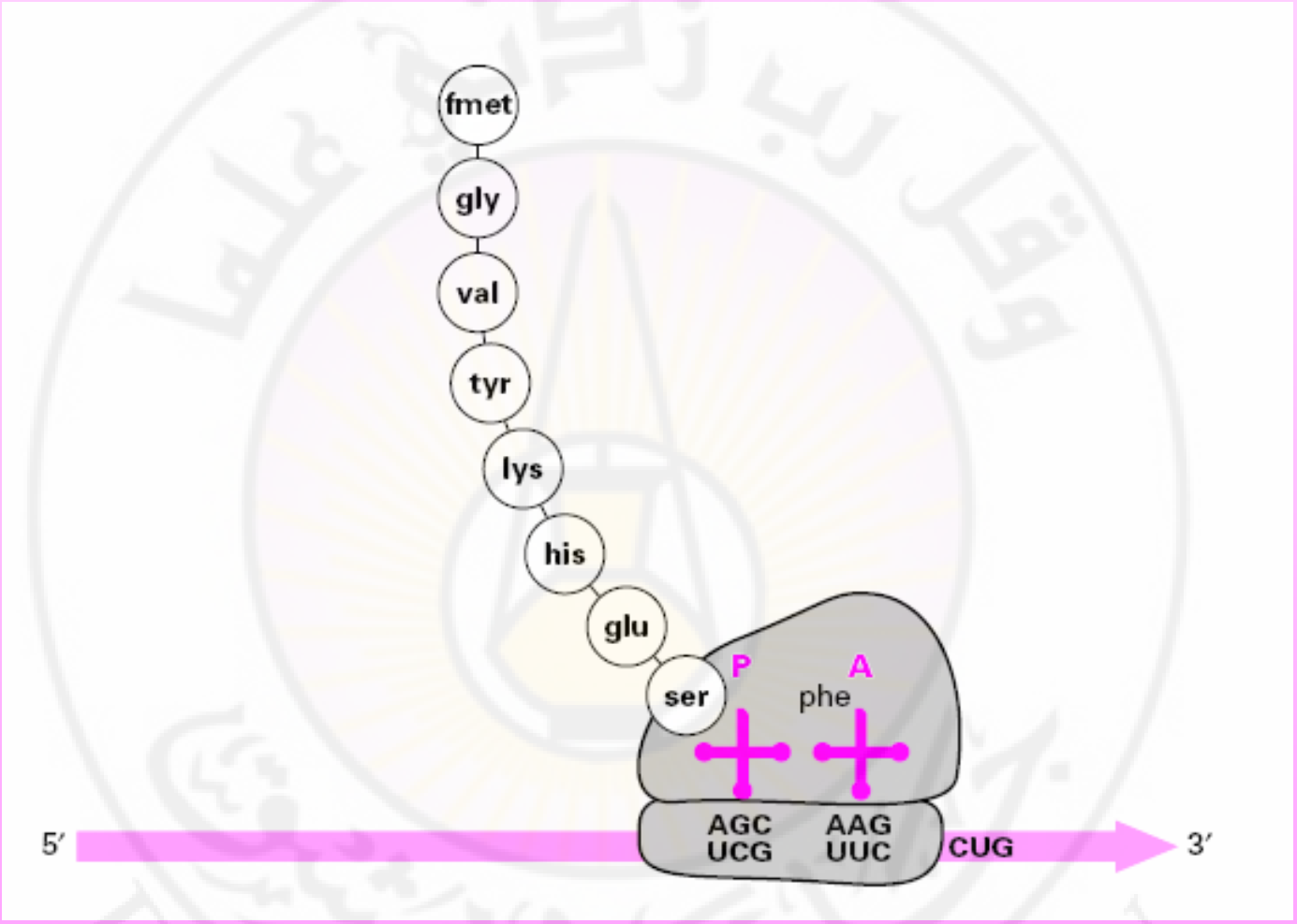
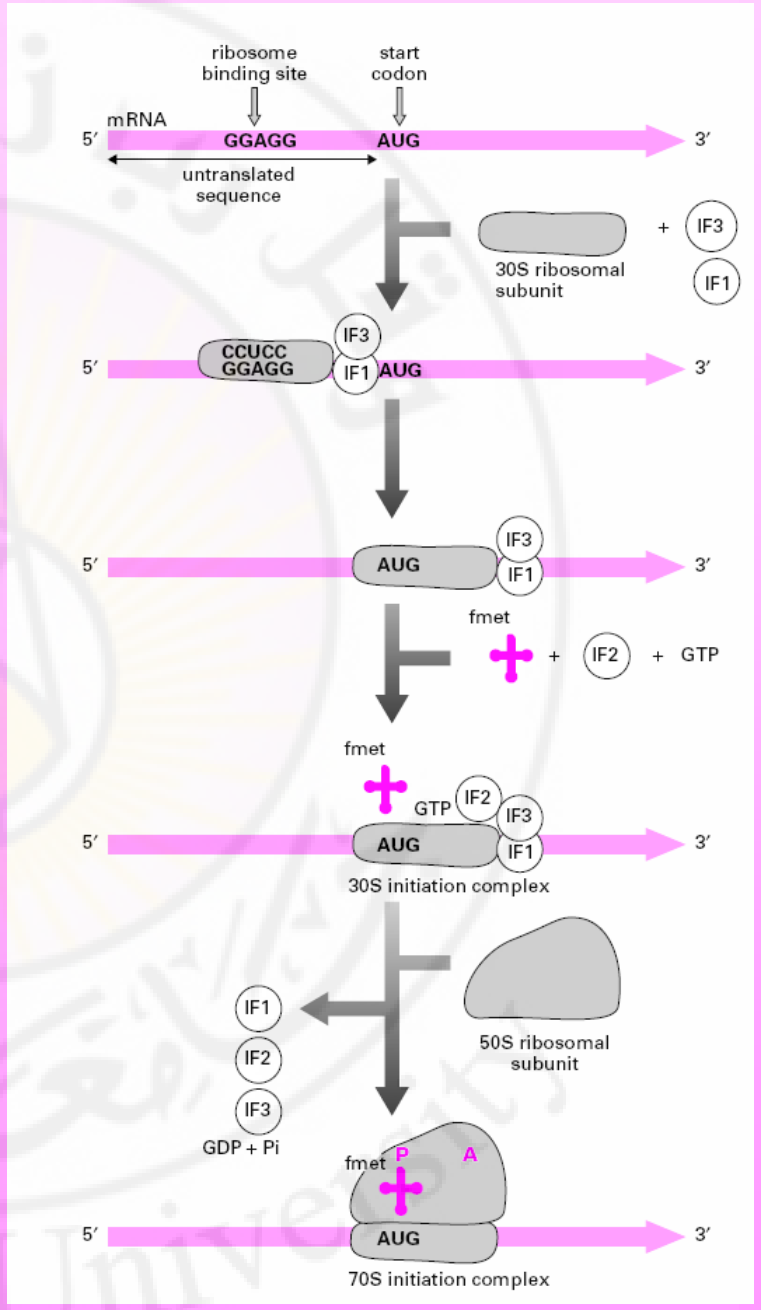


