Molecular Lab (1): Genomic DNA Extraction

-All downstream applications depend on the extraction of the genetic material (DNA,RNA).

-DNA Extraction: is a process of isolation and purification of DNA from a sample using a combination of physical and chemical methods.

A- Chemical Methods: The reagents that are used in the laboratories and help in the extraction.
B- Physical Methods: The equipments or instruments that are used in the laboratory such as (Centrifuge).

-Routine procedure widely used in:
1-Molecular biology labs (scientific research labs) for example to study a gene involved in a cancer.

-We can not study a gene without extraction of the genetic material.

Slide (2):

2- Genetic testing is used to diagnose or rule out suspected genetic or inherited diseases. Also to identify disease carriers "Such as prenatal check up" or to predict those at high risk for specific conditions.

3- Forensic analyses to gather evidences from the crime scene that can be used in the court.
-DNA fingerprinting (DNA profiling) is a technique to identify individuals by features of their DNA.

*Every person has a DNA identity that is different from any another person.

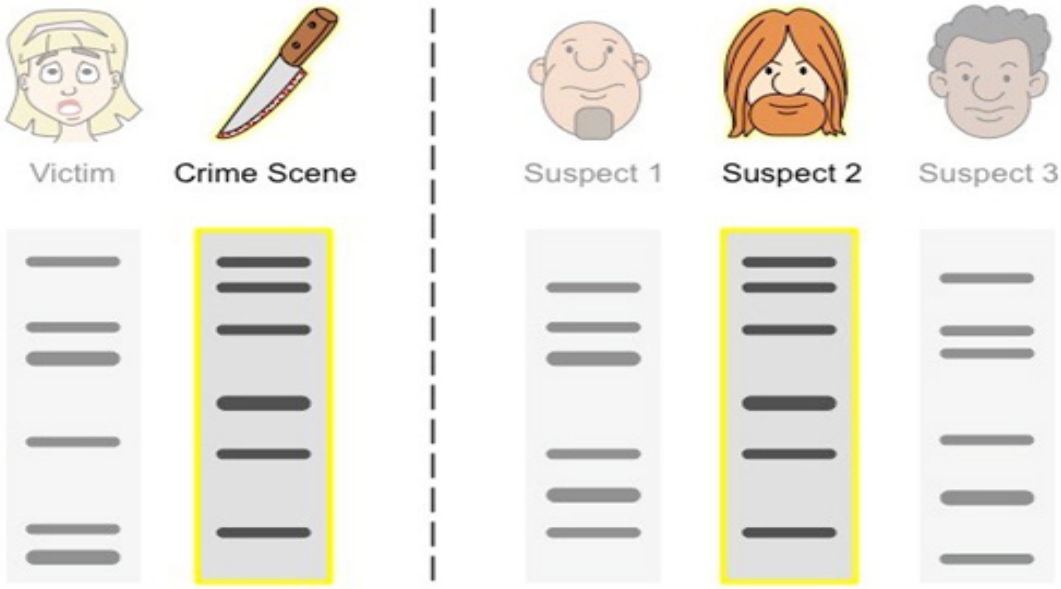
Slide (3):

-99% of the DNA in all people are identical , due to the similarity of the enzymes that work in our body.

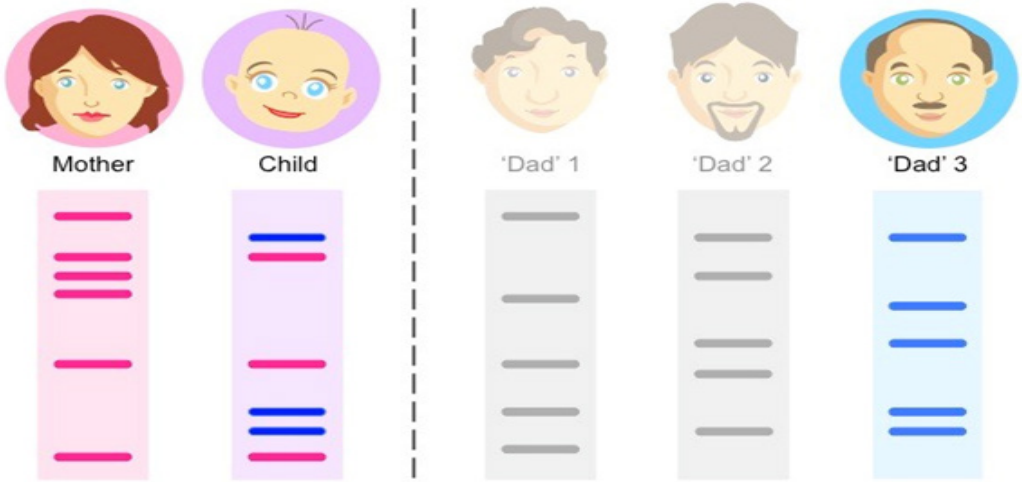
-DNA profiles: are small set (1%) of DNA variations that are very likely to be different in all unrelated individuals.

