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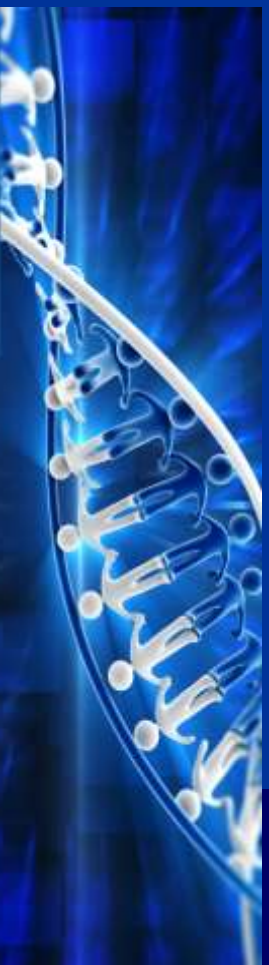


Extraction of DNA and RNA

By
Dr. Dalia
Fouad

Work shop of extraction of DNA and RNA

4-5/08/1438

What is DNA?

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA.

Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).

The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T).

Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people.

The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

What is DNA? (cont.)

DNA bases pair up with each other, A with T and C with G, to form units called base pairs.

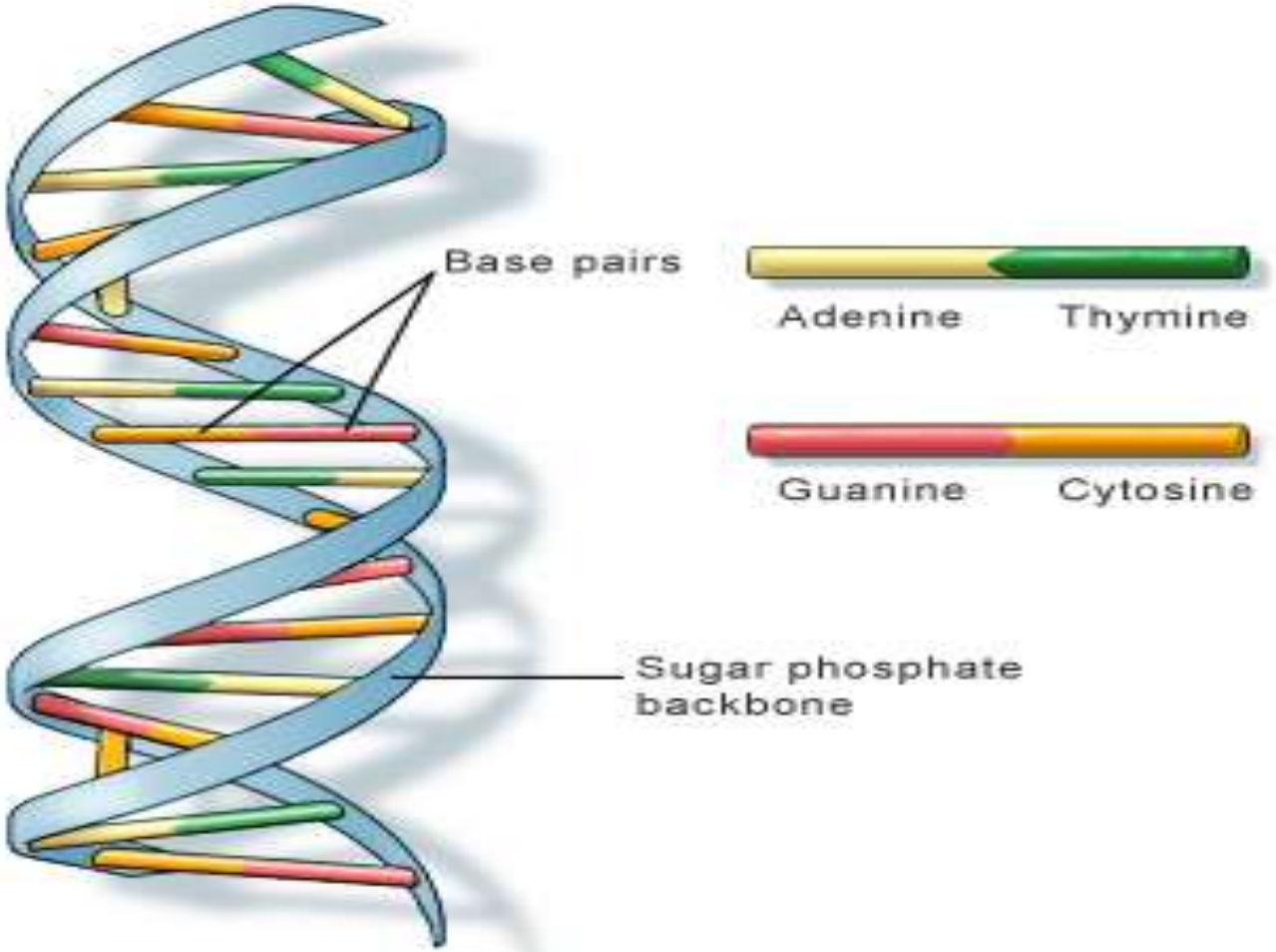
Each base is also attached to a sugar molecule and a phosphate molecule.

Together, a base, sugar, and phosphate are called a nucleotide.

Nucleotides are arranged in two long strands that form a spiral called a double helix.

The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

- **An important property of DNA is that it can replicate, or make copies of itself.**
- **Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases.**
- **This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.**


Base pairs
Adenine
Thymine
Guanine
Cytosine
Sugar phosphate backbone

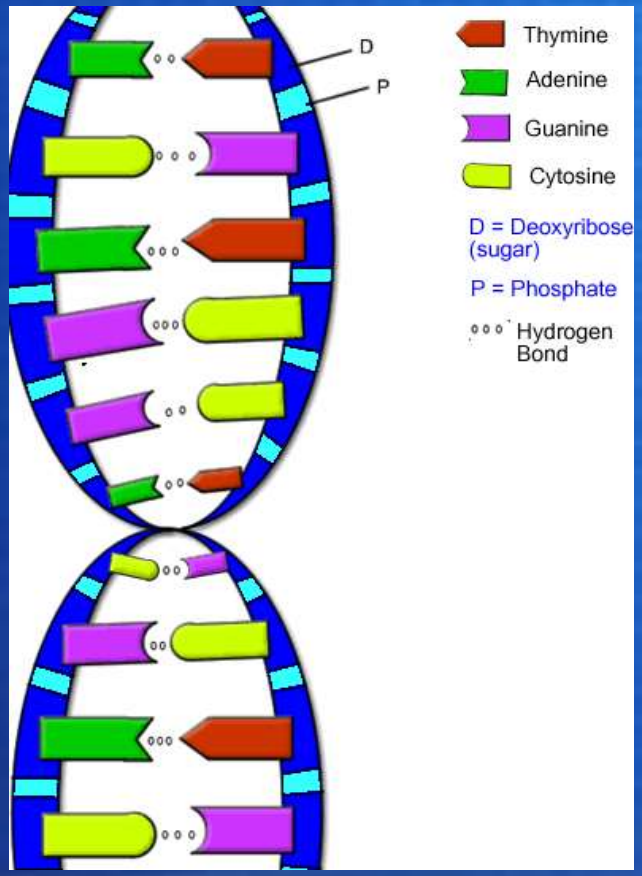
Why do people want to obtain genomic DNA ?

There is a number of reasons.

- For example, this DNA could be used to clone new genes or look for special regions of interest.

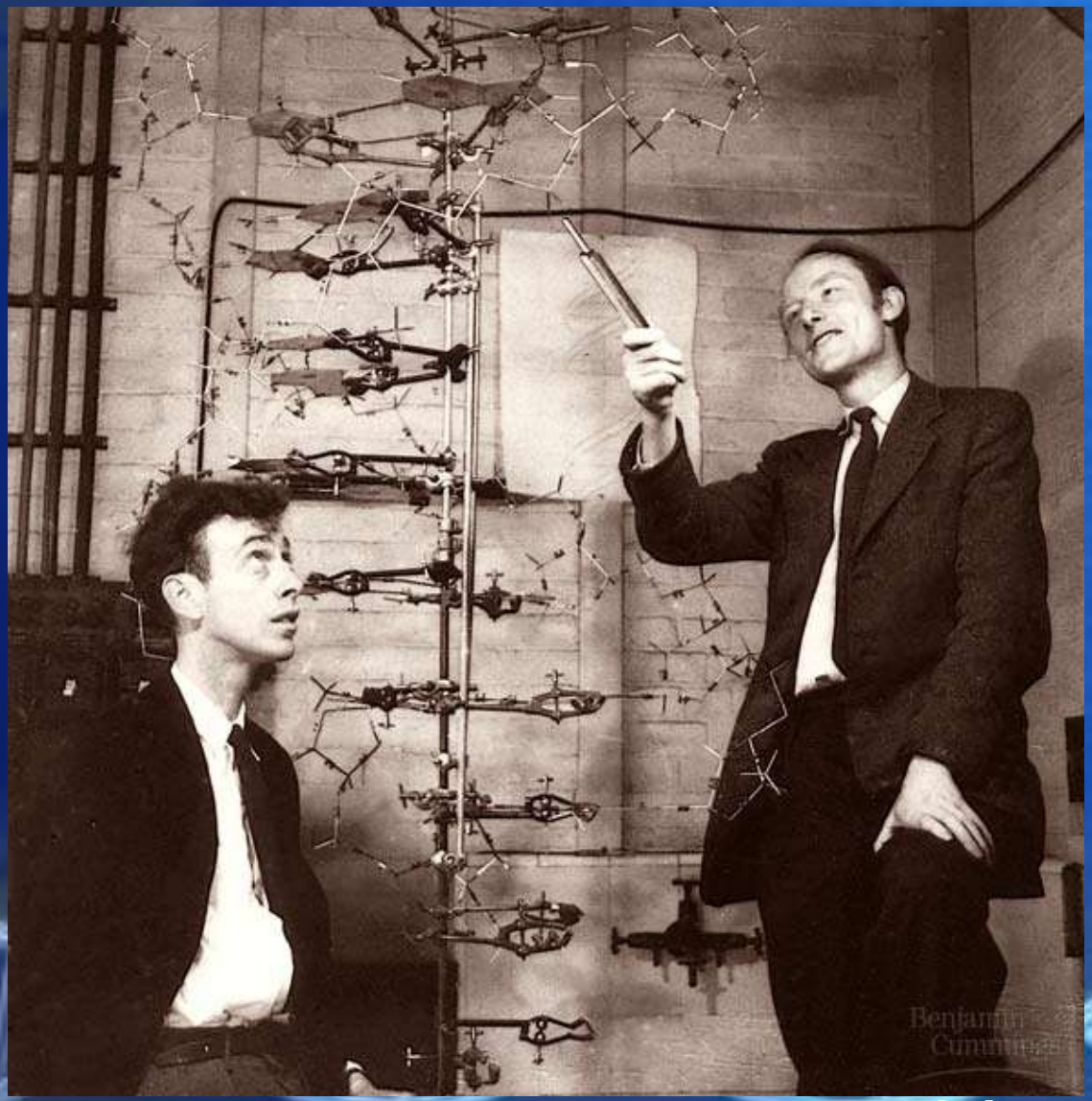
- You could also obtain a number of different genomic DNA and make comparisons with them to identify genetic diseases.

