Molecular Genetic

Importance of Molecular Genetics

- Genetics is playing an important role in the practice of clinical medicine.
- **- Medical genetics involves any application of genetics to medical practice, it thus includes:**
- **Studies of the inheritance of disease in families.**
- **Mapping of disease genes to specific locations on chromosomes**
- **Analysis of the molecular mechanisms through which genes cause disease**
- **Diagnosis and treatment of genetic disease (ex. Gene therapy)**

DNA Isolation

- **DNA isolation:** is an extraction process of DNA from various sources

- **The aim:** is to separate DNA present in the nucleus of the cell from other cellular components.

Application of DNA isolation

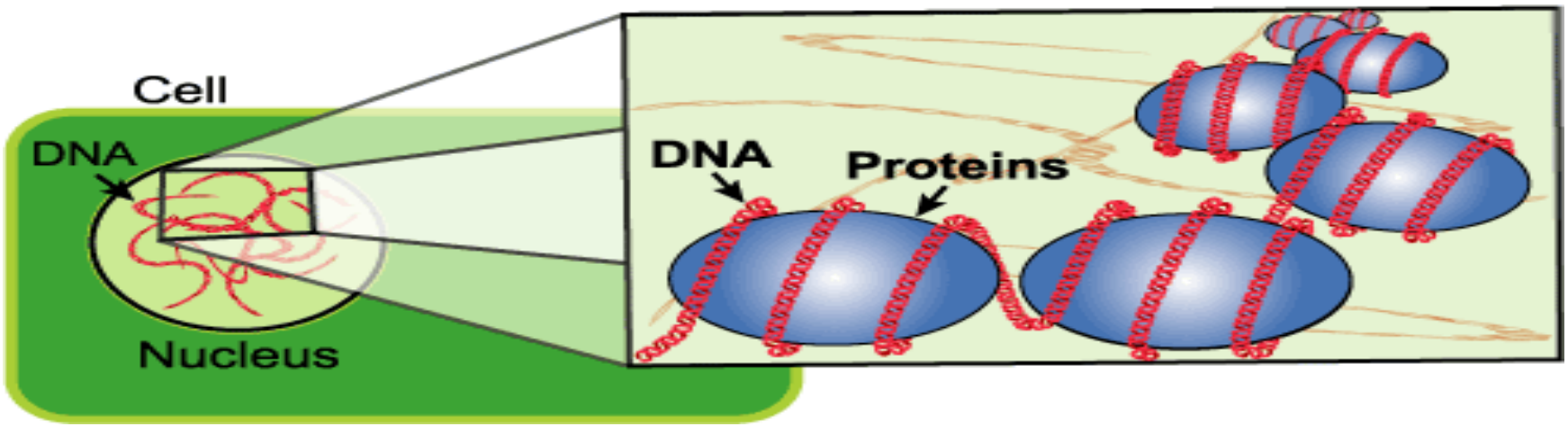
- **It is needed for genetic analysis which used for:**
- **1- scientific:** use DNA in number of Applications , such as introduction of DNA into cells & animals or plants for diagnostic purposes (gene clonining)
- **2- Medicine:** is the most common. To identify point sources for hospital and community-based outbreaks and to predict virulence of microorganisms
- **3- forensic science:** needs to recover DNA for identification of individuals ,(for example rapists, petty thieves, accident , or war victims) , and paternity determination.

- **Many different methods and technologies are available for the isolation of genomic DNA.**
- **All methods involve:**

➢ **A. disruption and lyses of the starting material followed by**

➢ **B. Removal of proteins and other contaminants and finally**

➢ **C. Recovery of the DNA**



- **To choice of a method depends on many factors:**

- **A. The quantity and molecular weight of the DNA**
- **B. The purity required for application**
- **C. The time and expense**

Sample Collection

A- Source: Sample can be isolated from any living or dead organism

Common sources for DNA isolation include:

- **Whole blood**
- **Buffy coat**
- **Bone material**
- **Buccal cells**
- **Cultured cells**
- **Amniocytes or amniotic fluid**
- **Sputum, urine, CSF, or other body fluids**

DNA Purification & Quantification

- **Separating DNA from other cellular components** such as proteins, lipids, RNA, etc.
- **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.**

Extraction of DNA

- **Key Steps**
- **Lysis of the cells**
- **Removal of contaminants includes**
  - **Proteins**
  - **RNA**
  - **Other macromolecules**
- **Concentration of purified DNA**