-DNA fingerprinting is used in criminal investigations and paternity testing.

- Criminal investigation: they took the small set of DNA variations and cut it by restriction enzymes and put it on Gel Electrophoresis and compare between the sample in the crime scene and the suspected person's one.



- Patrenity testing: The sample of the DNA of the child is a mix between the DNA sequence of their parents.



-Slide (4):

- We need cells to isolate DNA. In eukaryotes like human (Homo sapiens), genomic DNA is found inside the nucleus of every cell (exceptions like the anucleated RBCs). Also, DNA is found within mitochondria (mitochondrial DNA), the energy (ATP) producing organelle.
-Genomic DNA: is formed of the genetic data of the organism.
-Genomic DNA: found in the nucleus of cells (Haploid-->46, Diploid--> 23).
-Genomic DNA: is called chromosomal DNA and it carries the information that specify all proteins that are responsible of the function of the organs.
-Mitochondrial DNA is 16Kbp small circular dsDNA (2-10 copies) code for 37 genes (inherited from mother).
-The source of mitochondrial DNA in the zygote is from the mother (egg), but the chromosomal DNA is from both (egg and sperm.
-Mitochondria produces it's own protein to do it's own function.
-Somatic cell (diploid) consists of 23 pairs of chromosomes code for different 25,000 genes. If DNA is laid out end to end, the total length will be approximately 2 meters.
-The size of genetic material is 6 million base pair.
-Each 23 chromosomes code for 25,000 genes.
when we take a sample of DNA from the blood it's taken from UBCs and RBCs.
The overall length of the DNA inside our body is enough to cover the distance between the Earth and the Sun 7 times going and coming back.

Side (5): DNA Packaging

-DNA is arranged into three different levels of organization:

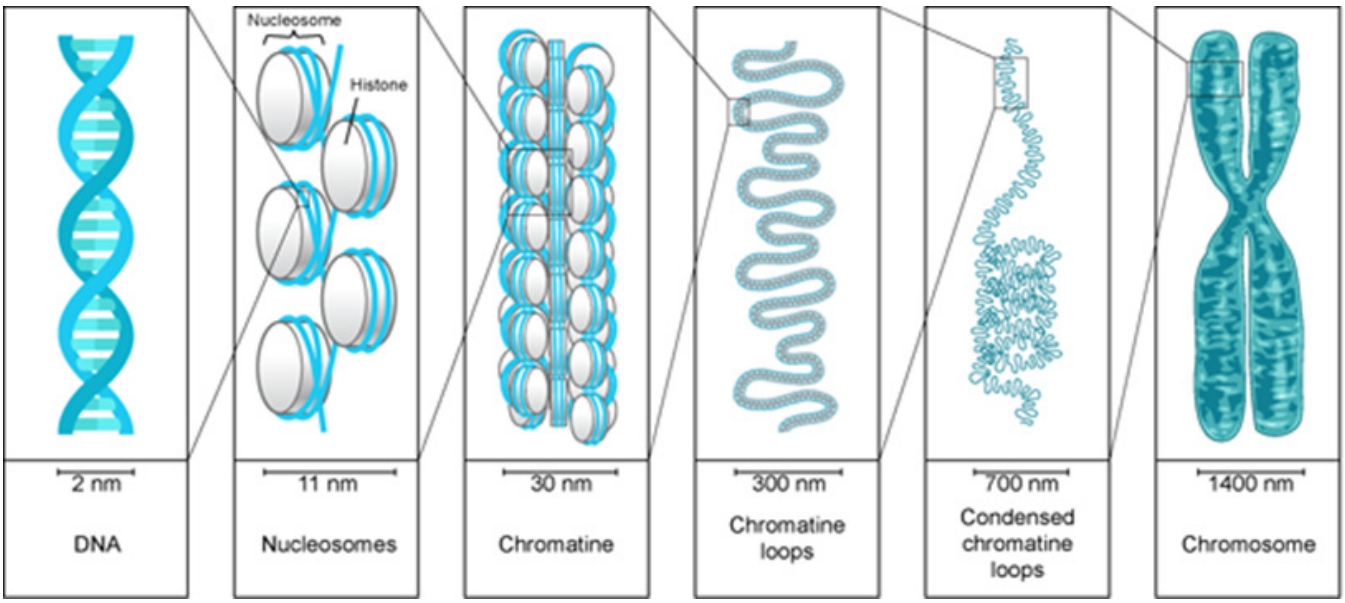
1-Nucleosome: DNA is wrapped around histone proteins (octamer).

-Each nucleosome is formed from 8 proteins called histones which form Octamer surrounded by two and half turns (beads and string).

2-Chromatin fibers: nucleosomes coil and form loops.

3-Chromosomes: chromatin fiber is further condensed.

-When DNA is extracted it should be intact.



Slide (6): Bacterial DNA

-In prokaryotes like bacterial cells, there are a single circular chromosome (bacterial DNA) found in the cytoplasm.