DNA Double Helix

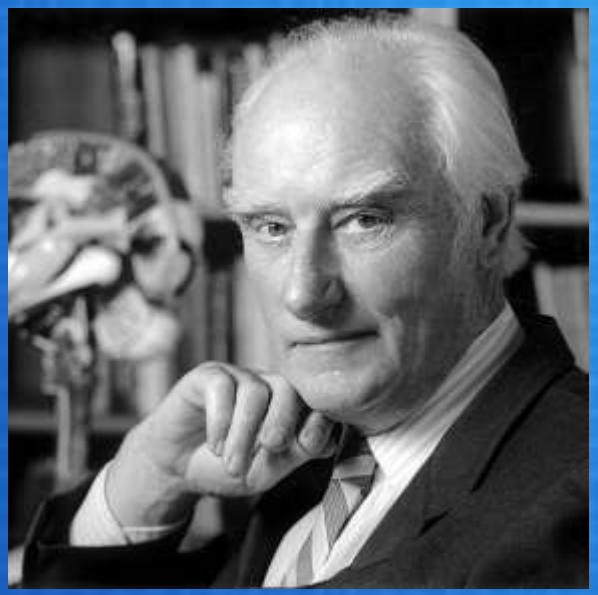


Watson and Crick

1953 article in Nature



Watson



Crick

The Roles of Nucleic Acids in heredity :

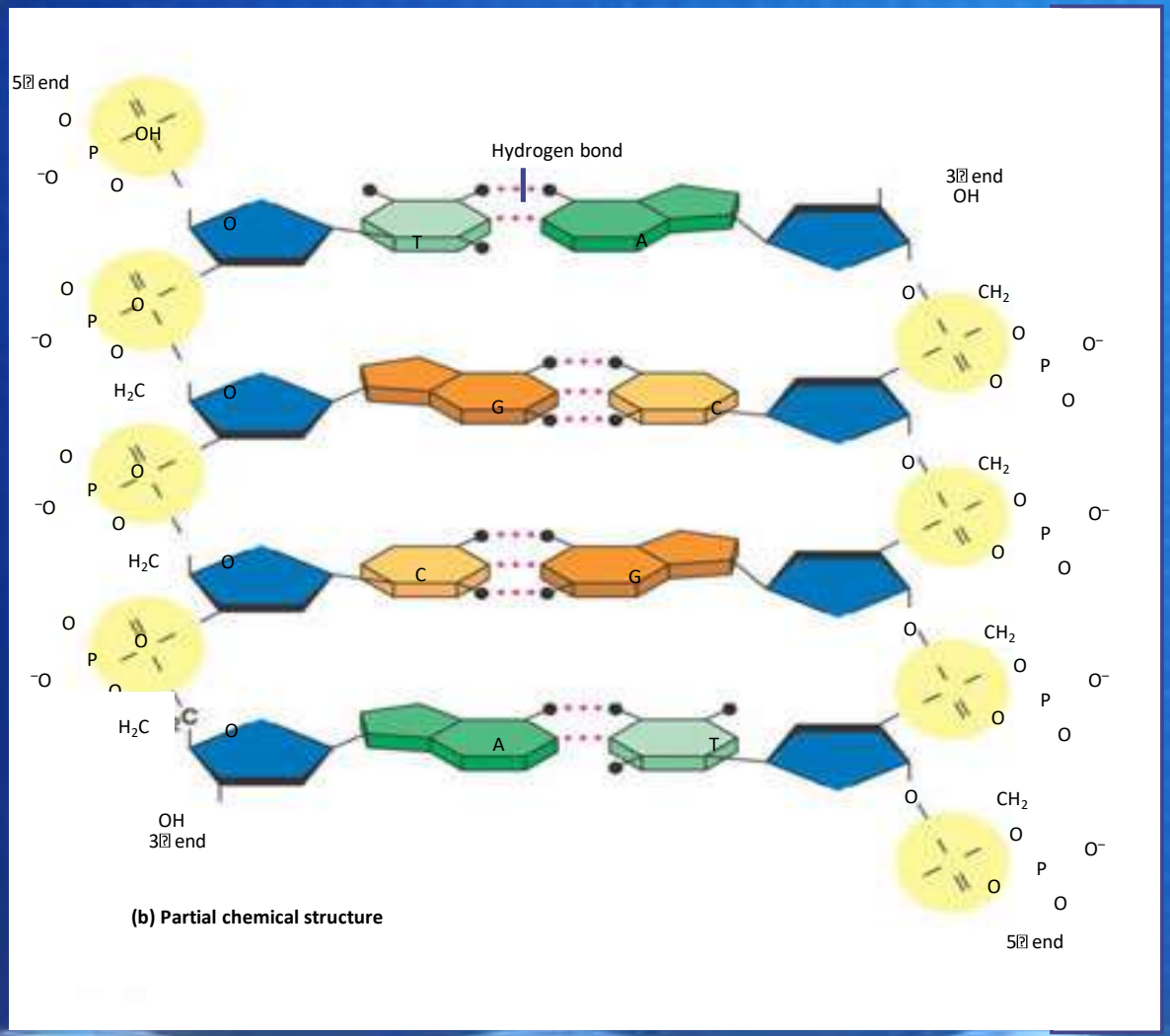
- There are two types of nucleic acids: Deoxyribo Nucleic Acid (DNA) and Ribo Nucleic Acid (RNA).
- DNA provides direction for its own replication.
- DNA also directs RNA synthesis and, through RNA, controls protein synthesis.

Organisms inherit DNA from their parents:

- When a cell divides, its DNA is copied and passed to the next generation of cells.

DNA Structure

- Prior to the 1950s, it was already known that DNA is a polymer of nucleotides, each consisting of three components:
  - a 5-carbon sugar called deoxyribose
  - a phosphate group (PO_4)
  - a nitrogenous base
    - adenine, thymine, cytosine, guanine


5′ end
Hydrogen bond
3′ end
OH
T
A
G
C
C
G
A
T
OH
3′ end
5′ end
(b) Partial chemical structure

- **Watson and Crick reasoned that there must be additional specificity of pairing**
- **Each base pair forms a different number of hydrogen bonds**
  - **Adenine (A) and thymine (T) form two bonds, cytosine (C) and guanine (G) form three bonds**

Figure 4.6 2-Deoxyribose is the sugar in DNA and ribose is the sugar in RNA. The carbon atoms are numbered as indicated for deoxyribose. The sugar is connected to the nitrogenous base via position 1'.

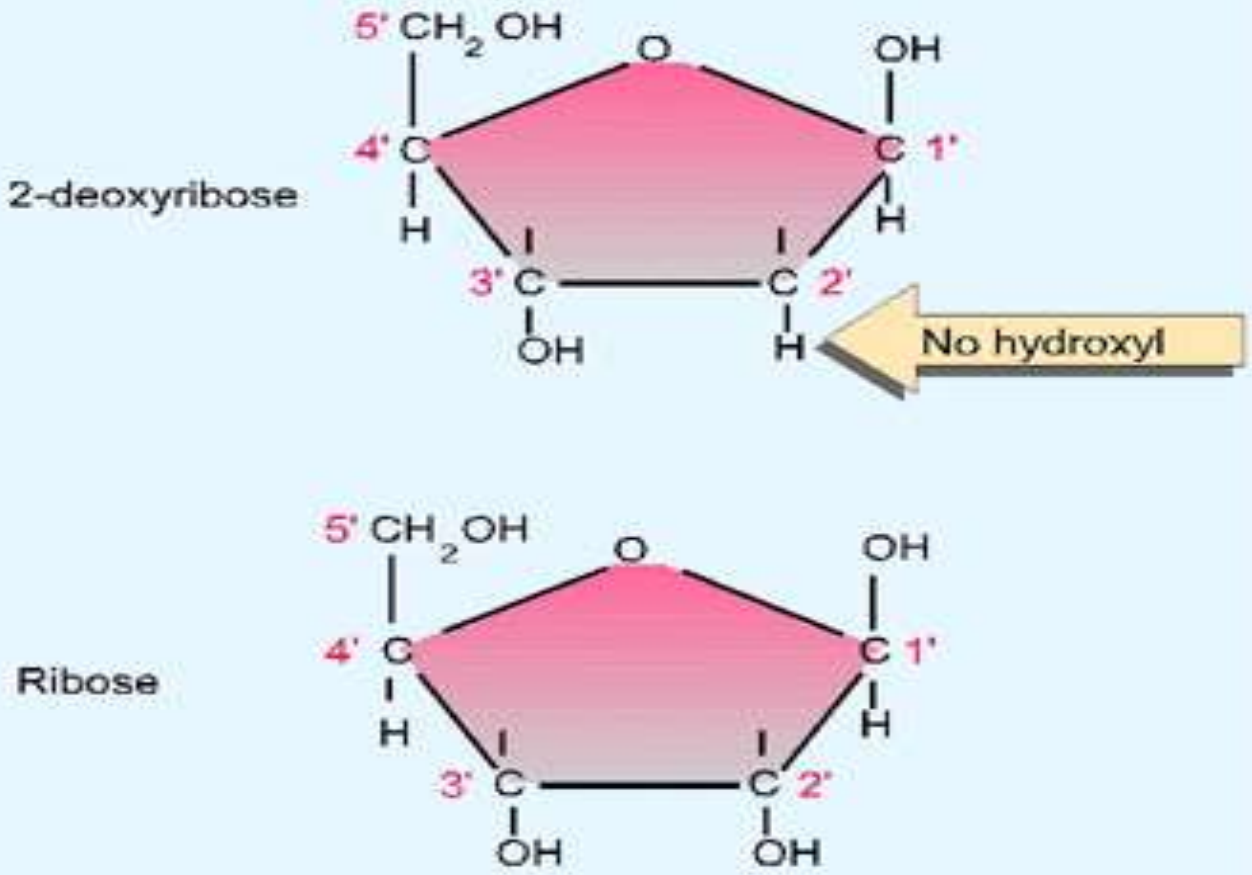
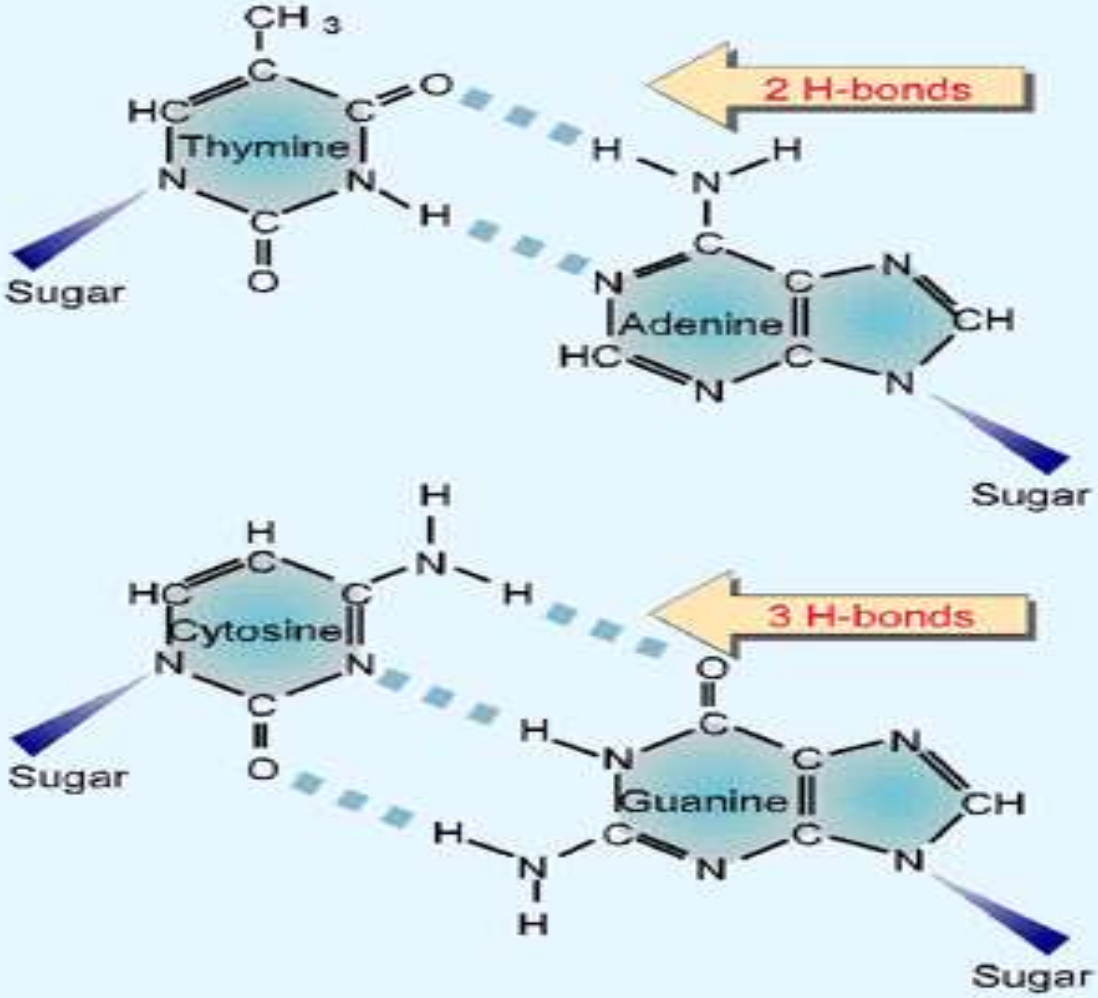


Figure 4.11 Complementary base pairing involves the formation of two hydrogen bonds between A and T, and of three hydrogen bonds between G and C. No other pairs form in DNA.