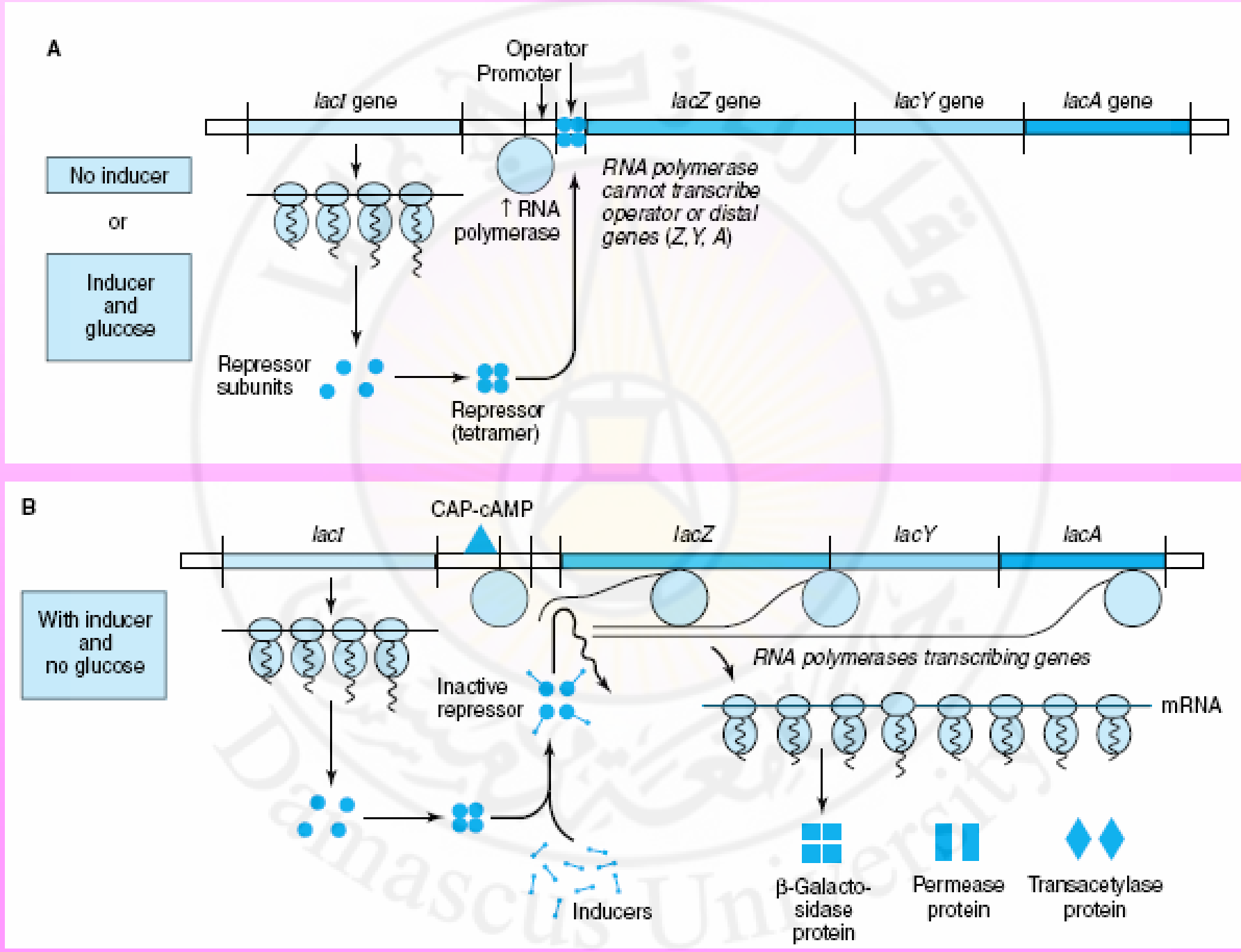
Fig: The P and A sites on the ribosome.

Fig: Formation of the 70S initiation complex.



Regulation in prokaryotes

Figure 39–2. The positional relationships of the structural and regulatory genes of the *lac* operon. *lacZ* encodes β-galactosidase, *lacY* encodes a permease, and *lacA* encodes a thiogalactoside transacetylase. *lacI* encodes the *lac* operon repressor protein.


A
Operator
Promoter
lacI gene
lacZ gene
lacY gene
lacA gene
No inducer
or
Inducer and glucose
↑ RNA polymerase
RNA polymerase cannot transcribe operator or distal genes (Z, Y, A)
Repressor subunits
Repressor (tetramer)
B
CAP-cAMP
lacI
lacZ
lacY
lacA
With inducer and no glucose
RNA polymerases transcribing genes
mRNA
Inactive repressor
Inducers
β-Galacto-sidase protein
Permease protein
Transacetylase protein

Mutations

Mutations occur in all organisms at random places in the genome with an approximately similar rate. Mutations can be **simple base substitutions, inversions, deletions, or insertions**. The length of insertions and deletions is variable.

Each individual has undergone some sort of genetic recombination and/or mutation to become unique in **physical and emotional constitution.** Many genetic mutations happen throughout all the cells of our bodies.

Most of the defective cells **die** and undergo **apoptosis**.

When a mutation occurs in the **somatic cells**, the children or offspring do not inherit the mutation, only when a mutation occurs in the **germline or sex cells** are the mutations passed on to the next generation.

Wild Types and Mutants

- Wild type strain is a strain of an organism isolated from nature
- Is the usual (native) form of the organism
- Any organism may undergo a heritable change in the base sequence of its nucleic acid genome and become a MUTANT

Some Definitions

Mutant	organism or strain whose genome carries a mutation
Mutation	inheritable change in the base sequence of the genome of an organism
Genotype	the precise genetic makeup of an organism
Phenotype	the observable characteristics of an organism Figure 1

GENETIC VARIATION

The human genome encodes for all the genetic elements necessary for normal cellular function. Variation of the DNA sequence can therefore impact the normal functioning of cells and organs, and contributes to the individual variability for a wide range of characteristics.