-Bacterial DNA: it carries the information that specify protiens that required for the functions of bacterial cell itself.

-Extrachromosomal DNA called plasmid also found in the cyto-plasm:

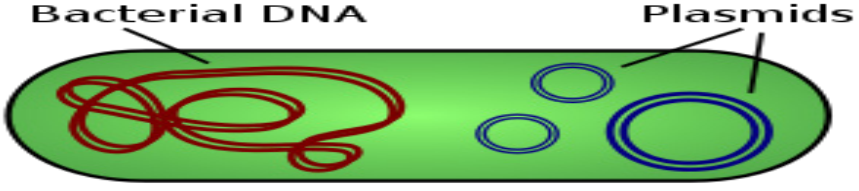
1-Small circular dsDNA.

2-Replicate independently of chromosomal DNA.

3-Hundred of copies of single plasmid.

4-Synthetically modified plasmids are used as vectors.

-Plasmid DNA: has simple genes such as antibiotic resistance of bacteria.

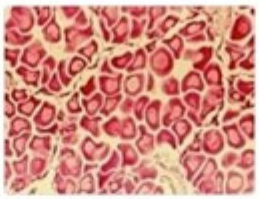


Slide (7): DNA Isolation from Various Samples.

-DNA can be extracted from variety of samples:

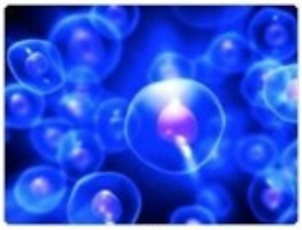
1-Human Samples:

Forensic Sample

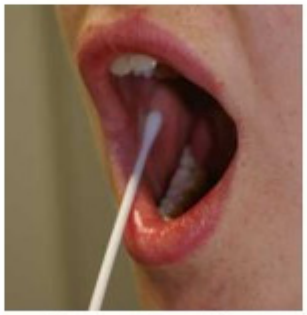


Tissues

Blood



Cells



Buccal swab

Prenatal genetic testing

-Foresic sample: any sample can be used.

Slide (8):

-Genomic material can be extracted from different human samples like cells or tissues (extraction from the liver tissue is easier than brain tissue depending on the biochemical nature of that tissue).

-Because there is a difficulty in purification the DNA.

-Buccal swab (cheek cells) is the most convenient and easiest way (non-invasive) because squamous epithelial cells divide every 24 hours

-Fetal sample: cells of the placenta (in late first trimester) or from amniotic fluid (in second trimester).

Slide (8):

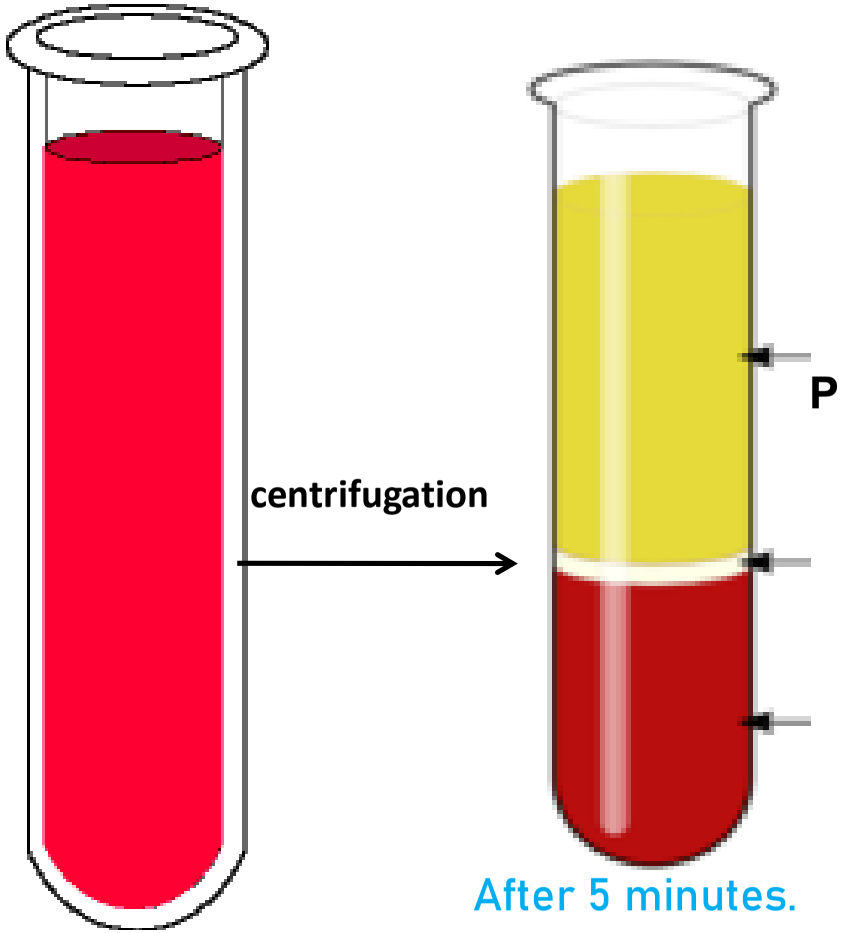
-In vitro fertilization (IVF): is a technique of doing fertilization of eggs in the lab.