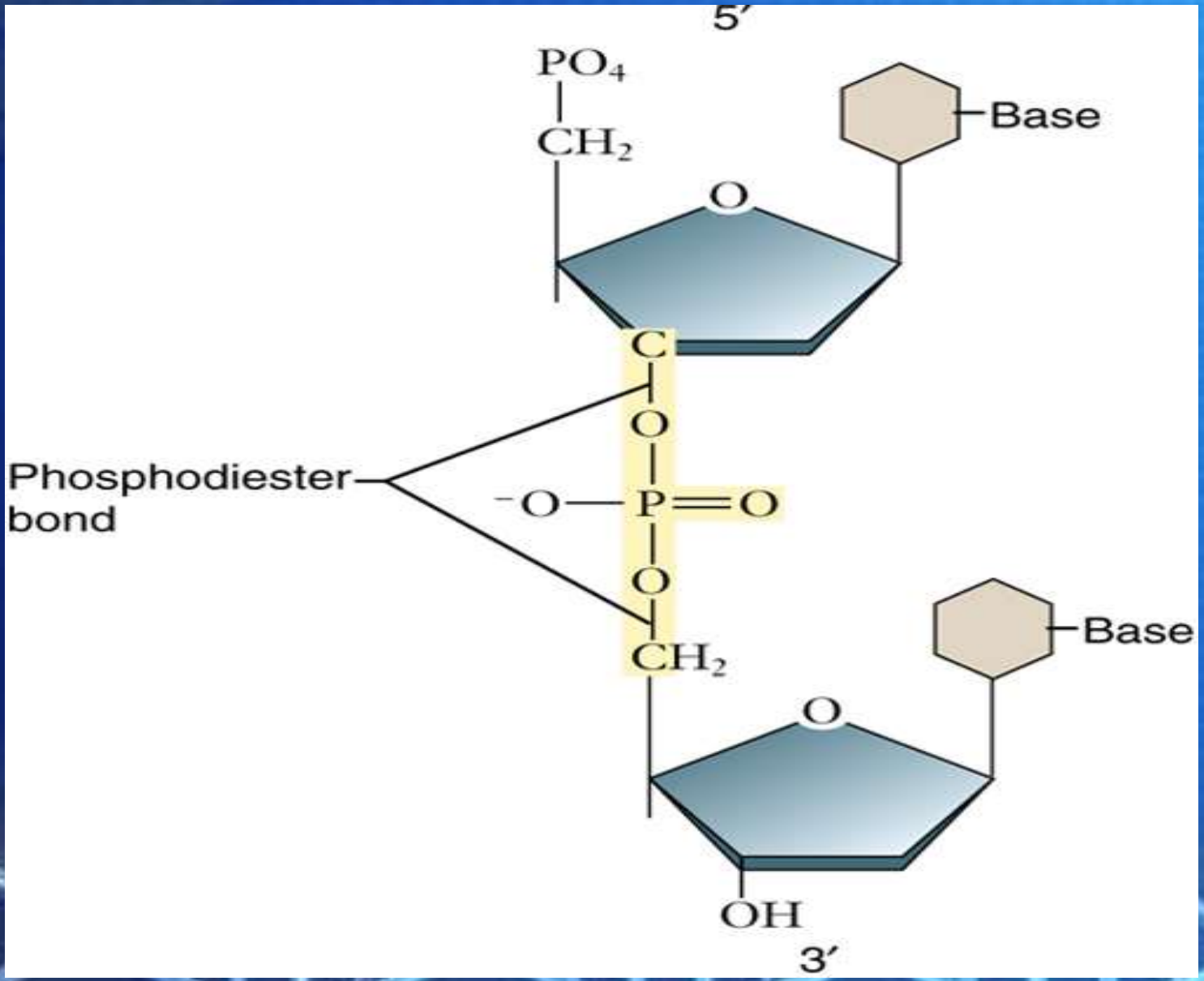
DNA Structure

Nucleotides are connected to each other to form a long chain

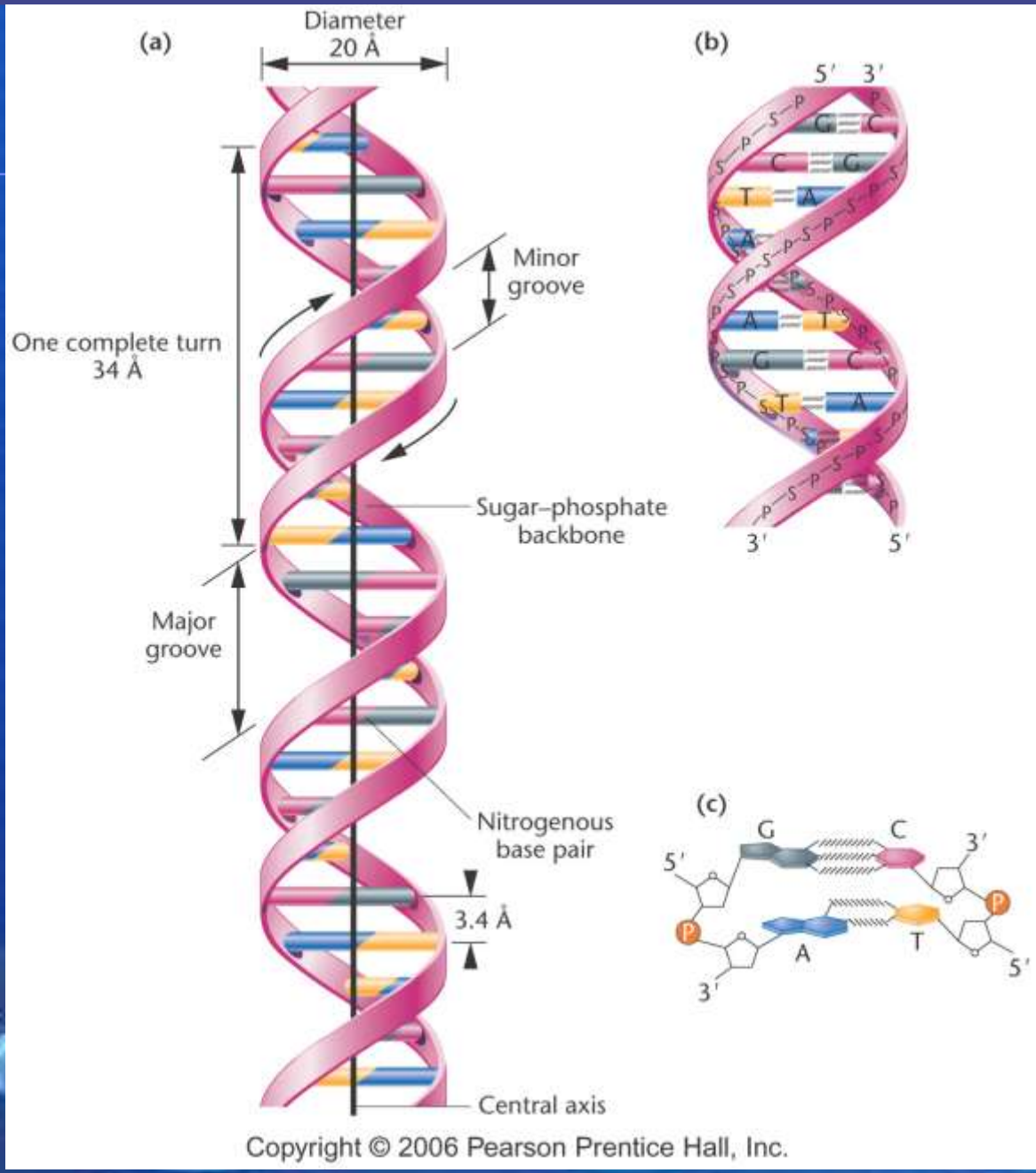
phosphodiester bond: bond between adjacent nucleotides

- **formed between the phosphate group of one nucleotide and the 3' –OH of the next nucleotide**

The chain of nucleotides has a 5' to 3' orientation.


5′
PO4
CH2
Base
O
C
O
Phosphodiester
bond
⁻O—P=O
O
Base
CH2
O
OH
3′

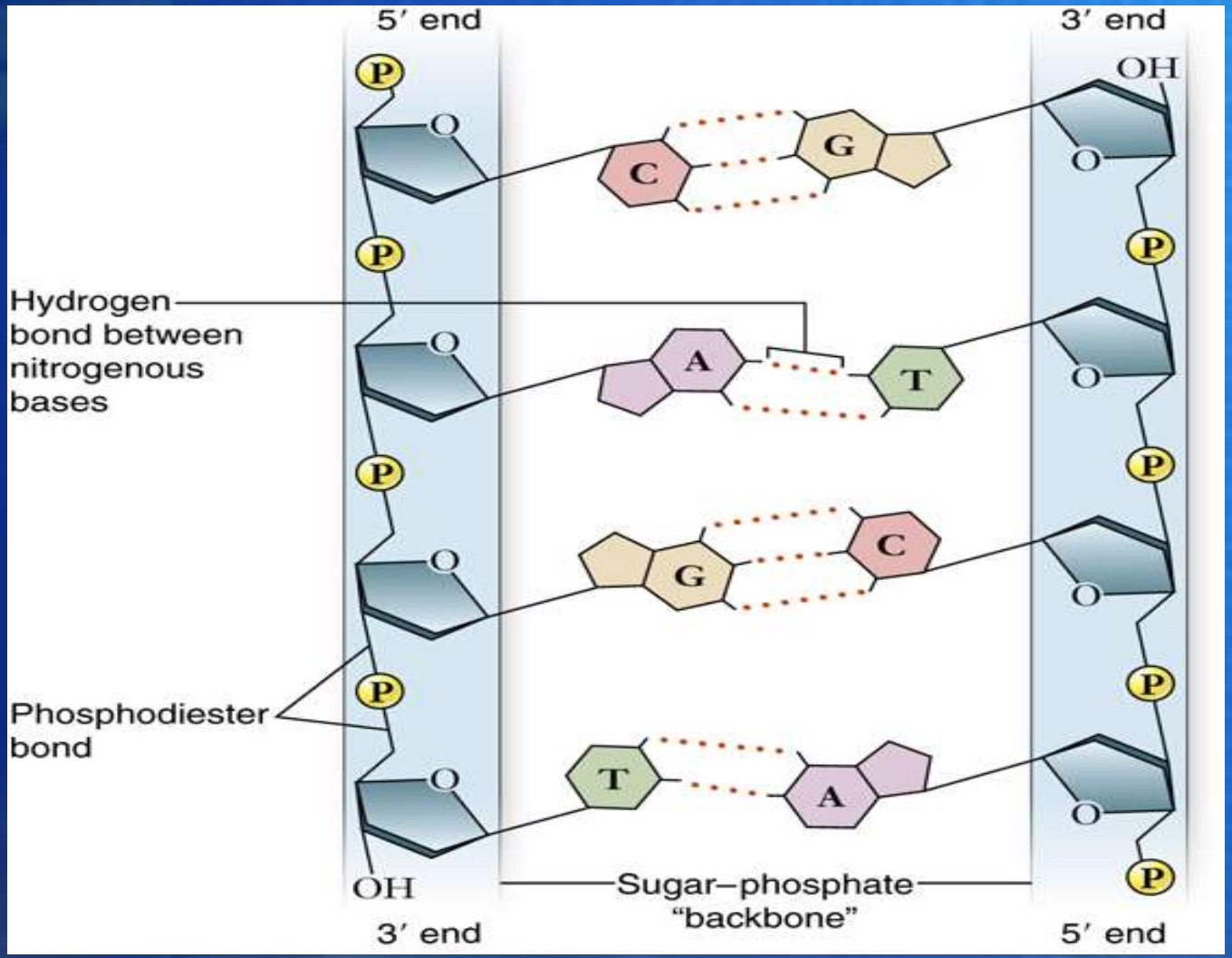
DNA Double Helix



DNA Structure

The double helix consists of:

- 2 sugar-phosphate backbones
- nitrogenous bases toward the interior of the molecule
- bases form hydrogen bonds with **complementary bases** on the opposite sugar-phosphate backbone
- G binds C by three hydrogen bonds
- A binds T by two hydrogen bonds


5′ end
3′ end
OH
P
O
C
G
A
T
Hydrogen bond between nitrogenous bases
Phosphodiester bond
OH
3′ end
Sugar–phosphate "backbone"
5′ end

- General tips for conducting a safe and successful experiment.
- 1. Always keep the work area clean of any unwanted tubes, beakers and dirty dishes.
- 2. All reagents should be marked clearly with reagent name and concentration.
- 3. All samples should be numbered and labeled correctly with the names and dates.
- 4. Make sure that after use the reagents and chemical are placed in the fridge or freezer as required.
- 5. In bacterial cultures make sure the reagents and dishes are autoclaved properly and label using autoclave taps.
- 6. Always mark the bottom of the bacterial culture dishes and not the lid, as the lids can easily be mixed up.

DNA extraction protocol

- There are different protocols and several commercially available kits that can be used for the extraction of DNA from whole blood. This procedure is one routinely used both in research and clinical service provision and is cheap and robust. It can also be applied to cell pellets from dispersed tissues or cell cultures.

Theory

- Successful nucleic acid isolation protocols have been published for nearly all biological materials. They involve the physical and chemical processes of tissue homogenisation, cell permeabilisation, cell lysis, protein degradation and removal of nucleases, protein precipitation, solubilisation of nucleic acids and finally various washing steps.
- Cell permeabilisation may be achieved with the help of non-ionic (non DNA-binding) detergents such as SDS and Triton.

Why do people want to obtain genomic DNA ?

There is a number of reasons.

- For example, this DNA could be used to clone new genes or look for special regions of interest.

- You could also obtain a number of different genomic DNA and make comparisons with them to identify genetic diseases.