There are three broad categories of genetic variation:

DNA sequence variation

Structural variation

Epigenetic variation

DNA sequence variation

Single nucleotide polymorphism: The most common form of DNA variation is the substitution of one base for another.

A **single nucleotide polymorphism (SNP)** refers to such a substitution, usually when seen in a population with a **frequency of more than 1 percent**.

Table 8.5 Types of Mutations

Base Changes	Normal	Mutant
Transitions	GAACGT	GAGCGT
Transversions	GAACGT	GATCGT
Missense mutation	GAA CGT Glu Arg	GAT CGT Asp Arg
Conservative substitution	ACT CGT Thr Arg	TCT CGT Ser Arg
Radical replacement	GAT CGT Asp Arg	GCT CGT Ala Arg
Nonsense mutation	GAA CGT Glu Arg	TAA CGT Stop
Insertions	GAACGT	GAAACGT
Deletions	GAACGT	GACGT

SNPs can confer phenotypic variation in one of several ways:

Nonsense mutation: A substitution that changes a codon for an **amino acid to a stop codon**, leading to termination of translation of the mRNA transcript and stop protein production.

Nonsense mutation	GAA CGT Glu Arg	TAA CGT Stop

Missense mutation: A substitution that changes the codon for one amino acid to the codon for another amino acid. The size of protein are not changed, but the sequence does change.

Some missense substitutions have little effect on protein structure or function because one amino acid is replaced by another with similar properties. This is known as a **conservative substitution.**

Missense mutation	GAA CGT Glu Arg	GAT CGT Asp Arg
Conservative substitution	ACT CGT Thr Arg	TCT CGT Ser Arg

Splice site mutation: A substitution in one of the DNA sequences flanking **intron-exon** boundaries that alters normal **pre-mRNA splicing**. Such mutations can result in intron retention (either partial or complete) or exon skipping.

Silent mutation: A change in one base that results in no change in the amino acid sequence of the protein because the most amino acids have more than one codon.

Transition and Transversion: The most common are base substitutions, where one nucleotide is exchanged for another. When a purine base is replaced by another purine, or a pyrimidine is replaced by another pyrimidine, this is called a **transition**.

If a pyrimidine is exchanged for a purine, or vice versa, this is a **transversion.**

Transitions	GAACGT	GAGCGT
Transversions	GAACGT	GATCGT

Indels (insertions and deletions): Insertions or deletions of one or more nucleotides represent the second most abundant form of sequence variation. When situated within the coding sequence of a gene, these variants can result in significant alterations in protein sequence.

Insertions	GAACGT	GAAACGT
Deletions	GAACGT	GACGT

As an example, a single base insertion of cytosine at nucleotide position 3020 of the NOD2 gene results in increased susceptibility to **inflammatory bowel disease**, with a frequency of 4 percent of Caucasians.

Structural genetic variation

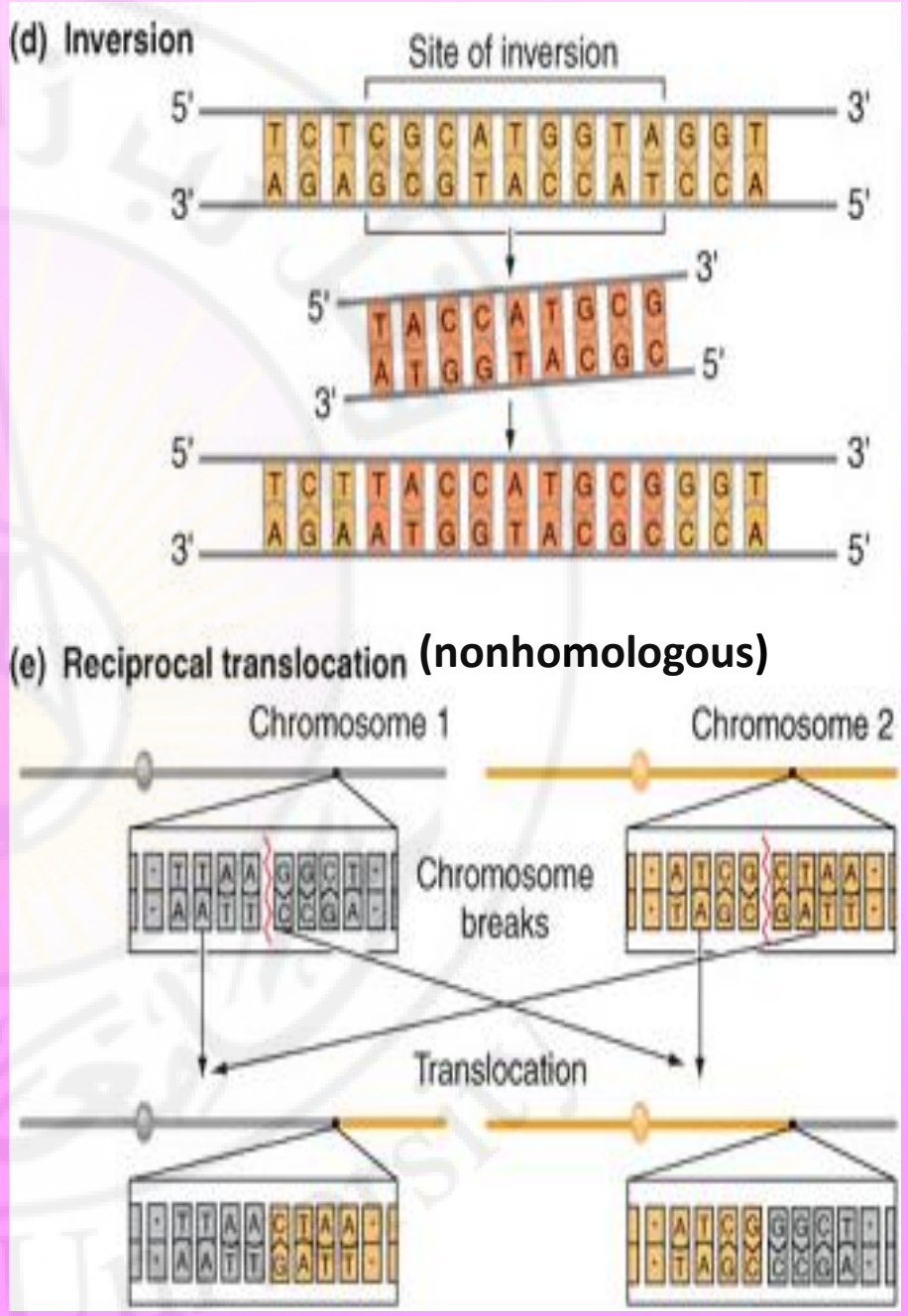
Structural genetic variants are those that affect large segments of DNA (>1000 bases) or whole chromosomes, often affecting the function of one or more genes. This class includes **copy number variation, translocations, and inversions.**

Copy number variation: Copy number variants (CNVs) refer to segments of DNA of more than 1000 bases in length that are either deleted (losses) or duplicated one or more times (gains).