-A one cell sample can be taken from the zygote for genetic testing. "At the second day after fertilization"

-Blood sample is more common although RBCs are anucleated

-After centrifugation of the sample, we take the buffy coat layer containing the WBCs.

Nowadays, there are kits available for extraction of DNA from whole blood sample.



After 5 minutes.

Plasma (55% of total blood)

Proteins, electrolytes, clotting factors, hormones , etc…

This layer is used in cases of determining the percentage of (Hormomone, proteins, Na, etc..)

Buffy coat (<1% of total blood)

WBC's and platelets

This layer has DNA.

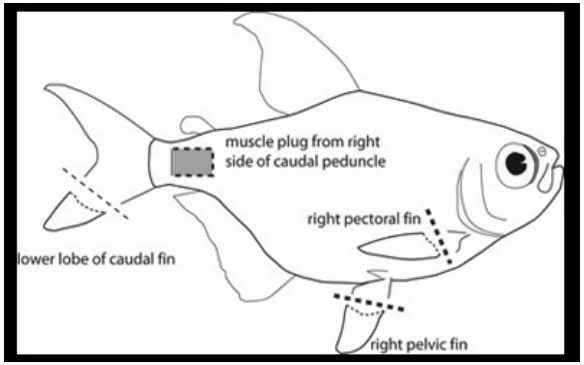
RBC's (45% of total blood)

This layer doesn't have DNA.

-The difference between plasma and the serum that the serum doesn't have clotting factors.

Slide (10): DNA Isolation from Various Samples

2-Animal cells/tissues (e.g. zebrafish fins)



The samples are taken from the fins of the fish without harming the fish.

Animal Cell

3-Plant material (e.g. banana and strawberry)

-Plant cell wall

-High levels of polysaccharides and polyphenols present in plant tissue so negatively affects the quality of extracted DNA and may inhibit downstream reactions.

-Isolation of DNA from plant material is more difficult than isolation it from animal cells due to the presence of cell wall.

Slide (11): DNA Isolation from Various Samples.

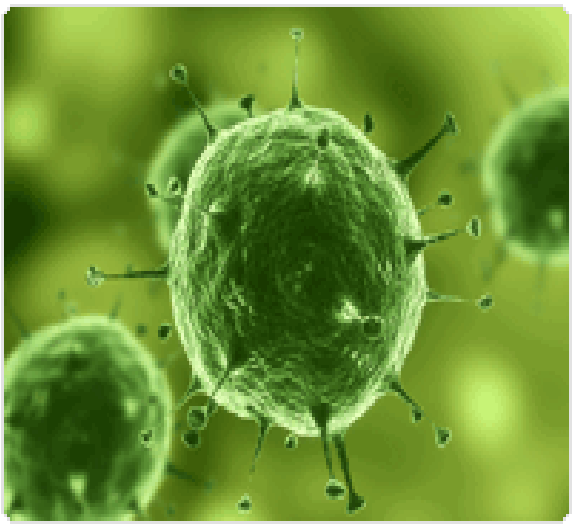
4-Viral and Bacterial cells "Isolating the genetic material from the viral or bacterial cells by a lot of methods":

-Nasal swab like COVID-19.

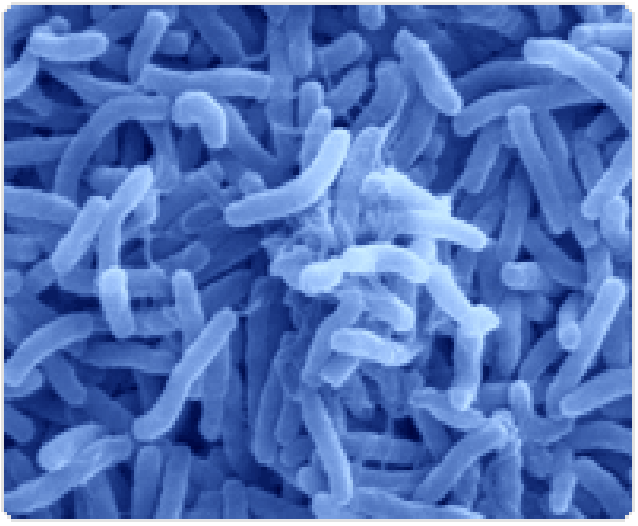
-Oral fluid.

-Blood sample (serum).

-Stool sample.



Viral cells



Bacterial cells

Slide (12): DNA Isolation from Various Samples.

5-Plasmid DNA (containing the gene of interest)

-After transformation of bacterial cells (competent cells), the amplified plasmid is extracted using different types of kits.

-The technique for using plasmid in DNA:

1-Isolation the gene of interest.

2-Insertion the gene into the vector (plasmid).