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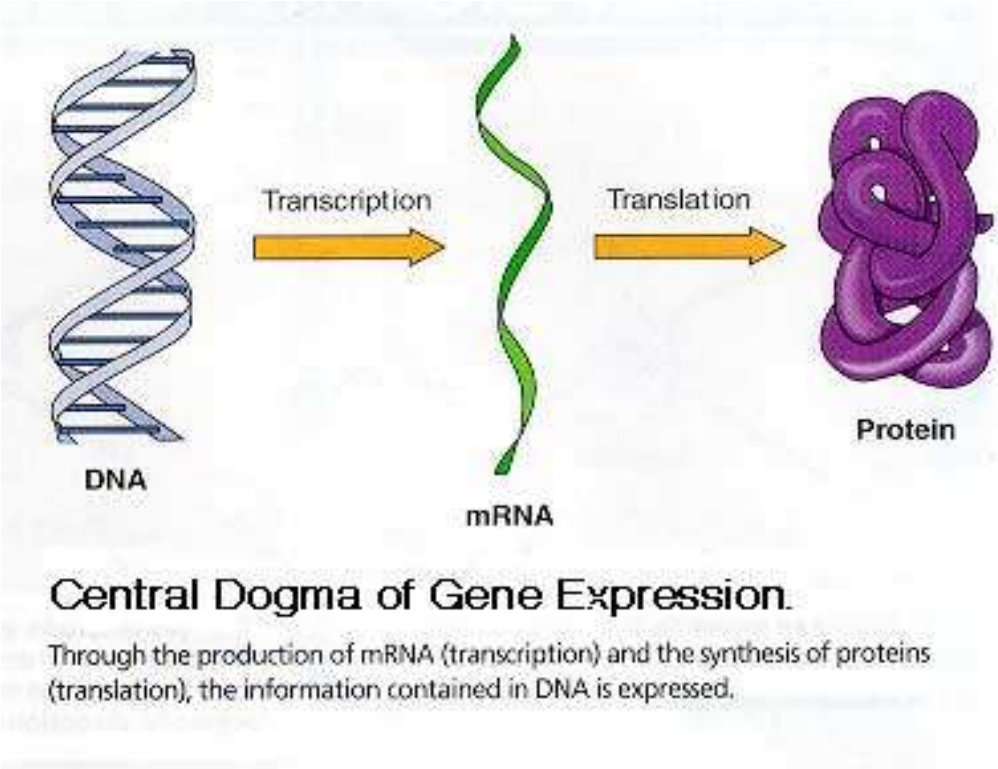
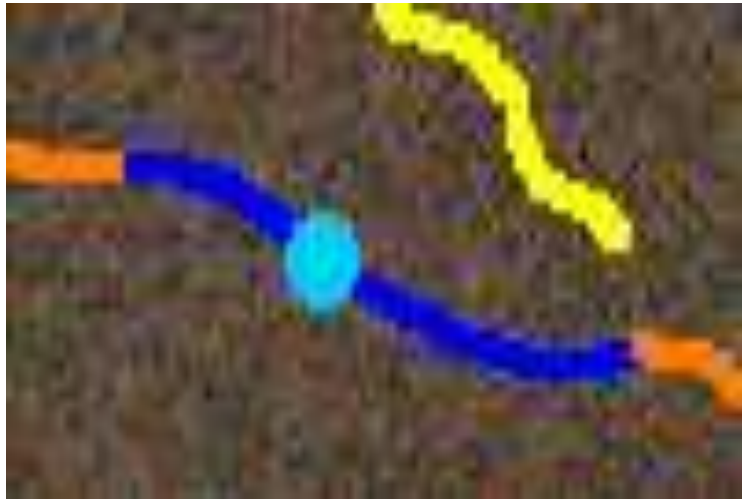
Transcription



Central Dogma of Gene Expression.

Through the production of mRNA (transcription) and the synthesis of proteins (translation), the information contained in DNA is expressed.



RNA (Ribonucleic acid)

Structure: Similar to that of DNA except:

1- it is single stranded polunucleotide chain.

2- Sugar is ribose

3- Uracil is instead of thymine

There are 3 types of RNA:

1- Ribosomal RNA (rRNA)

2- Messenger RNA (mRNA)

3- Transfer RNA (tRNA)

RNA are copies from DNA sequences formed by a process called " transcription". After transcription some modifications occur to obtain the three types of RNA.

Types of cellular RNA molecules

TABLE 5.2 RNA molecules in *E. coli*

Type	Relative amount (%)	Sedimentation coefficient (S)	Mass (kd)	Number of nucleotides
Ribosomal RNA (rRNA)	80	23	1.2×10^3	3700
		16	0.55×10^3	1700
		5	3.6×10^1	120
Transfer RNA (tRNA)	15	4	2.5×10^1	75
Messenger RNA (mRNA)	5		Heterogeneous	

- **All RNA in *E. coli* (a bacterium) is made by one RNA polymerase**
- **mRNA encodes proteins**

Types of RNA

There are three main types of RNA

1-messenger RNA

mRNA is transcribed from the DNA template and carries the coding informations to the site of protein synthesis .

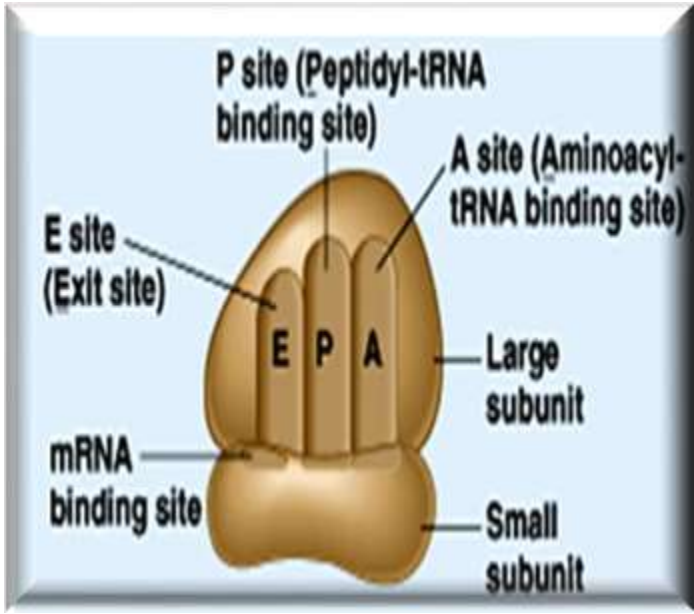
It is the RNA which helps to carry the informations produced by the DNA during protein synthesis in all higher organisms such as humans, animals etc

1- Ribosomal RNA (rRNA):

- 80 % of total RNA in the cells are rRNA.

- rRNA are found in combination with several proteins (about 82 proteins) as component of the ribosome
Which is the site of protein synthesis.

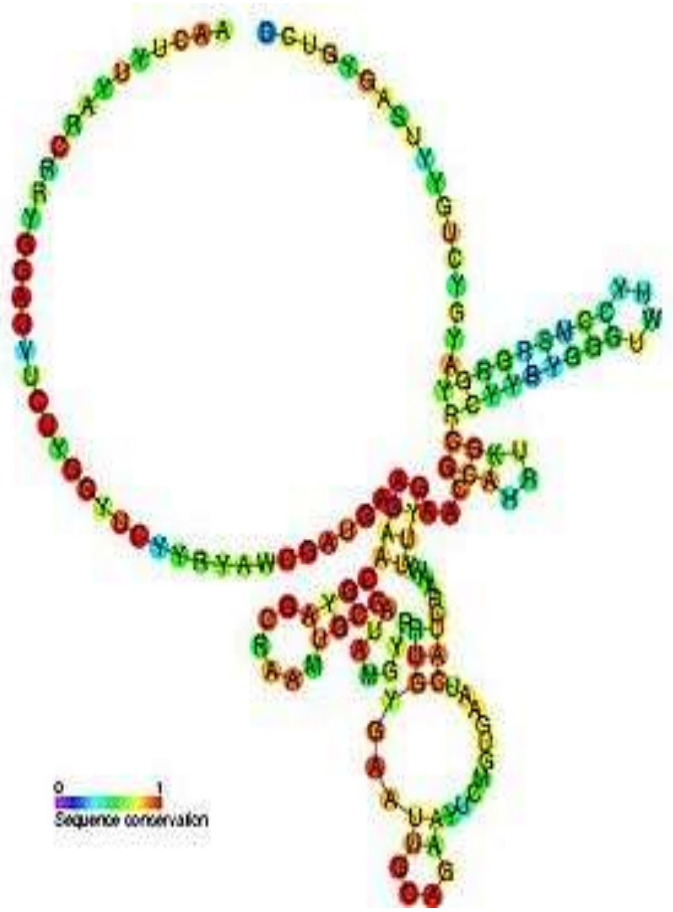
- In Eucaryotic (mammals). There are 4 size types of rRNA (5S, 5.8S, 18Ss and 28S)



Schematic model of Ribosome

- **rRNA** :- **(Ribosomal RNA)** is the central component of ribosomes. The important function of rRNA is to decode the mRNA to form amino acids and then relate to tRNA during translation

- It catalysis the ribosomal activity during translation phase of protein synthesis such as humans , animals etc .



3- Transfer RNA (tRNA):

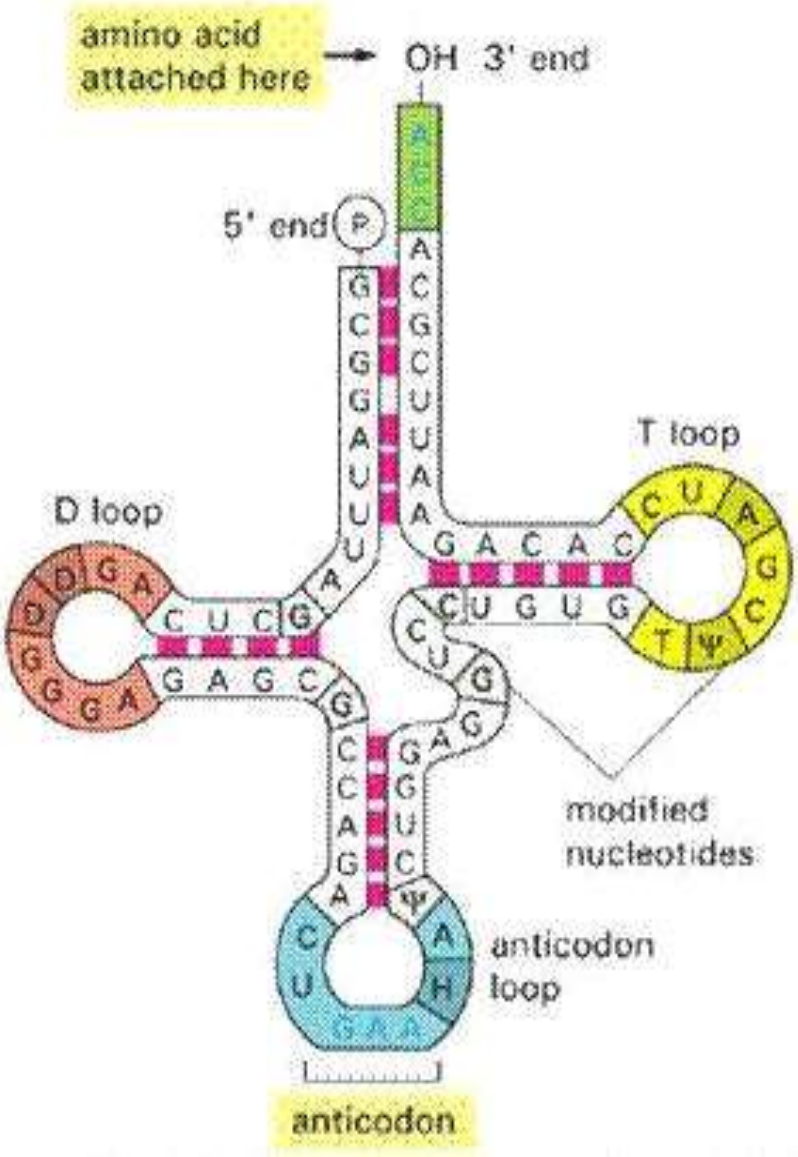
tRNA represents 15% of total RNA in the cell.