Chromosome translocations and inversions: A translocation occurs when there is exchange of genetic material between **non-homologous chromosomes.**

Chromosomal inversion occurs when a chromosome breaks in two places, inverts itself, and then reinserts itself.

In either case the gene may be disrupted or a new fusion gene may result. **Often these fusion genes are oncogenes**.



Epigenetic variation

Epigenetic variation refers to inherited changes of **phenotypes** without directly altering the **genotypes** DNA sequence. These forms of variation can have dramatic effects on protein expression.

Examples of epigenetic variation include:
Histone modifications (acetylation or deacetylation) of histone proteins, the DNA packaging proteins.

In progeria, the genes are normal, but the **abnormally short telomere length** in only certain cells lines causes an abnormal pattern of gene expression. The senescent pattern of gene expression in specific tissues results in the observed clinical disease of progeria.

Most epigenetic modification is either programmed in the gametes prior to fertilization, or is acquired over one's lifetime. In uterus and environmental exposures can influence epigenetic change.

As such, epigenetic modification represents one mechanism linking **genetic and environmental disease risk factors**.

Mutagens

- Any physical or chemical agent that raises the frequency of mutations above the spontaneous rate.

Main types of mutagens

- Chemical
  - Alkylating agents
  - Base analogs
  - Methylating Agents
  - DNA intercalating agents
  - DNA crosslinking agents
  - Reactive oxygen species
- UV radiation
- Ionizing radiation

Polymerase Chain Reaction (PCR)

The PCR reaction mix comprises of:

- **reaction buffer** (optimized for magnesium chloride)
- **deoxyribonucleotide triphosphates (dNTPs)**
- **oligonucleotide primers**
- **a thermostable DNA polymerase** (usually Taq DNA polymerase: isolated from ***Thermus aquaticus***), which synthesizes DNA by incorporating dNTPs and extending the annealed primers.
- **the template** to be amplified (DNA).

PCR is a method that allows **exponential amplification** of short DNA sequences.
PCR uses of a pair of **primers**, each about **20 nucleotides** in length, that are **complementary** to a defined sequence on each of the two strands of the DNA.

These primers are extended by a **DNA polymerase** so that a copy is made of the designated sequence.

The **primers** can be used to make a copy of the **input DNA strand** and of the **short copy** made in the first cycle of reaction. This leads to **exponential amplification.**

Steps Involved in a PCR Cycle

The initial step of PCR involves incubation of around **95°C for 3-5 min to denature** the target genomic DNA into single strands.

The first incubation in a cycle involves incubation at **95°C for 10 s to 1 min to denature** newly synthesized template DNA.

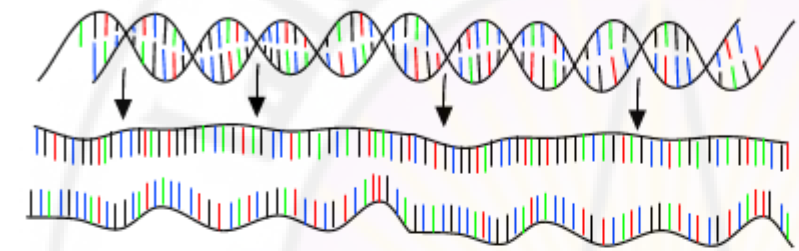
The second incubation is at a temperature determined by the melting properties of the primers, ideally around **55°C for 30 s**, optimized to allow them **to anneal** only to their specific target sequences.

The third incubation is usually **72°C for 30 s to 2 min**, during which Taq DNA polymerase **extends the primers** mediating the synthesis of DNA using the target sequence as the template.

The three incubations represent **one PCR cycle which repeated 30-40 times**

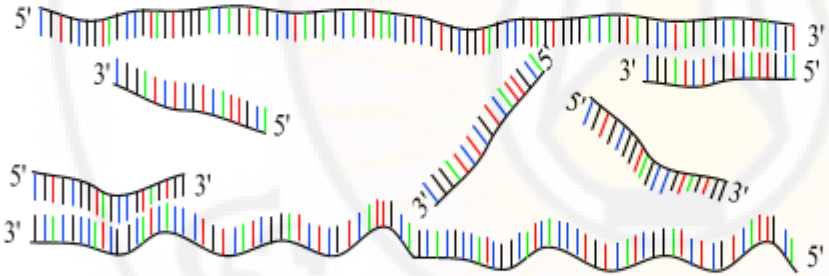
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

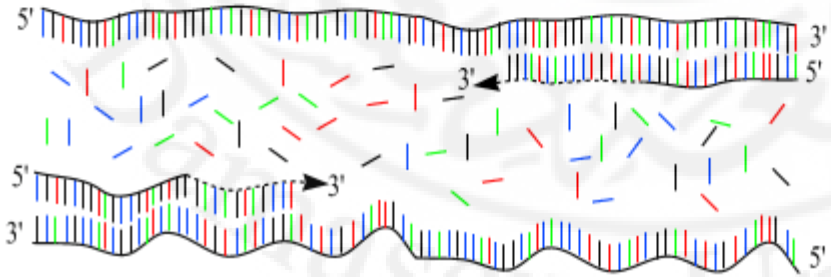
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