3-Transfer the vector into the bacterial cell (competent).

4-The bacterial cell will copy the vector.

5-amplified plasmid is extract

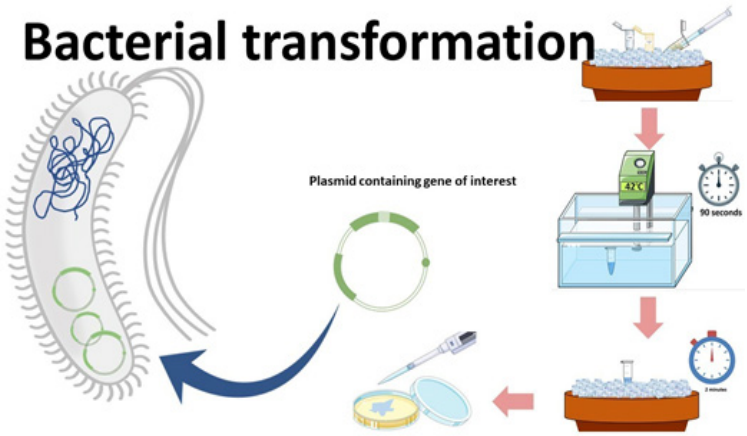
-Type of kits:

-Miniprep (50-100 µg) kits

-Midiprep (100-350 µg) kits

-Maxiprep (500-850 µg) kits

*The purified plasmid is stored as stock at -20°C



Slide (13): DNA Extraction Kits

-Obtaining high quality (purity) and quantity (concentration/ amount) of intact DNA is often the first and most critical step in many fundamental molecular biology applications, such as DNA cloning, sequencing, PCR and electrophoresis.

-Different kinds of kits are available from different companies: Qiagen, Invitrogen, Promega and Bio Basic Inc.

Slide (14): DNA Extraction Kits

QIAGEN®

BIO BASIC INC.

invitrogen™
by life technologies™

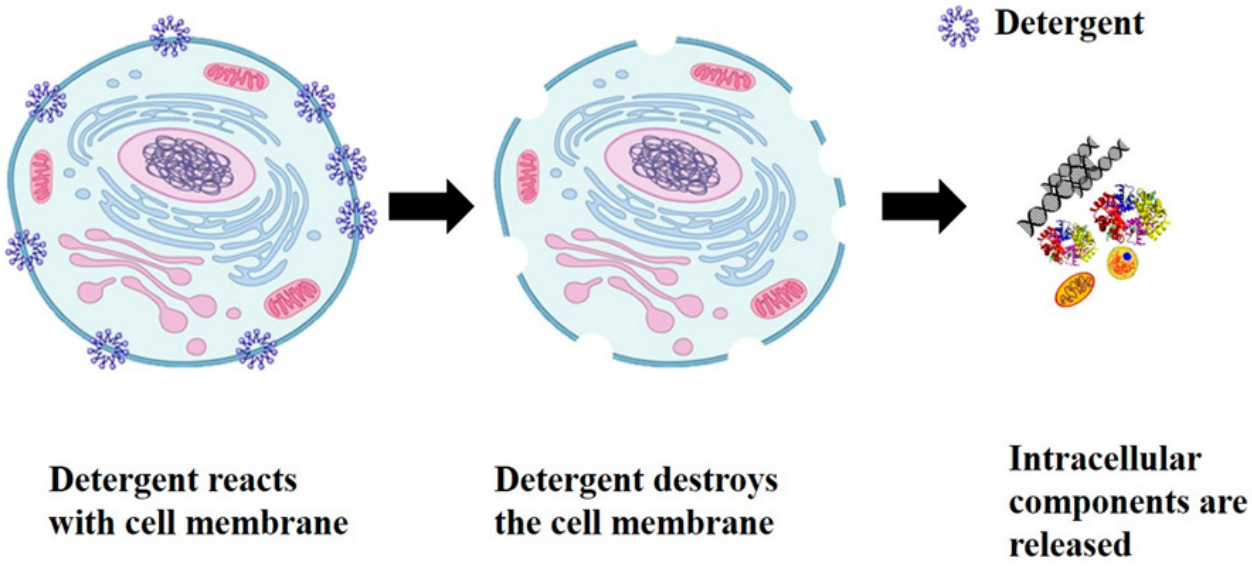
Promega

Slide (16): Part II The Principle of DNA Extraction

-There are three basic steps in DNA extraction:

"These steps are fixed regardless of the way that [Manual or Automated]"

1. Cell lysis "Degradation and Rupture" with digestive "Sloution lysis buffer" solution to expose the DNA. Lysis buffer contains detergents/surfactants such as SDS (sodium dodecyl sulphate) to disrupt both cellular and nuclear membranes (make holes in the membrane).



-The first step is done coinciding with the second one.

-The mixture of intracellular components is called cell lycate.