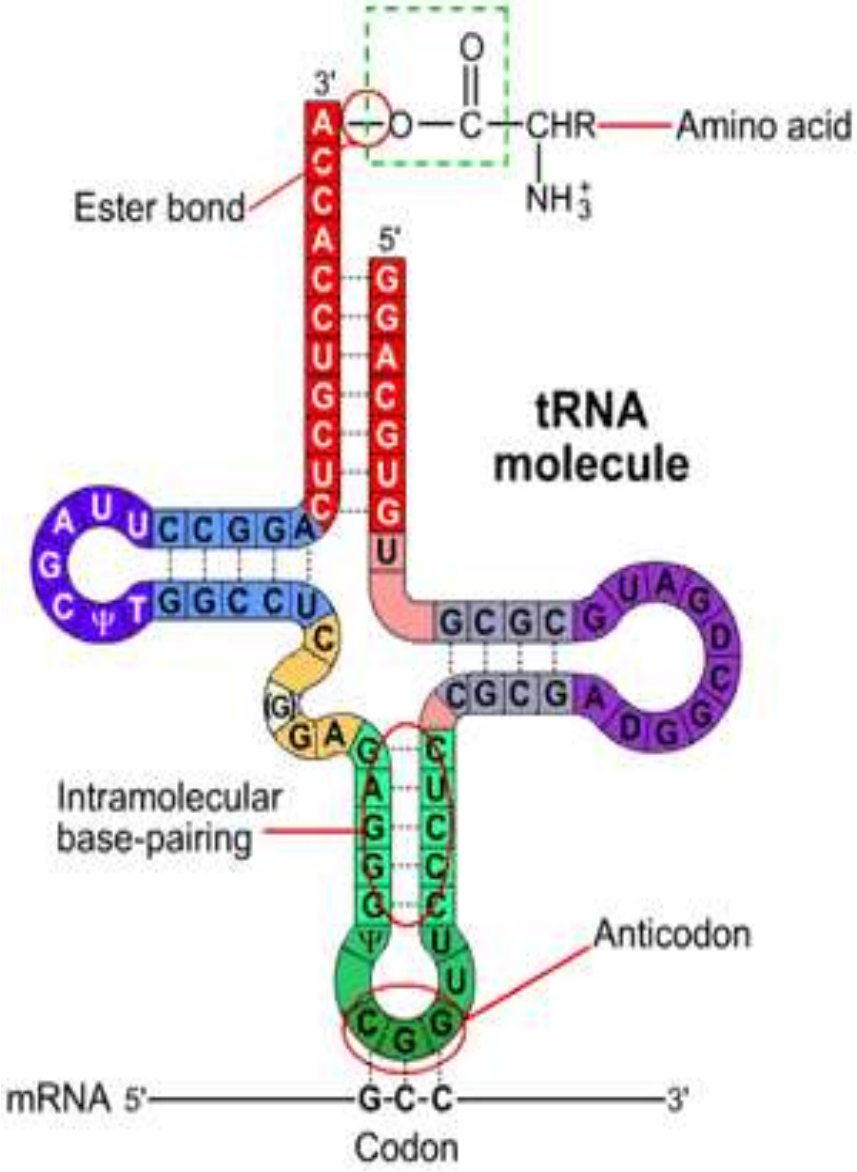
Structure:

1- Amino acid attachment site or amino acid acceptor: which terminates with the triplet CCA.

2- Anticodon loop or anticodon triplet

3- D loop and T loop: contain unusual bases e.g. dihydrouracil, ribothymidine or methyl guanine

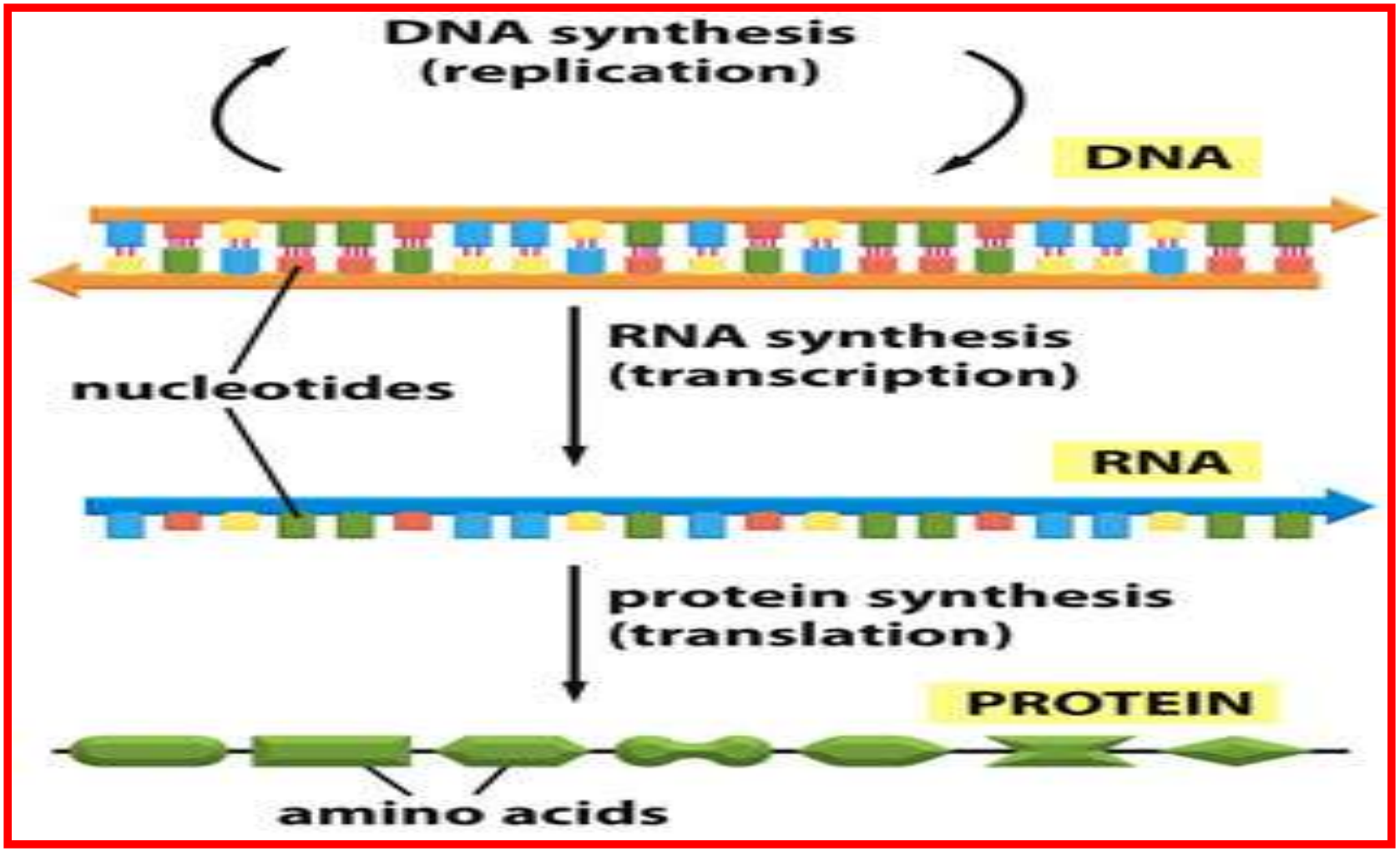




Structure of tRNA

Funtions of tRNA

RNA Synthesis



DNA & RNA

Purification

Quantitation

DNA Purification Requirements

- Many applications require purified DNA.
- Purity and amount of DNA required (and process used) depends on intended application.
- Example applications:

➢ Tissue typing for organ transplant

➢ Detection of pathogens

➢ Human identity testing

➢ Genetic research

DNA Purification Challenges

1. Separating DNA from other cellular components such as proteins, lipids, RNA, etc.
2. Avoiding fragmentation of the long DNA molecules by mechanical shearing or the action of endogenous nucleases.

   Effectively inactivating endogenous nucleases (DNase enzymes) and preventing them from digesting the genomic DNA is a key early step in the purification process. DNases can usually be inactivated by use of heat or chelating agents.

Quality is Important

- Best yields are obtained from fresh or frozen materials.
- Blood/Tissues must be processed correctly to minimize destruction of DNA by endogenous nucleases.
- DNA yield will be reduced if endogenous nucleases are active.
- Prompt freezing, immediate processing or treatment with chelating agents (such as EDTA) minimizes nuclease effects.

Nucleic Acid Purification

There are many DNA purification methods. All must:

1. Effectively disrupt cells or tissues
   (usually using detergent)
2. Denature proteins and nucleoprotein complexes
   (a protease/denaturant)
3. Inactivate endogenous nucleases
   (chelating agents)
4. Purify nucleic acid target away from other nucleic acids and protein
   (could involve RNases, proteases, selective matrix and alcohol precipitations)

Disruption of Cells/Tissues

Most purification methods disrupt cells using lysis buffer containing:

- **Detergent** to disrupt the lipid bilayer of the cell membrane
- **Denaturants** to release chromosomal DNA and denature proteins

Additional enzymes are required for lysis of some cell types:

- Gram-positive bacteria require lysozyme to disrupt the bacterial cell wall.
- Yeasts require addition of lyticase to disrupt the cell wall.
- Plant cells may require cellulase pre-treatment.

Inactivation of Nucleases

- Chelating agents, such as EDTA, sequester Mg^{2+} required for nuclease activity.
- Proteinase K digests and destroys all proteins, including nucleases.

- Some commercial purification systems provide a single solution for cell lysis, protein digestion/denaturation and nuclease inactivation.

Removal of RNA

- Some procedures incorporate RNase digestion during cell lysate preparation.
- In other procedures, RNase digestion is incorporated during wash steps.

Separation of DNA from Crude Lysate

DNA must be separated from proteins and cellular debris.

Separation Methods

- Organic extraction
- Salting out
- Selective DNA binding to a solid support

Separation by Organic Extraction

- DNA is polar and therefore insoluble in organic solvents.
- Traditionally, phenol:chloroform is used to extract DNA.
- When phenol is mixed with the cell lysate, two phases form. DNA partitions to the (upper) aqueous phase, denatured proteins partition to the (lower) organic phase.

- DNA is a polar molecule because of the negatively charged phosphate backbone.
- This polarity makes it more soluble in the polar aqueous phase.

Separation by Salting Out

- Salts associate with charged groups.
- At high salt concentration, proteins are dehydrated, lose solubility and precipitate. Usually sodium chloride, potassium acetate or ammonium acetate are used.
- Precipitated proteins are removed by centrifugation.
- DNA remains in the supernatant.

Ethanol Precipitation of DNA

- Methods using organic extraction or salting-out techniques result in an aqueous solution containing DNA.
- The DNA is precipitated out of this solution using salt and isopropanol or ethanol.
- Salt neutralizes the charges on the phosphate groups in the DNA backbone.
- The alcohol (having a lower dielectric constant than water) allows the sodium ions from the salt to interact with the negatively charged phosphate groups closely enough to neutralize them and let the DNA fall out of solution.

Separation by Binding to a Solid Support

Most modern DNA purification methods are based on purification of DNA from crude cell lysates by selective binding to a support material.

Support Materials

- Silica
- Anion-exchange resin

Advantages

- Speed and convenience
- No organic solvents
- Amenable to automation/miniaturization

DNA purification column containing a silica membrane

Silica

- DNA binds selectively to silica in the presence of high concentrations of chaotropic salts (e.g., guanidinium HCl).
- Protein does not bind under these conditions.
- Silica membranes or columns are washed with an alcohol-based solution to remove the salts.
- DNA is eluted from the membrane with a low-ionic-strength solution, such as a low-salt buffer or water.

Advantages

- Fast purification
- Amenable to automation
- No centrifugation required (can use vacuum)
- No organic solvents or precipitation steps

DNA Quantitation

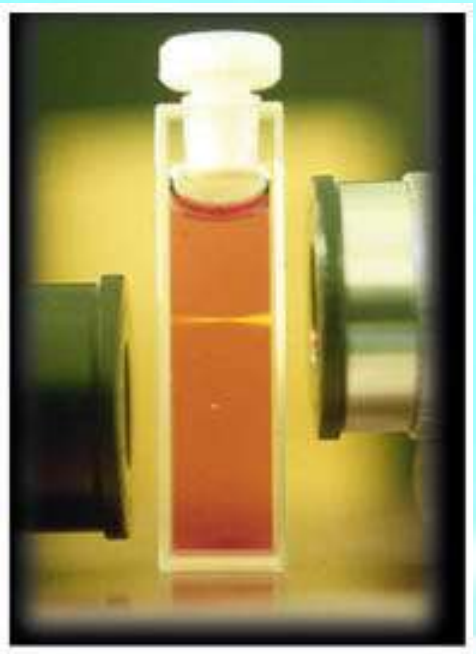
Once DNA is purified, it is usually quantified. Typical quantities are in the milligram-picogram range

- gram (g)
- milligram (mg) = 10^{-3} g; 0.001g
- microgram (µg) = 10^{-3} mg; 0.000001g
- nanogram (ng) = 10^{-3} µg; 10^{-6} mg; 0.000000001g
- picogram (pg) = 10^{-3} ng; 10^{-6} µg; 10^{-9} mg; 0.000000000001g

Quantification Methods

Spectrophotometry: Use of light absorbance to measure concentration. Many biological substances absorb light. The spectrophometer measures absorbance of light at specific wavelengths

- Most commonly used method
- DNA concentration can be calculated absorbance at 260 nm:
  $A = \varepsilon \times c \times l$ (Beer-Lambert Law)
  A = absorbance
  ε = extinction coefficient
  c = concentration
  l = path length