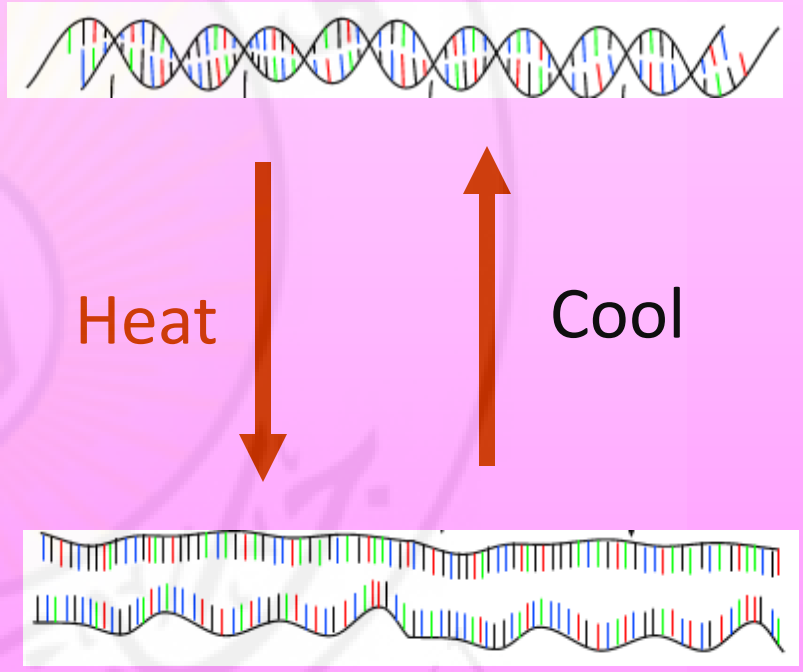
2 minutes 72 °C

only dNTP's

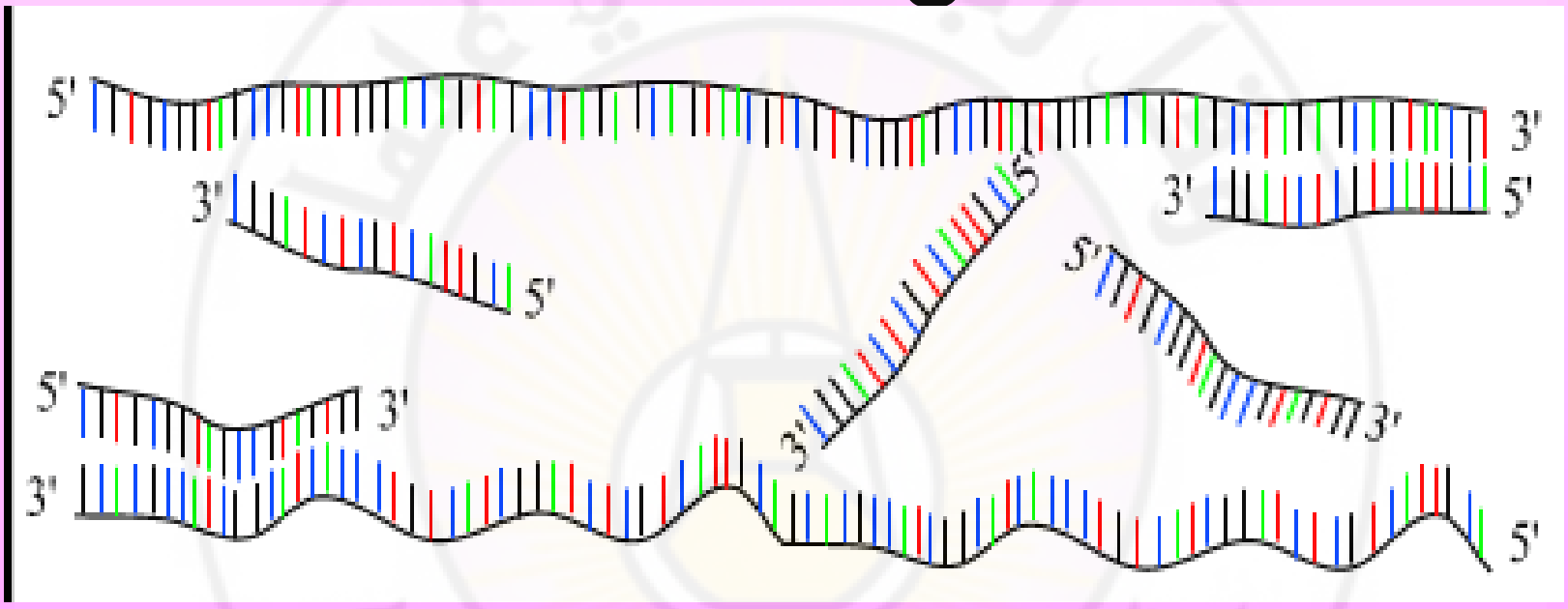
(Andy Vierstraete 1999)

Denaturation

- Heating separates the double stranded DNA (Denaturation)
- Slow cooling anneals the two strands (Renaturation)