Slide (17): Part II The Principle of DNA Extraction

2. Inactivate endogenous nucleases like DNases. Actually, this is can be done by adding proteases like proteinase K as well as chelating agents (e.g. EDTA) which sequester Ca+2 and Mg+2 required for nuclease activity. On the other hand RNases are usually added to the sample to get rid of RNA.

-Proteases: Type of enzymes degradate any protein such as DNases).

-DNases and RNAses require Ca+ and Mg+ to function.

-The final sample should not have DNA and RNA at the same stime.

Slide (17): Part II The Principle of DNA Extraction

3-Purification of DNA from proteins, RNA, detergents, salts and reagents found in cell lysate:

-Ethanol precipitation using ice-cold ethanol or isopropanol.
-Phenol/chloroform extraction.
-Minicolumn purification.
-Magnetic beads.

Slide (18): Part II The Principle of DNA Extraction

1-Ethanol precipitation:
-DNA is insoluble in ethanol or isopropanol (anti-solvent) so it will aggregate together forming a pellet (at the bottom of the tube) upon centrifugation.
-DNA is polar molecule, it's negativity charged due to (phosphate)
-To enhance precipitation of DNA in presence of 95-100% ethanol (absolute), the solution should contain positive ions such as sodium acetate (use the right concentration !!!!).
-Too much sodium acetate, the salt will co-precipitate with DNA and too little will result in incomplete recovery of DNA.
-The right concentration is determined by experiment.
-After centrifugation, a pellet of crude DNA is formed.

Slide (19): Centifuge

-It goes around 8000 cycles per minute.
-The sample in centrifuge should be balanced, how?
-Every sample should has another sample opposite of it, at the same size. Due to the high speed of the centrifuge.
-If there is no balance the centrifuge will damage.

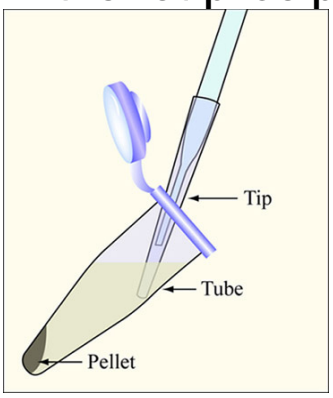
Centrifuge

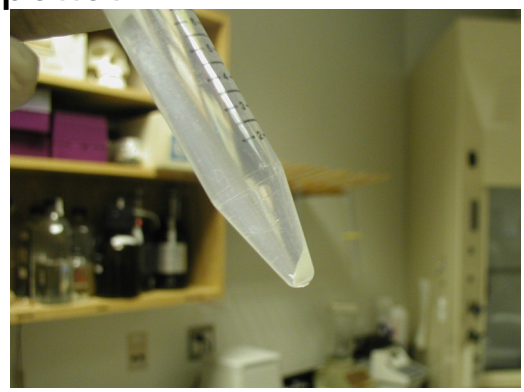
Slide (19): Centifuge

-Centrifuge has many types based on the size of the samples, the size of the tubes.
-Each type work at specific tempreature.

Slide (19): The principle of DNA Extraction

-Ethanol precipitation: formation of DNA pellet.

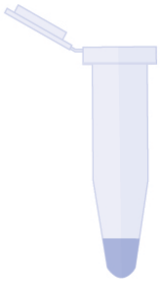




-As the sample is pure,it appears more transparent.

Slide (19): The principle of DNA Extraction

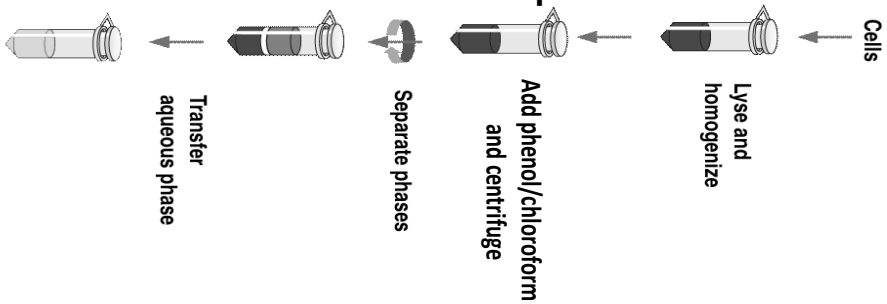
1-Ethanol precipitation:
The pellet is washed with 70% ethanol to remove some salts present in the left over supernatant and bound to DNA Air dry the pellet (5-10 min) then redissolve in ultrapure or Milli-Q water (desalinated water) (DNase/ RNase free water).