Summary

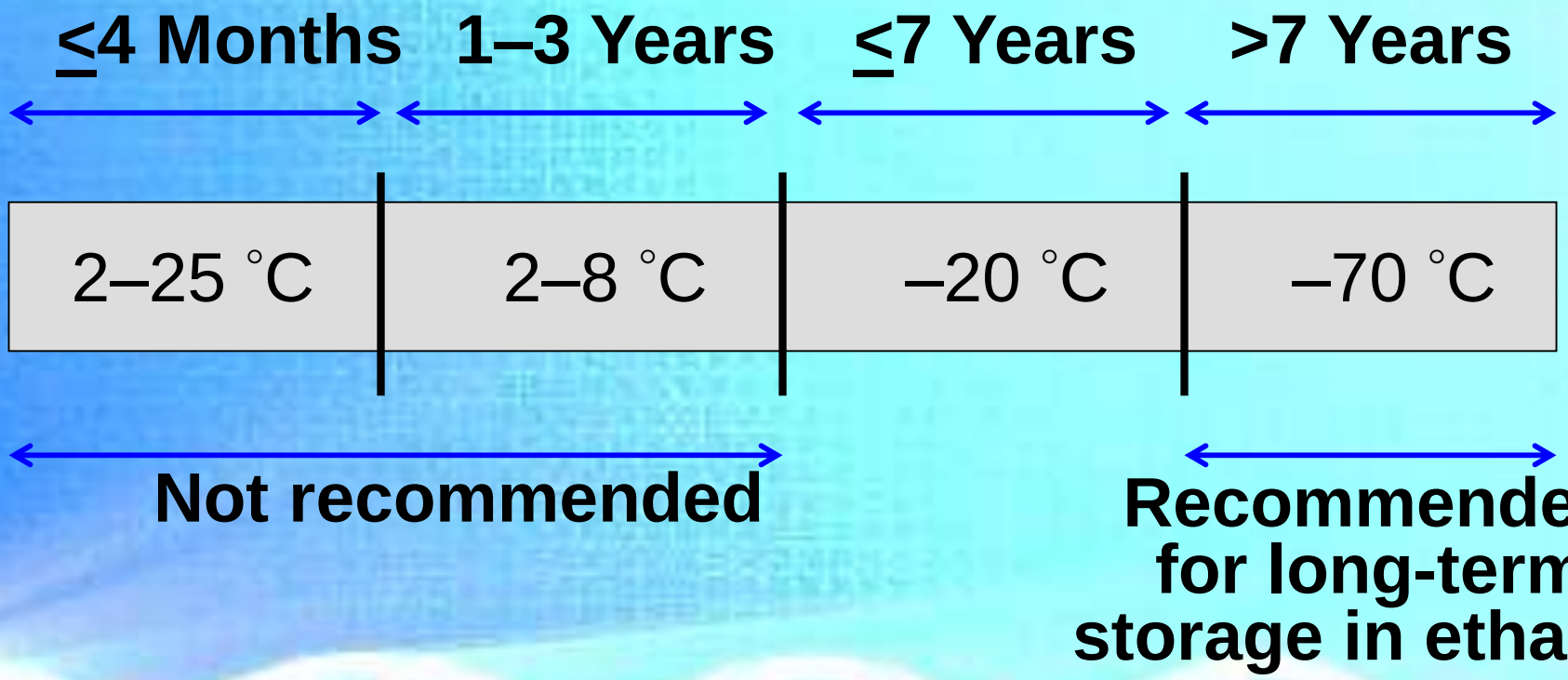
DNA purification methods all do the following:

- Disrupt cells and denature/digest of proteins
- Separate DNA from proteins, RNA and other cellular components
- Prepare a purified DNA solution

Older methods relied on laborious organic extraction and precipitation procedures.

Newer methods are faster, using selective binding of DNA to silica or magnetic beads, and are amenable to automation and miniaturization.

Nucleic Acid Storage Requirements: Storage of DNA Specimens



Quantity from UV Spectrophotometry

- DNA and RNA absorb maximally at 260 nm.
- Proteins absorb at 280 nm.

Quality from UV Spectrophotometry

A_{260}/A_{280} = measure of purity

$(A_{260} - A_{320})/(A_{280} - A_{320})$

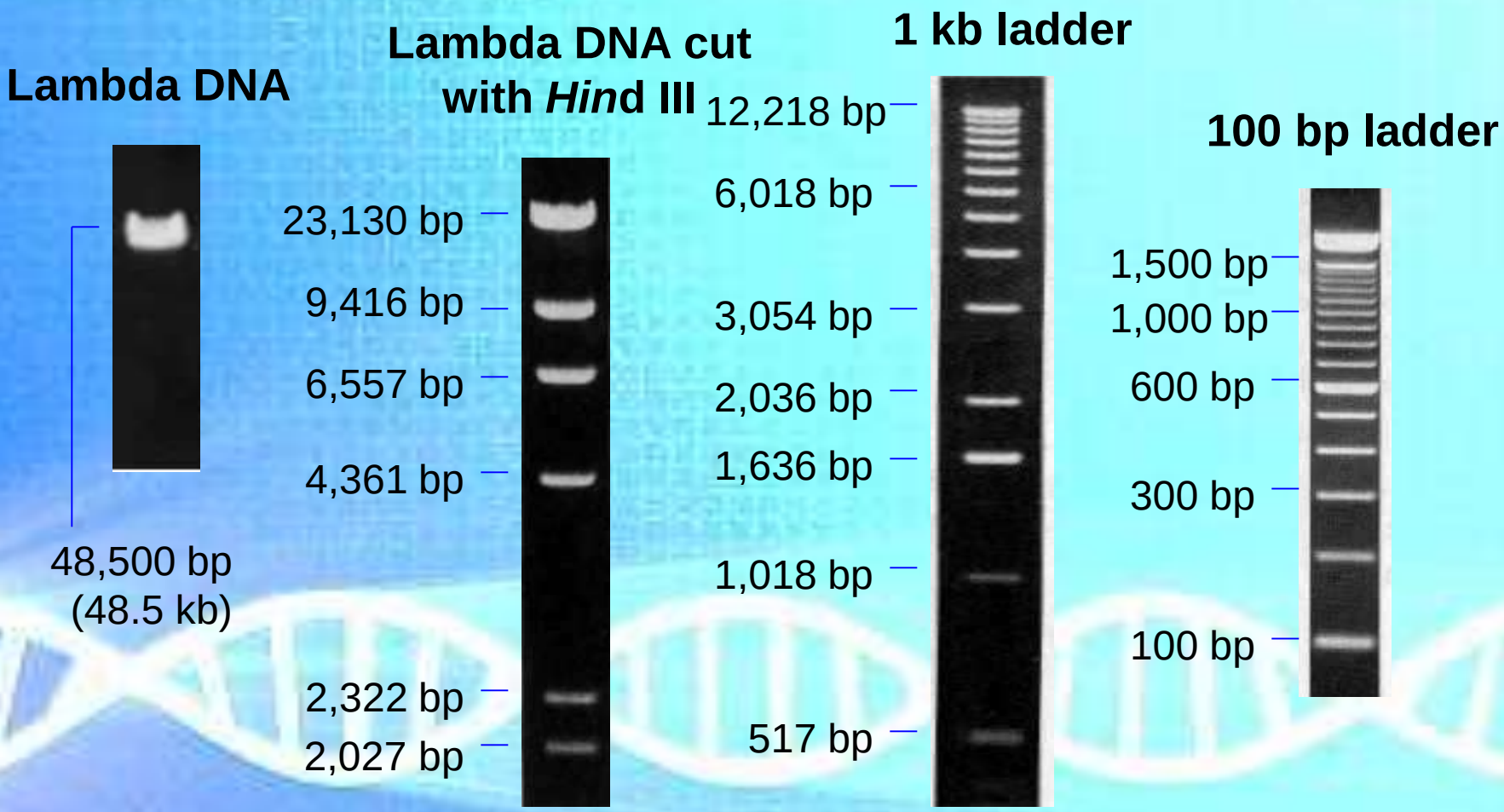
1.7 – 2.0 = good DNA or RNA

<1.7 = too much protein or other contaminant (?)

Quality from Agarose Gel Electrophoresis

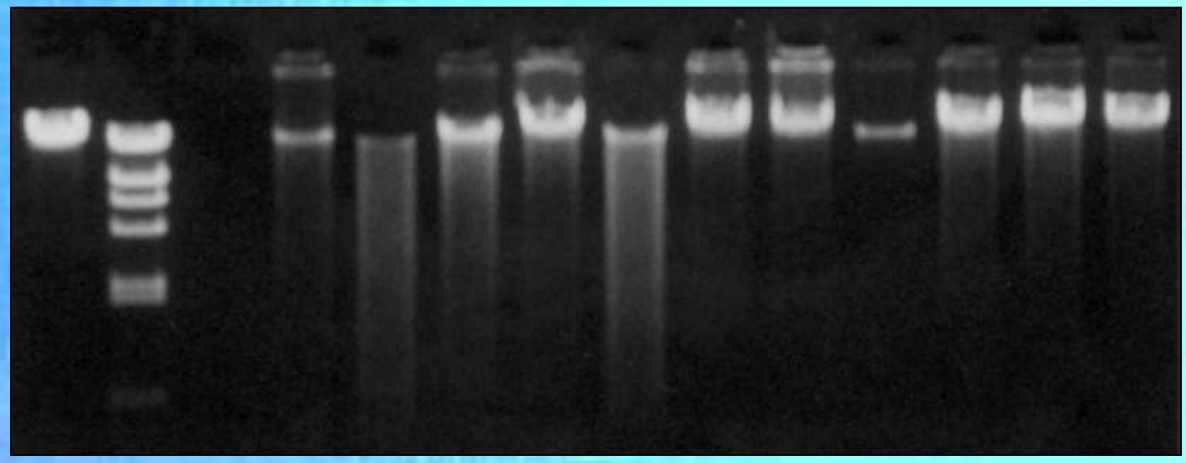
- Genomic DNA:
  - 0.6% to 1% gel, 0.125 µg/mL ethidium bromide in gel and/or in running buffer
  - Electrophorese at 70–80 volts, 45–90 minutes.
- Total RNA:
  - 1% to 2% gel, 0.125 µg/ml ethidium bromide in gel and/or in running buffer
  - Electrophorese at 80–100 volts, 20–40 minutes.

DNA Size from Agarose Gel Electrophoresis: Compares unknown DNA to known size standards



DNA Quality from Agarose Gel Electrophoresis

Human Whole Blood DNA

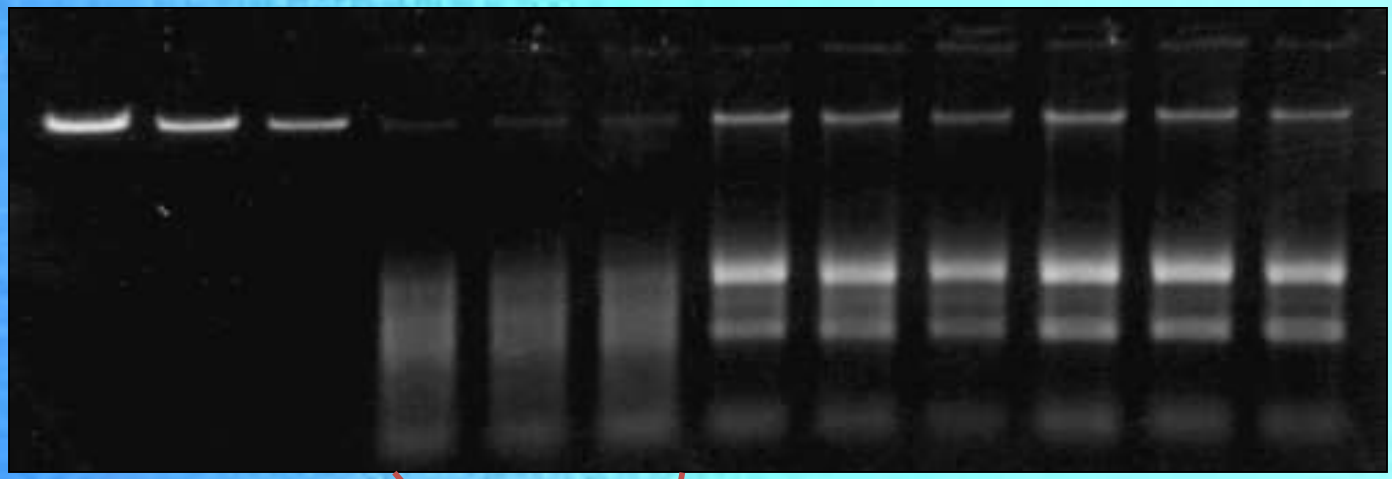


Lambda DNA marker

Lambda DNA cut with *Hin*d III marker

Whole blood genomic DNA

Cultured Cell DNA and RNA



DNA

28S

18S

100 50 25 ng
Genomic DNA markers

Degraded RNA

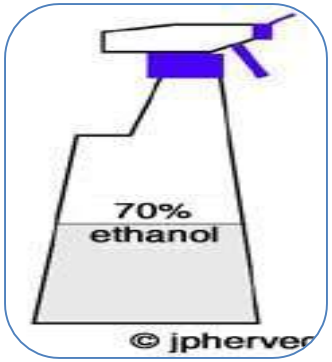
5S rRNA, tRNA, and other small RNA molecules

mRNA = background smear
high → low MW

Materials

Gloves

Cotton pad for working area



Ethanol 70% to sterilize the area

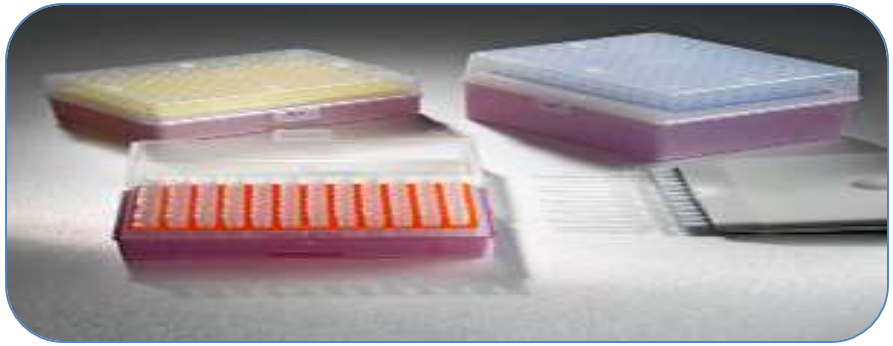
Lavender or purple top tube, it contains EDTA (the potassium salt, or K_2EDTA). This is a strong anticoagulant. Lavender top tubes are generally used when whole blood is needed for analysis.