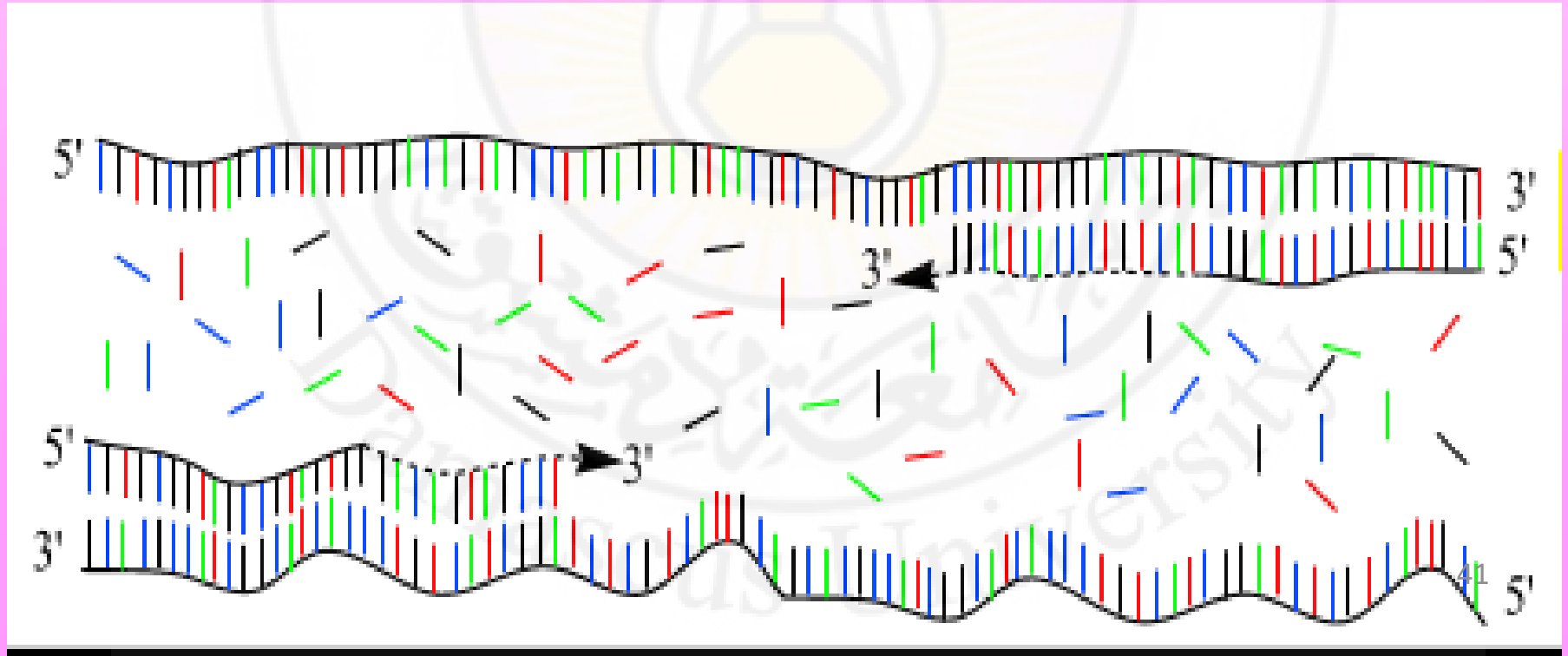
Annealing



- Two primers bind to the complementary region
- As the DNA cools, they wedge between two template strands
- Optimal temperature varies based on primer length. Typical temperature from 40 to 60 °C

Extension

- DNA polymerase replicates DNA
- Optimal temperature 72 °C



The PCR product has to be checked if :

1)There is a product formed.

There is a possibility that the quality of the **DNA is poor**, or that there is too much **starting template**.

1)The product has right size. It is possible that there is a product, for example a band of 300 bases, but the expected product should be 600 bases long. In that case, one of **the primers probably fits on a part of the gene closer to the other primer**. It is also possible that both primers fit on a **totally different gene**.

3)Only one product is formed. it is possible that the primers fit on the desired locations, and also on other locations. In that case, you can have **several products**.

Properties of DNA polymerase

- Needs a pre-existing DNA to duplicate (**template DNA**). Cannot assemble a new strand from components
- Can only extend an existing piece of DNA (**primers**)
- DNA polymerase needs **Mg^{++} as cofactor**
- DNA polymerase needs **dNTPs**
- Each DNA polymerase works best under **optimal temperature, pH and salt concentration** which are provided by **PCR buffer**
- DNA polymerase always moves in one direction (**from 5'→3'**)

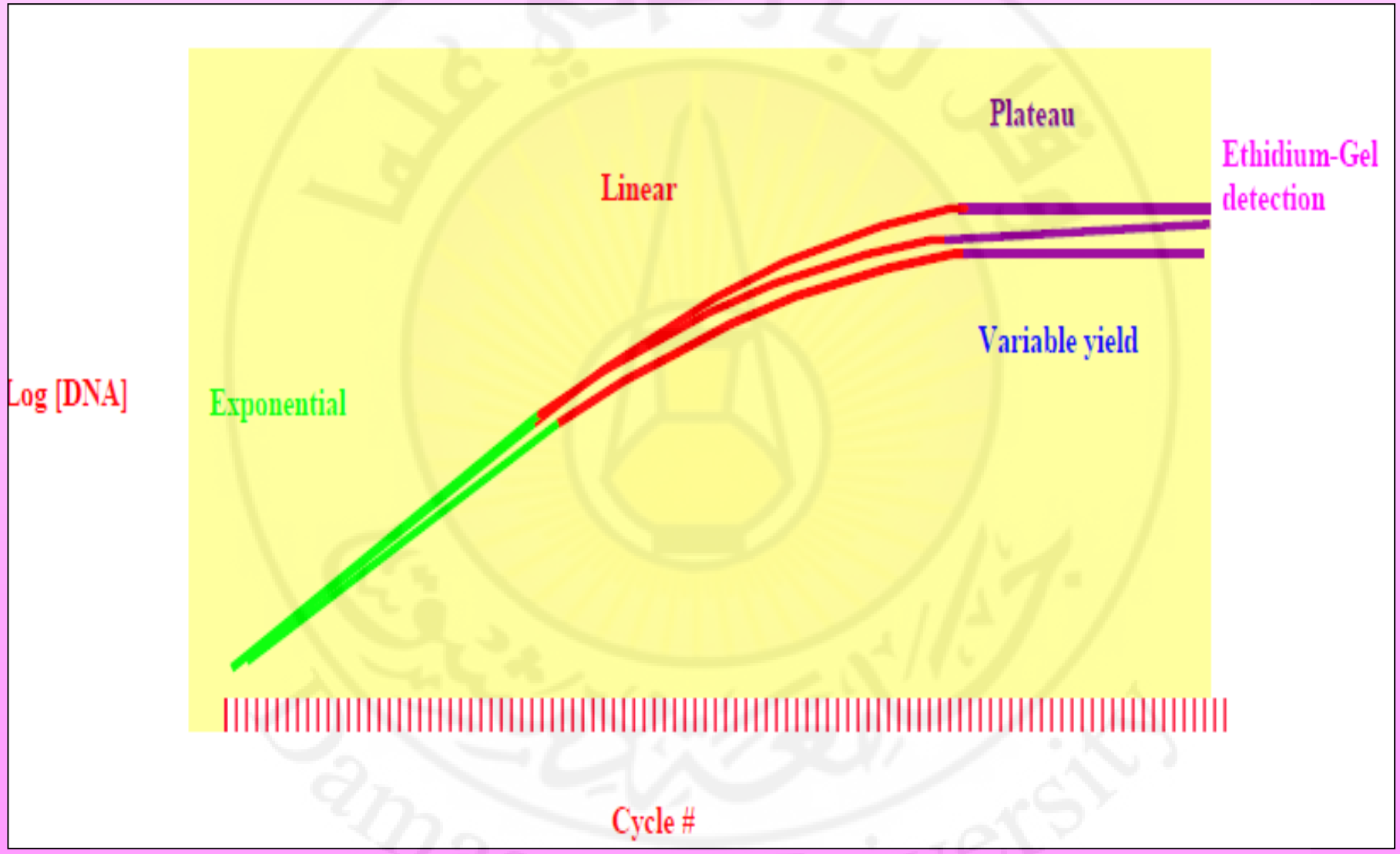
A basic PCR run has three phases:

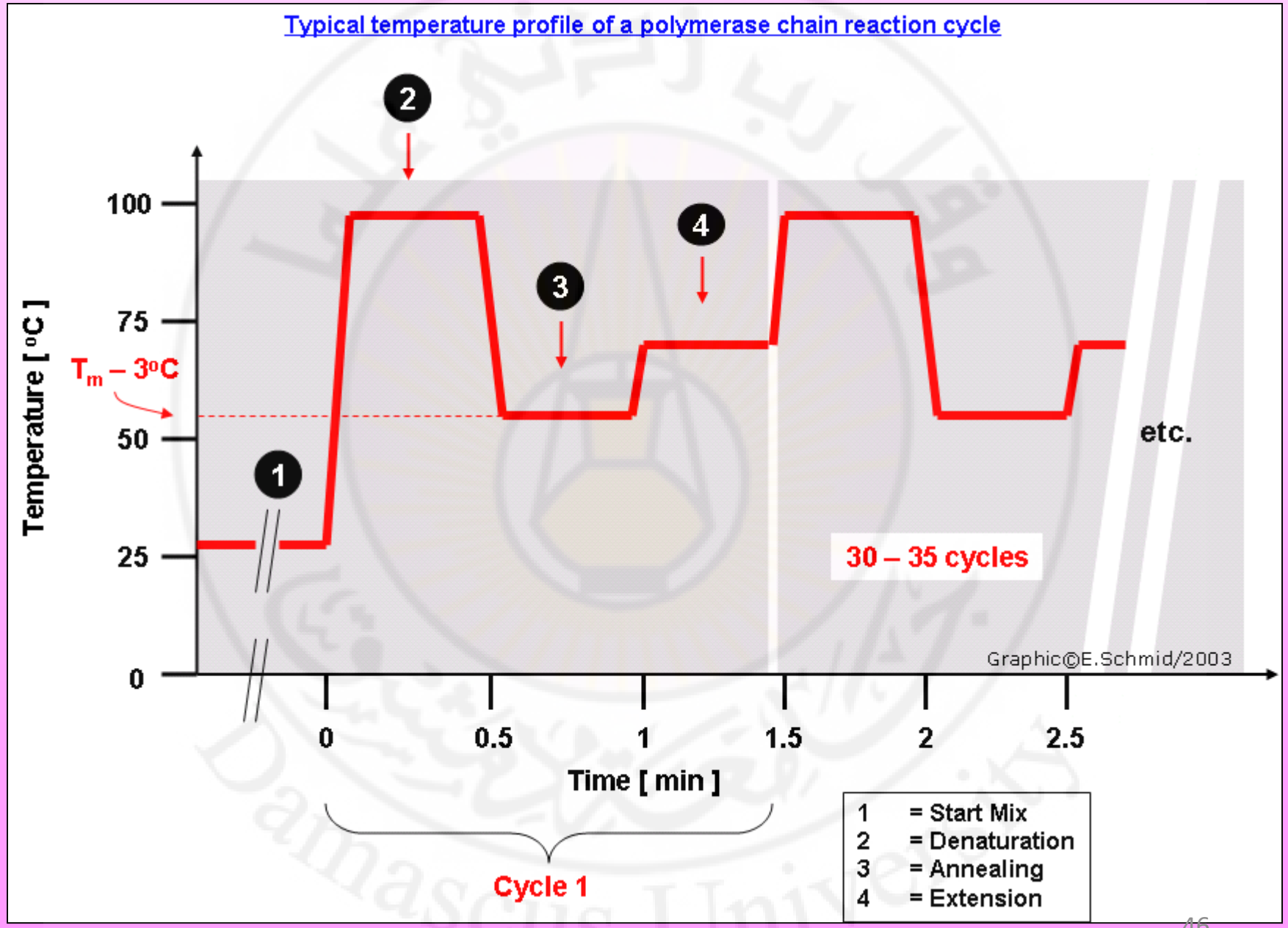
Exponential: Exact doubling of product is accumulating at every cycle. The reaction is very specific and precise.

Linear: The reaction components are being consumed, the reaction is slowing, and products are starting to degrade.

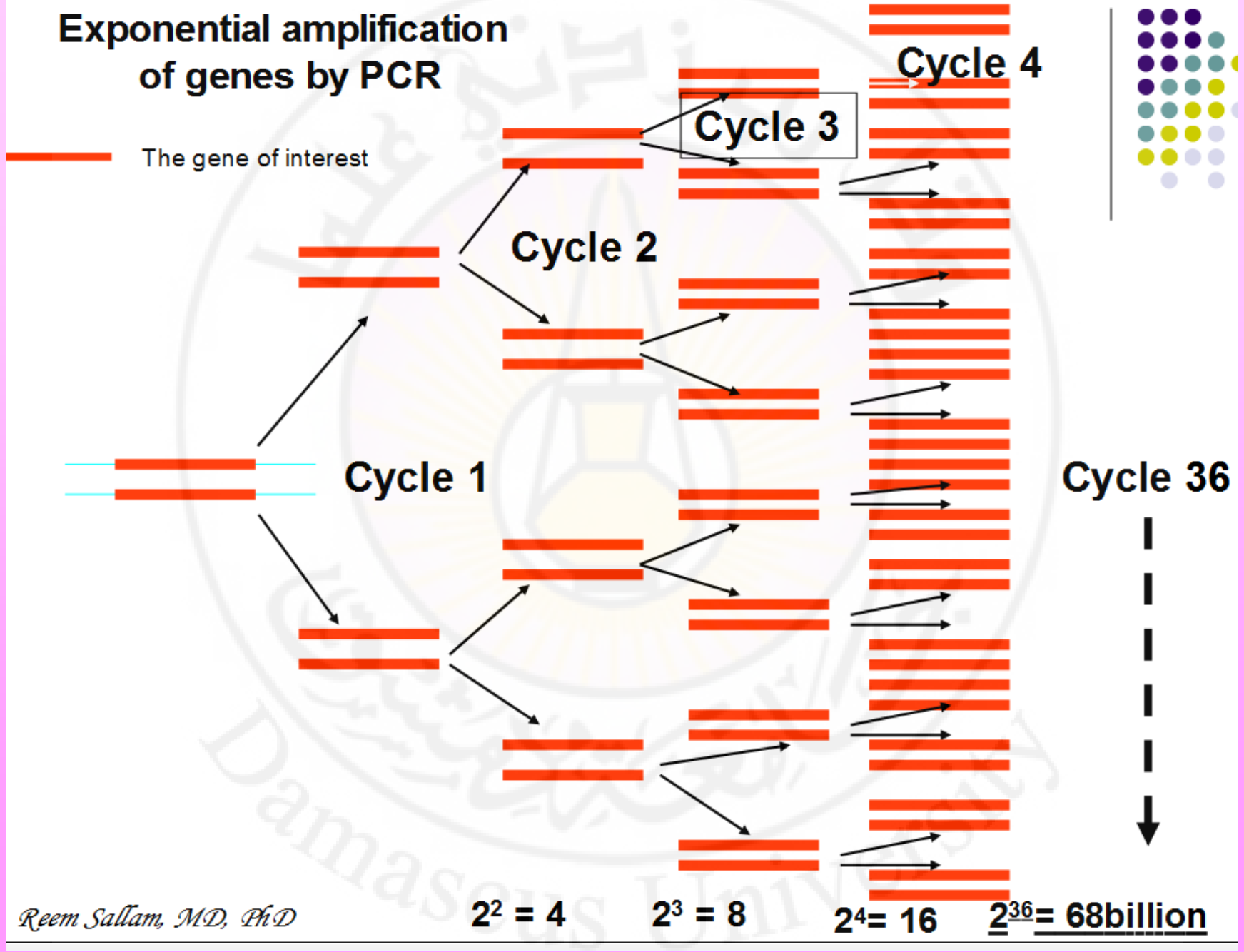
Plateau (End-Point): Gel detection for traditional methods. The reaction has stopped, no more products are being made and if left long enough, the PCR products will be degraded.