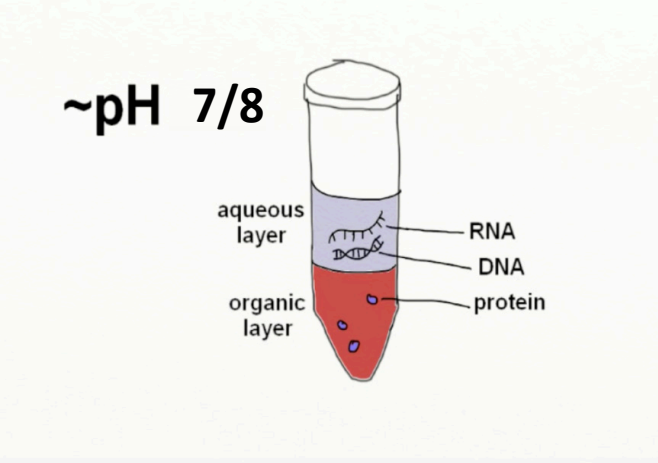
Store purified DNA at -20C

Slide (20): The principle of DNA Extraction

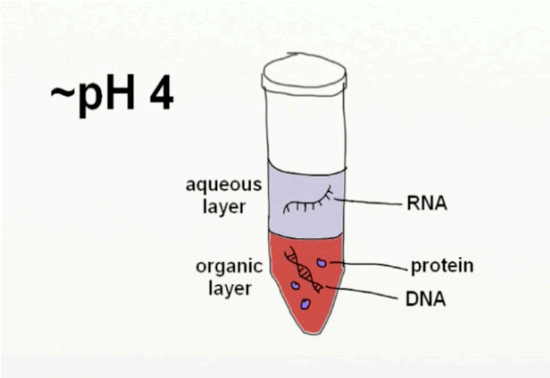
2-Phenol/ chloroform extraction: (Liquid extraction)
-Equal volume of phenol/chloroform added to an aqueous solution of lysed cells.
-Centrifugation yields two phases: the upper aqueous phase (containing the nucleic acids DNA and RNA) and the lower organic phase (containing the lipids and denatured proteins.
-Phenol/Chloroform dissolve all the component of the solution except nuclues acid.
-Why to use both phenol and chloroform?
1.Chloroform prevents the retention of water by phenol and forces a sharper separation of the two phases.
2.Protein denaturation is more efficient in presence of both organic solvents.



Slide (21): Effect of pH on Partitioning



Both DNA and RNA are in the aqueous phase



DNA molecules are neutralized and start to leave to the organic phase but RNA molecules remain in the aqueous layer due to H-bonds between water and exposed nitrogenous bases in ssRNA

Slide (22): The principle of DNA Extraction

3-Minicolumn purification:
(Spin-column-based nucleic acid purification)
-which depends on the binding and adsorption of nucleic acids to a solid phase (e.g. silica, SiO2)

-The kit is bought and it should be specific to the sample.

Slide (23): The principle of DNA Extraction

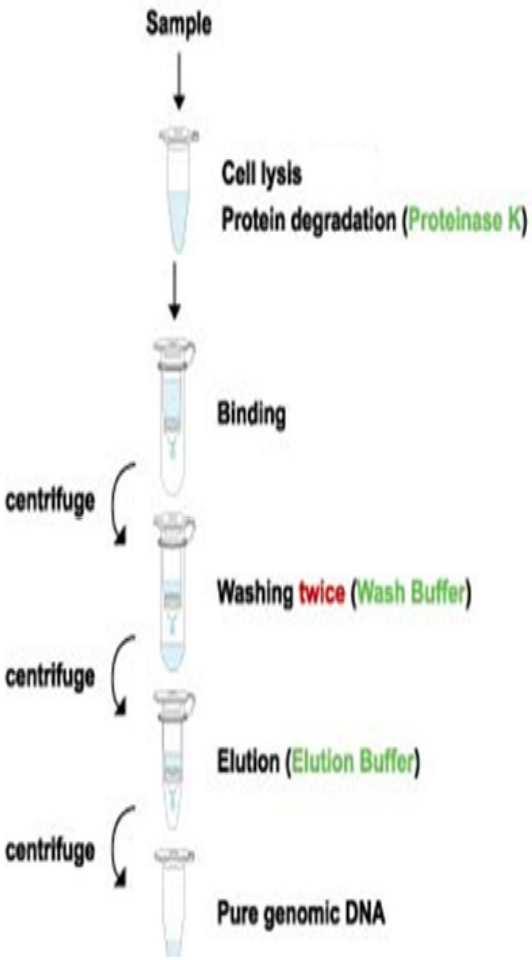
3-Minicolumn purification:

-After cell lysis, inactivate endogenous nucleases (e.g. DNases) with proteinase K enzyme and chelating agents (e.g. EDTA).

-Add binding solution to cell lysate, mix and centrifugate.