Pasteur Pipette

Permanent Marker

Centrifuge Tube 50 ml

Rack



Pipettes and Tips

Biohazard Bags

Tissues

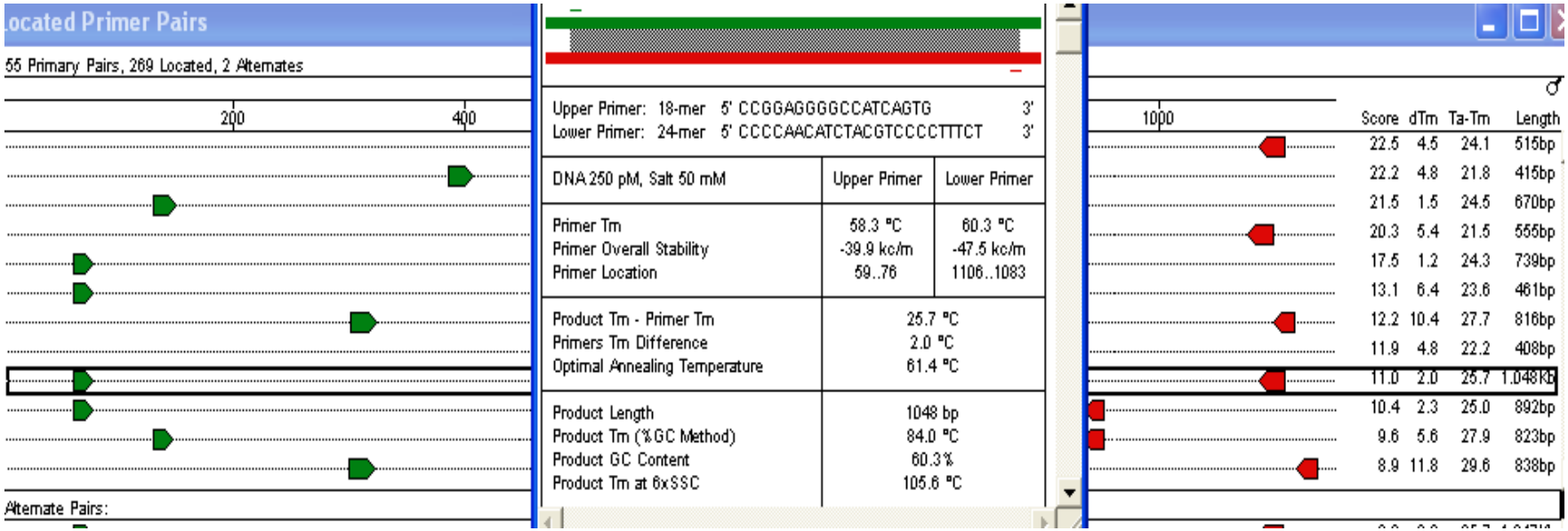
DNA Blood Kit- Qiagen

Machines

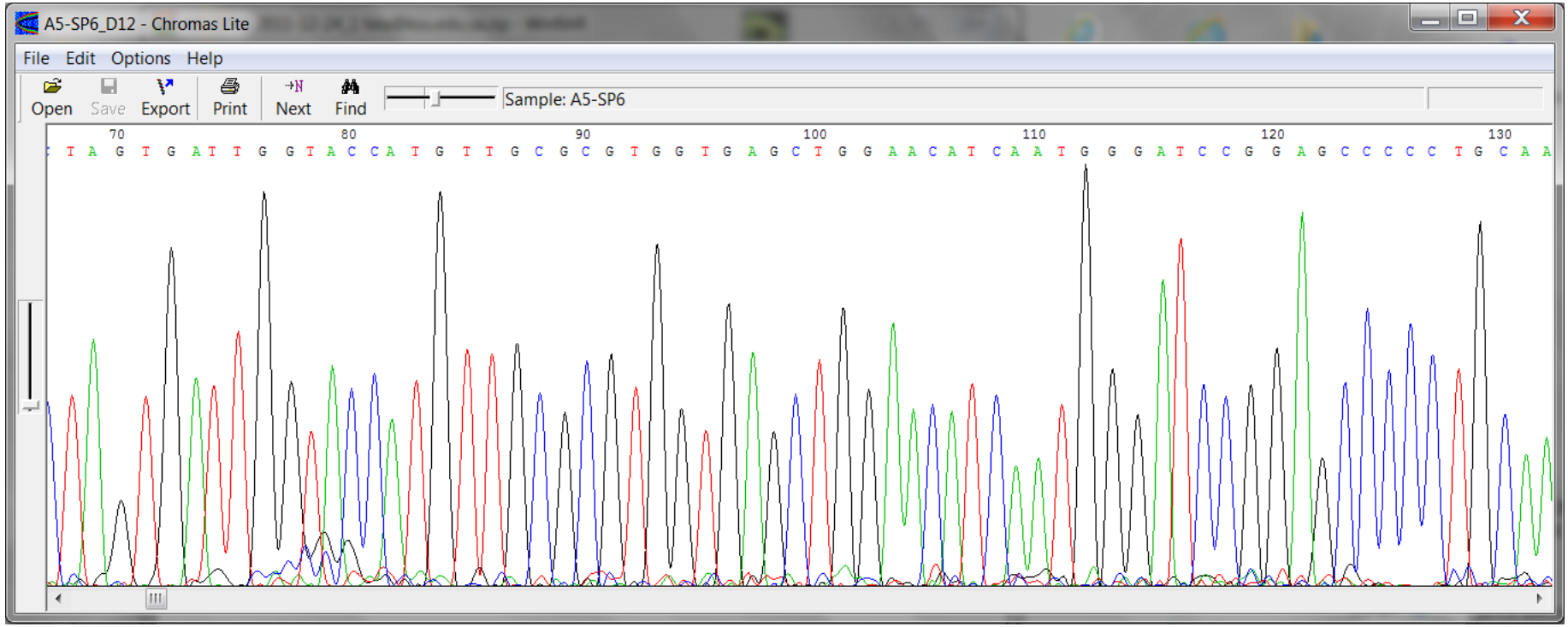
Centrifuge

Vortex

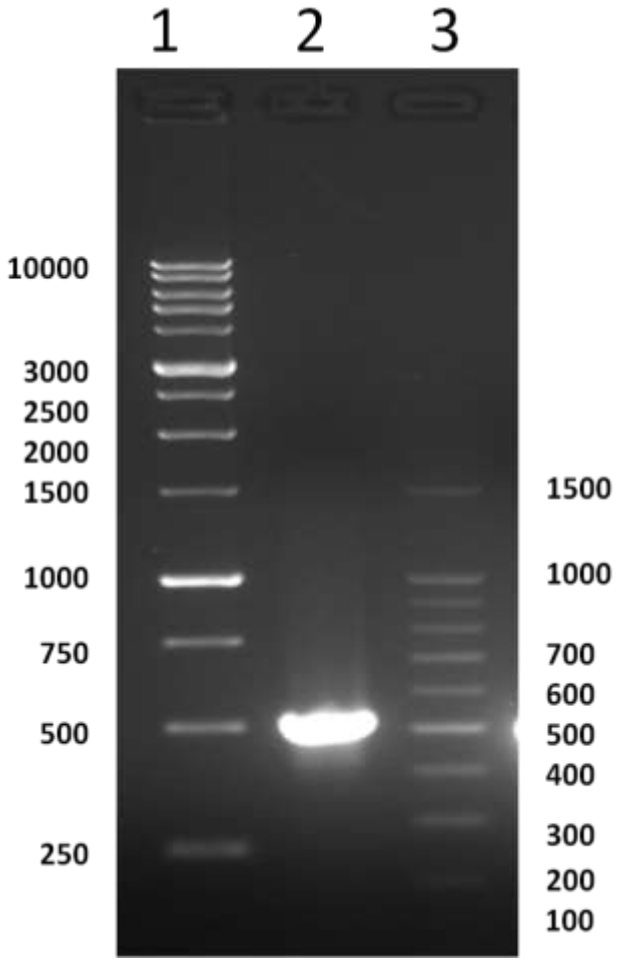
Shaker



Primer Select program of DNA-Star Package



Sequencing data using Chromas program

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1     ATG GCG TTG AAG GCT GTG TGC GTG CTG AAG GGC GAC GGC CAG GTT   45
1      M   A   L   K   A   V   C   V   L   K   G   D   G   Q   V   15

46    CAG GGC ACC ATC CAC TTC GAG CAG AAG GAA AAT GGG CCA GTC ATG   90
16     Q   G   T   I   H   F   E   Q   K   E   N   G   P   V   M   30

91    GTA TCG GGG TCC ATT TCA GGA TTG GCC GAA GGC GAT CAT GGA TTC   135
31     V   S   G   S   I   S   G   L   A   E   G   D   H   G   F   45

136   CAT GTC CAT CAG TTT GGA GAT AAC ACA CAA GGC TGT ACC AGT GCA   180
46     H   V   H   Q   F   G   D   N   T   Q   G   C   T   S   A   60

181   GGT CCT CAC TTT AAT CCT CTG TCC AAA AAA CAT GGT GGG CCA AAG   225
61     G   P   H   F   N   P   L   S   K   K   H   G   G   P   K   75

226   GAT CAA GAG AGG CAT GTT GGA GAC CTG GGC AAT GTG ACT GCT GGC   270
76     D   Q   E   R   H   V   G   D   L   G   N   V   T   A   G   90

271   AAA GAT GGT GTG GCC ATT GTG TCT ATT GAA GAT CCT GTG ATC TCA   315
91     K   D   G   V   A   I   V   S   I   E   D   P   V   I   S   105

316   CTC TCA GGA GAC CAT TCC ATC ATT GGC CGC ACA ATG GTG GTC CAT   360
106    L   S   G   D   H   S   I   I   G   R   T   M   V   V   H   120

361   GAA AAA CCA GAT GAC TTG GGC AAA GGT GGA AAT GAA GAA AGT ACA   405
121    E   K   P   D   D   L   G   K   G   G   N   E   E   S   T   135

406   AAG ACG GGA AAT GCT GGA AGT CGT CTG GCC TGC GGC GTG ATT GGG   450
136    K   T   G   N   A   G   S   R   L   A   C   G   V   I   G   150

451   ATC GCC CAA TAA gca ttc cct agg acg tgg tct gag tcc tag taa   495
151    I   A   Q   *                                             165