PCR Phases


Plateau
Ethidium-Gel detection
Linear
Variable yield
Log [DNA]
Exponential
Cycle #


Typical temperature profile of a polymerase chain reaction cycle
2
100
75
T_m – 3°C
50
25
0
Temperature [°C]
1
3
4
etc.
30 – 35 cycles
Graphic©E.Schmid/2003
0
0.5
1
1.5
2
2.5
Time [min]
Cycle 1
1 = Start Mix
2 = Denaturation
3 = Annealing
4 = Extension

Exponential amplification of genes by PCR



Reem Sallam, MD, PhD

Reverse transcription polymerase chain reaction (RT-PCR)

The mRNA strand is reverse transcribed into DNA complement (complementary DNA, or cDNA) using the enzyme reverse transcriptase, and the resulting cDNA is amplified using PCR. Reverse transcription PCR is not to be confused with real-time polymerase chain reaction (Q-PCR or qRT-PCR), which is also sometimes abbreviated as RT-PCR.

RT-PCR is widely used in the diagnosis of genetic diseases and on determination the abundance of mRNA molecules as a measure of gene expression.

RT-PCR is commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as Influenza virus A and HIV.

Real-time polymerase chain reaction

Also called quantitative real time polymerase chain reaction (Q-PCR / qPCR / qrt-PCR). It is a laboratory technique based on the PCR, which is used to amplify and simultaneously quantify a targeted DNA molecule.

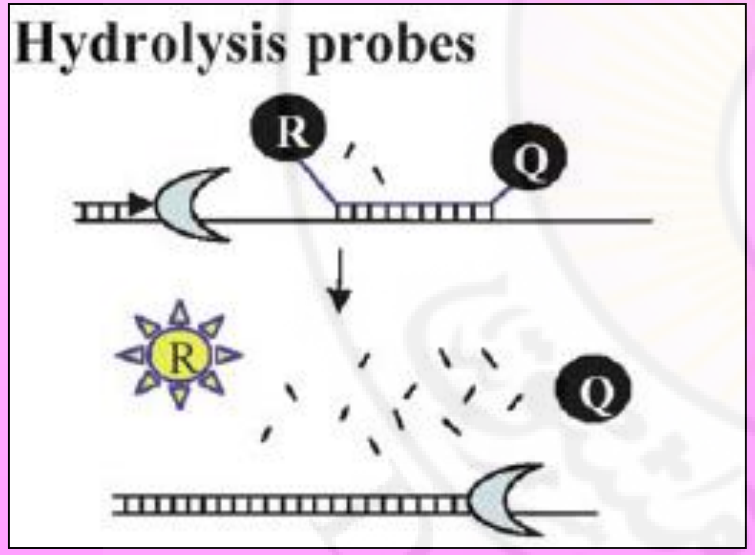
Real Time-PCR enables both detection and quantification.

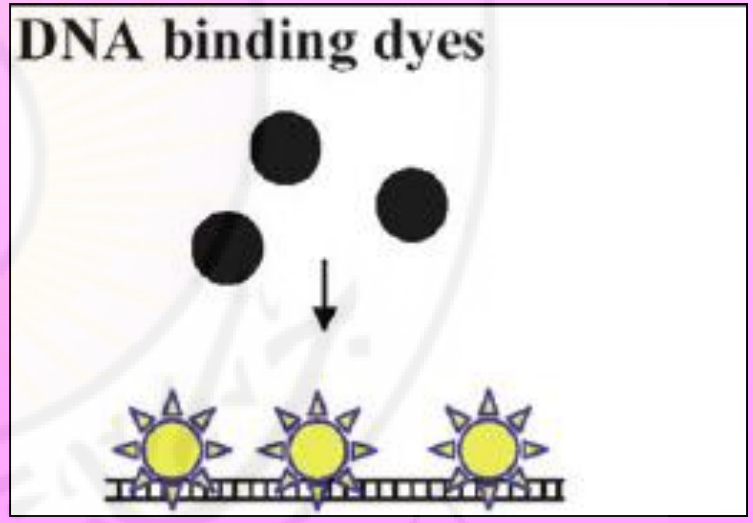
Quantitative comparisons or estimates of PCR products should be based on the exponential phase of the reaction using "real time PCR".

Real-Time PCR Principles

Two general methods for the quantitative assays:

1. Hydrolysis probes (TaqMan)
2. DNA-binding agents (SYBR Green)





Q Quencher fluorophore
R Reporter fluorophore

Principles of Real-Time Quantitative PCR Techniques

SYBR Green I technique

SYBR Green I fluorescence is enormously increased upon binding to double-stranded DNA. During the extension phase, more and more SYBR Green I will bind to the PCR product, resulting in an increased fluorescence. Consequently, during each subsequent PCR cycle more fluorescence signal will be detected.

Hydrolysis probe technique

The hydrolysis probe is conjugated with a **quencher fluorochrome**, which absorbs the fluorescence of the **reporter fluorochrome** as long as the probe is intact. However, upon amplification of the target sequence, the hydrolysis probe is displaced and subsequently hydrolyzed by the Taq polymerase. This results in the separation of the reporter and quencher fluorochrome and consequently the fluorescence of the reporter fluorochrome becomes detectable. During each consecutive PCR cycle this fluorescence will further increase because of the progressive and exponential accumulation of free reporter fluorochromes.