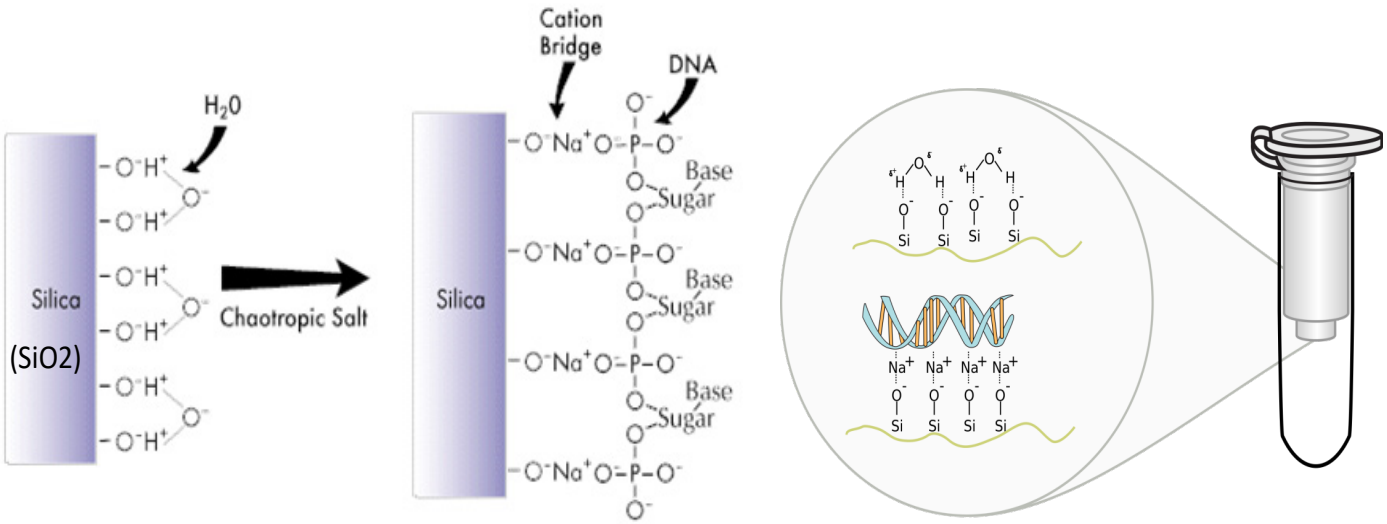
*After centifugation all components will pass through the solid phase except DNA will bind to the solid phase (Silica Membrane)

-Washing and column elution(with TE buffer or DNase/ RNase free water).



-The elution buffer damage the bond between DNA and the silica membrane such as Tris Edta Buffer.

Slide (24): Binding DNA to Silica membrane



Spin column-based nucleic acid purification

Binding solution consists of :

1. Chaotropic salt like guanidinium chloride

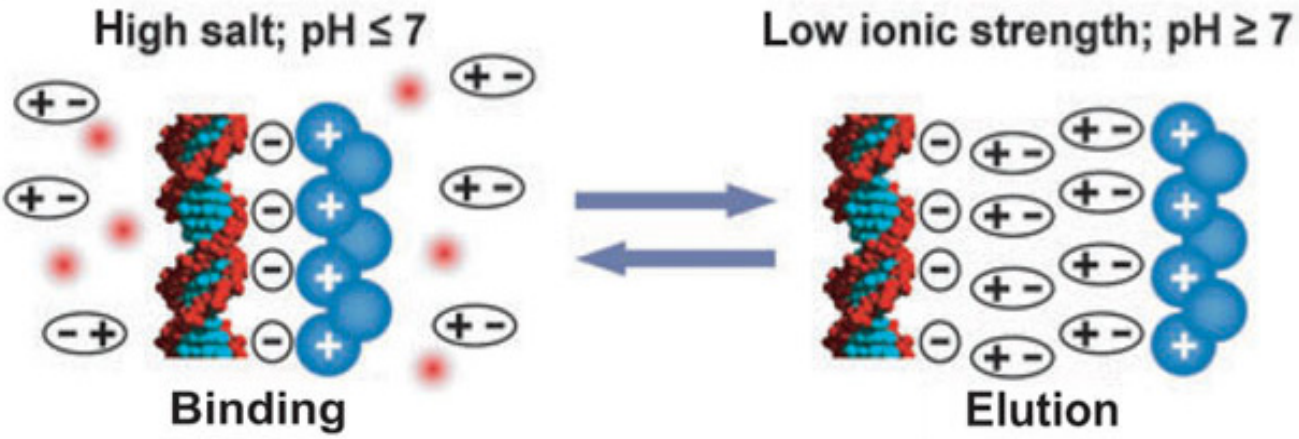
-Chaotropic: the molecule that distribute a structure and enhance activity.

2. Sodium acetate salt (act as bridge)

-Both DNA and Silica are hydrated because they are negatively charged, so, if we tried to mix them without binding solution they would not bind to ezch other.

-By adding binding solution, this solution will remove water by distrubtion H-bonds from DNA and Silica, then Na+ will act as a bridge between DNA and Silica.

Slide (25): Binding and Elution



Slide (26): The principle of DNA extraction

-Used in isolation Corona-Virus

4-Magnetic Beads-based DNA/RNA extraction:

-Quick and efficient for direct separation of crude DNA or RNA from sample.

-No need for centrifugation, separation by applying of magnetic field.

-Avoid the risk of cross-contamination during the traditional methods.

-Various types of magnetic particles are commercially available working in manual or automated mode.

-Save time and money in case of large numbers of samples.