496   ctc atc tgt tgt ctt gct   513
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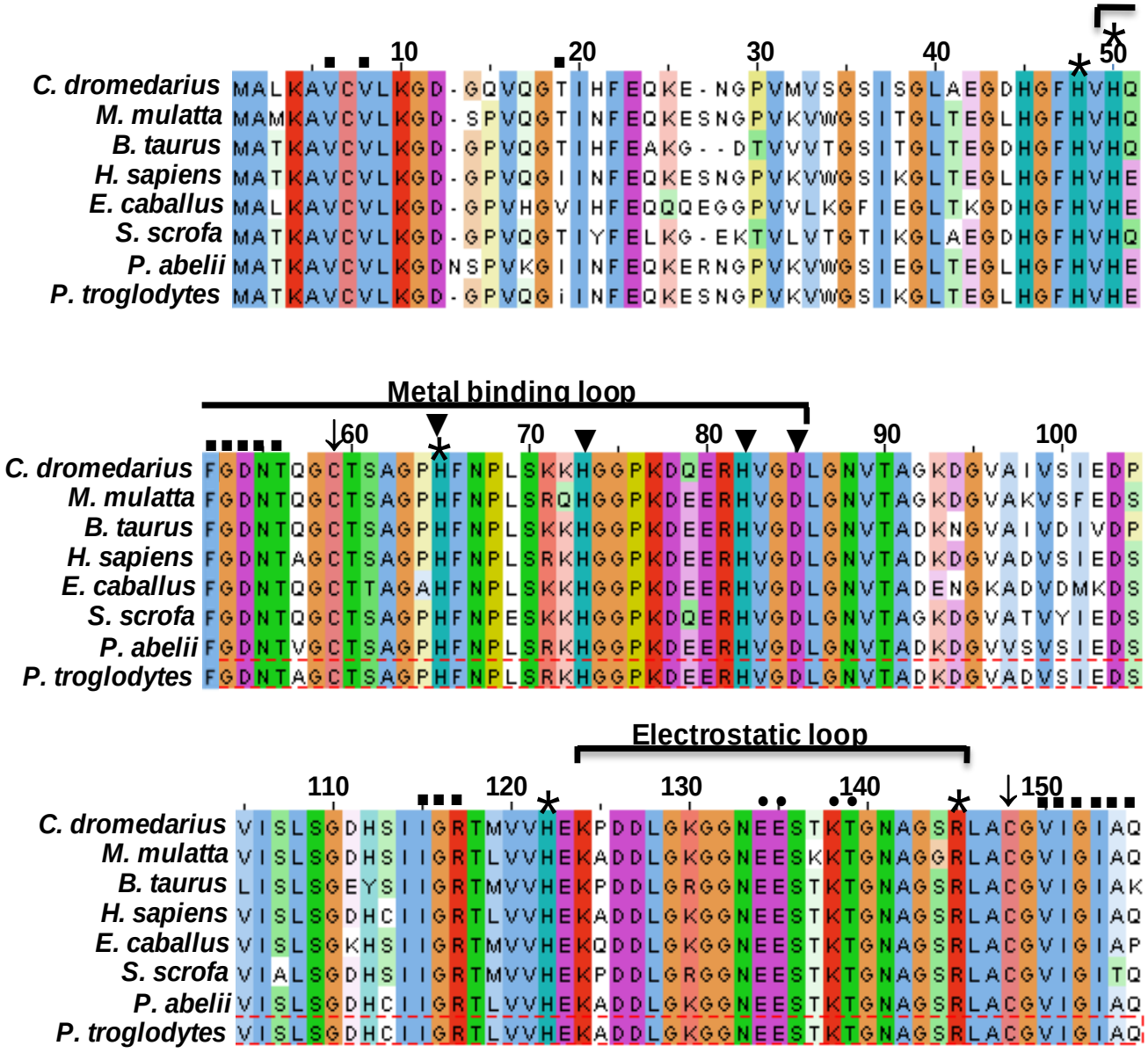
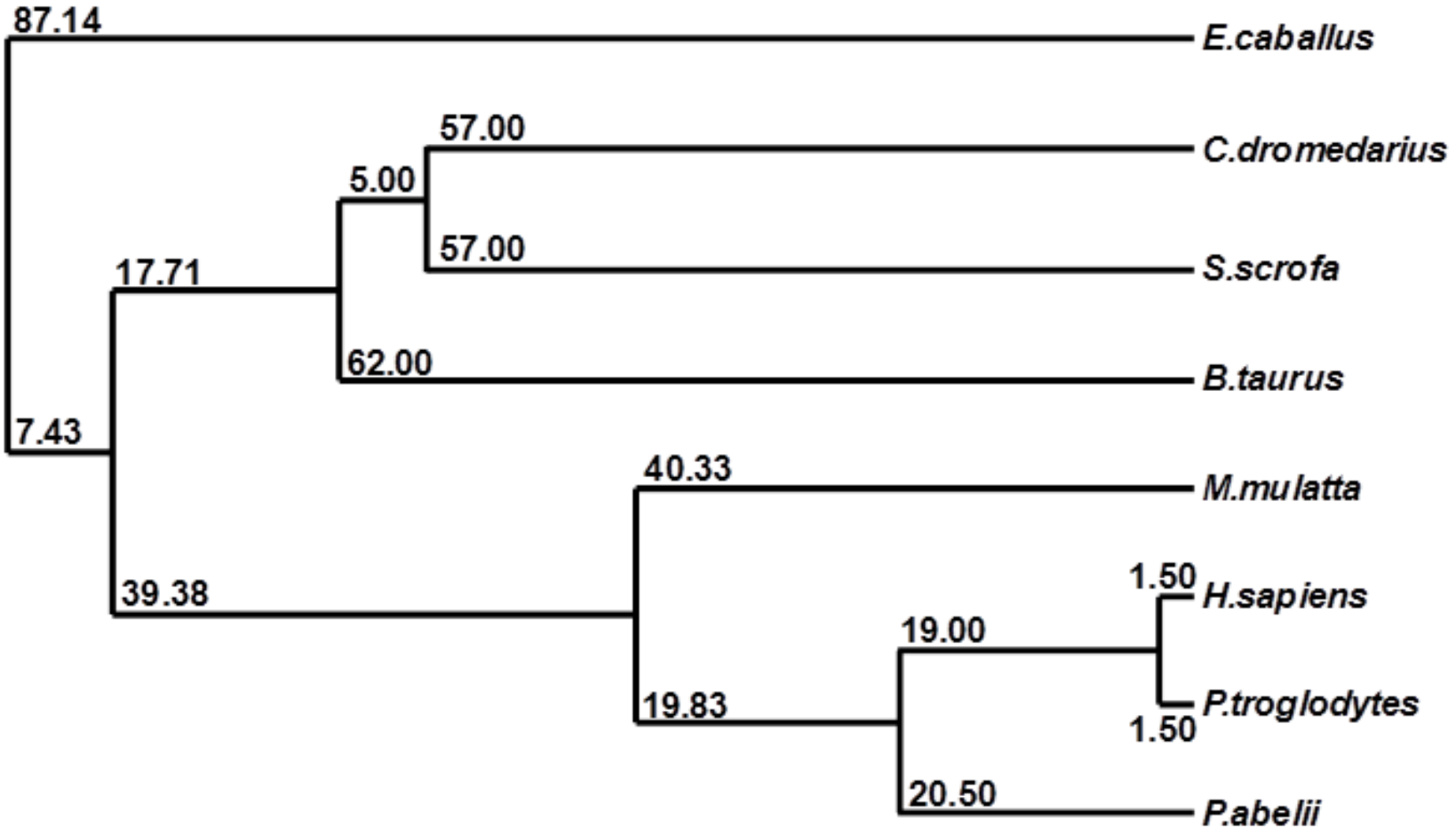
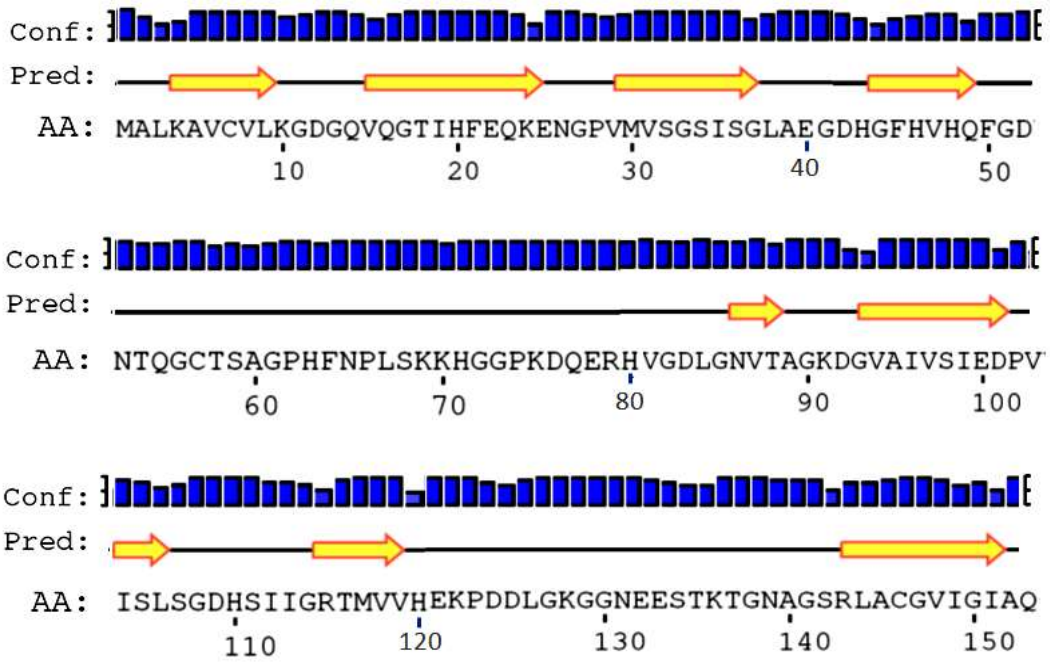
PCR and Nucleotide sequence of the cloned gene and the predicted amino acid sequence and primary protein structure

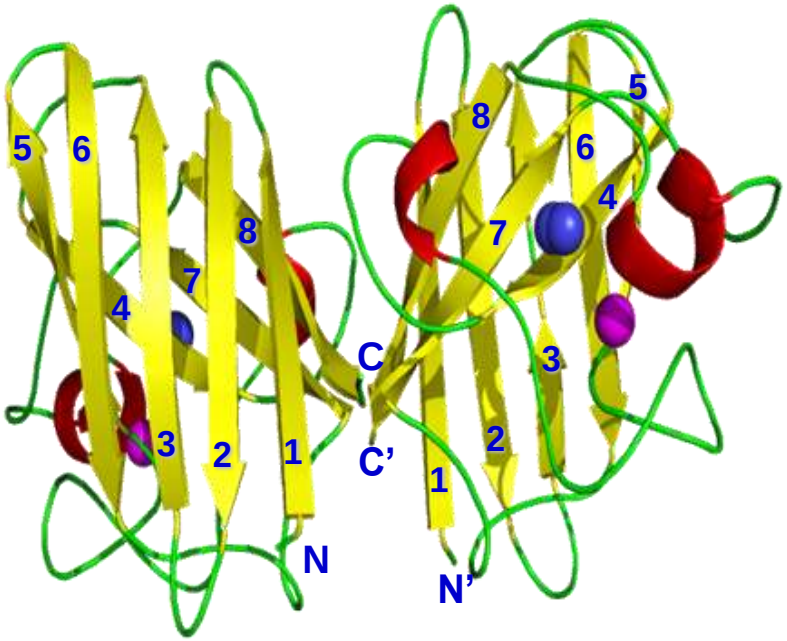

Metal binding loop
Electrostatic loop
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
C. dromedarius
M. mulatta
B. taurus
H. sapiens
E. caballus
S. scrofa
P. abelii
P. troglodytes

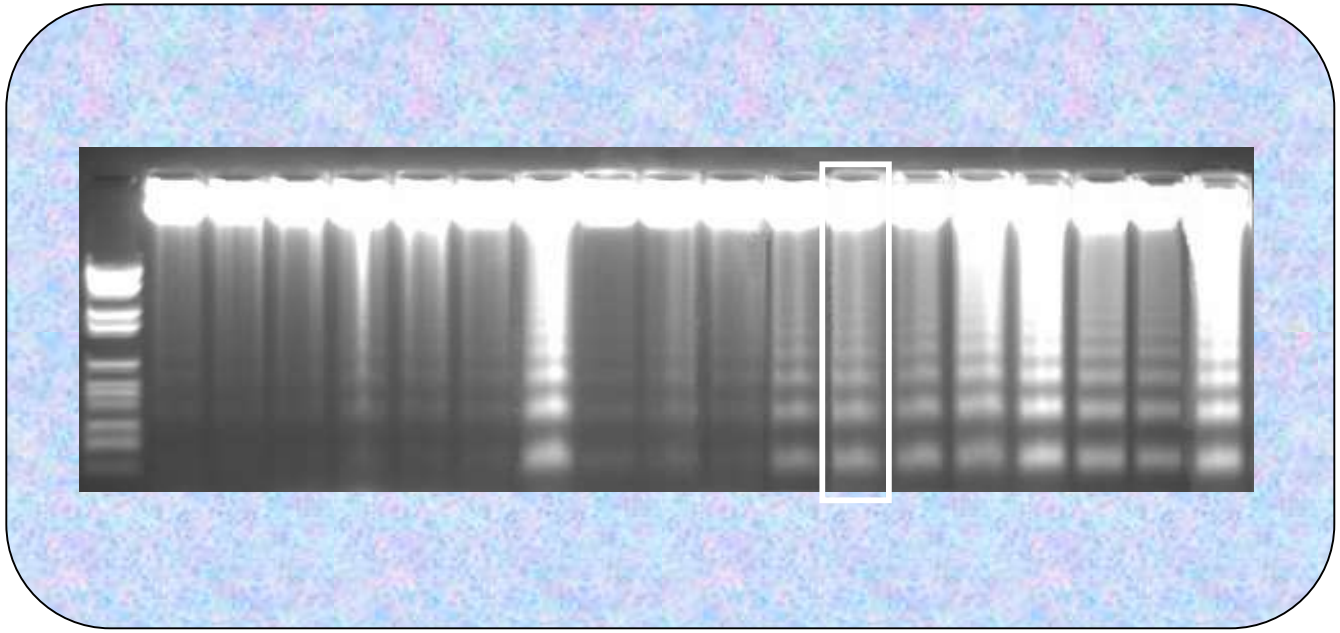

87.14
E.caballus
57.00
C.dromedarius
5.00
57.00
S.scrofa
17.71
62.00
B.taurus
7.43
40.33
M.mulatta
1.50
H.sapiens
39.38
19.00
19.83
P.troglodytes
1.50
20.50
P.abelii

2ry structure annotation sites of the cSOD1 sequence



Predicted 3D structure of the cSOD1






Thank you for
your attention