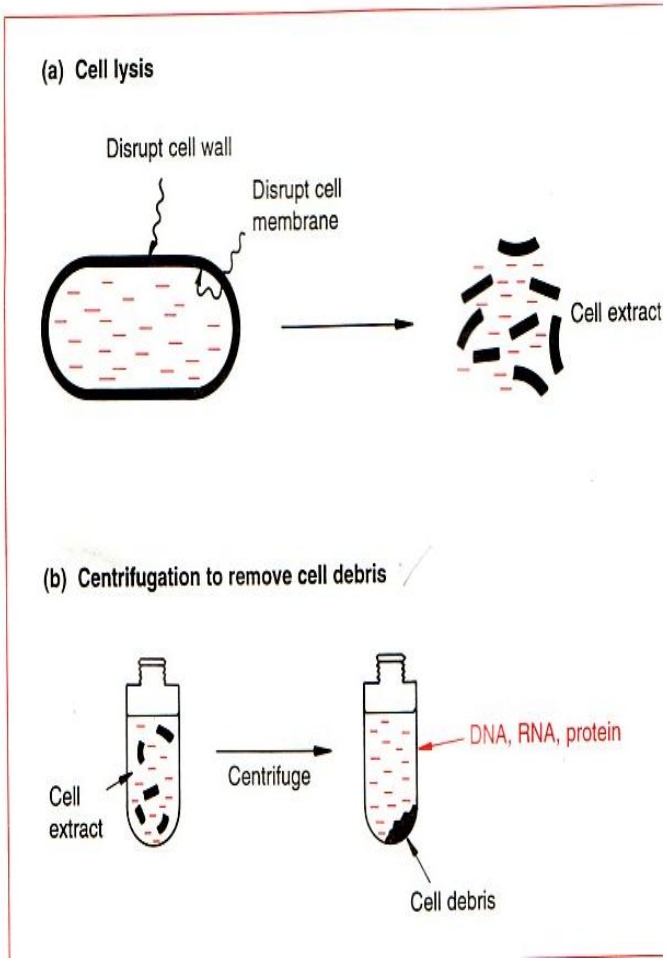
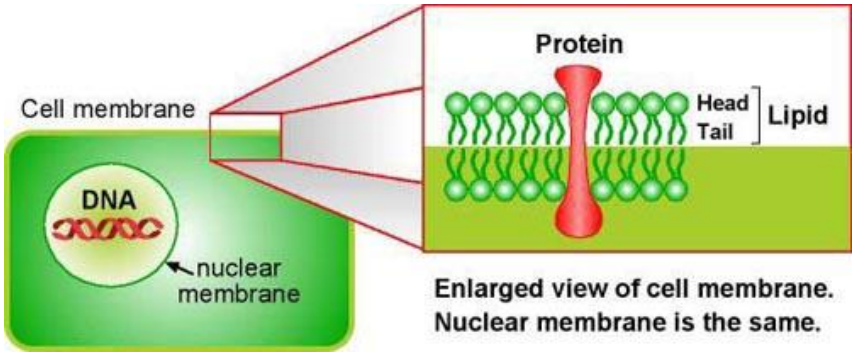
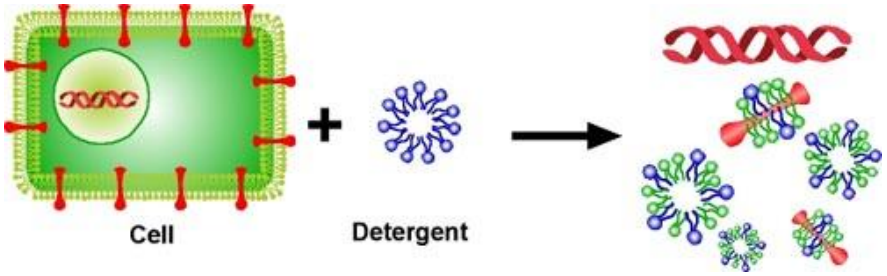


Figure 3.4 Preparation of a cell extract. (a) Cell lysis. (b) Centrifugation of the cell extract to remove insoluble debris.

1. Lysis of the Cell

Use Detergent to solubilize the membrane lipid.





2. Separate DNA From Crude Lysate

- DNA must be separated from proteins and cellular debris.

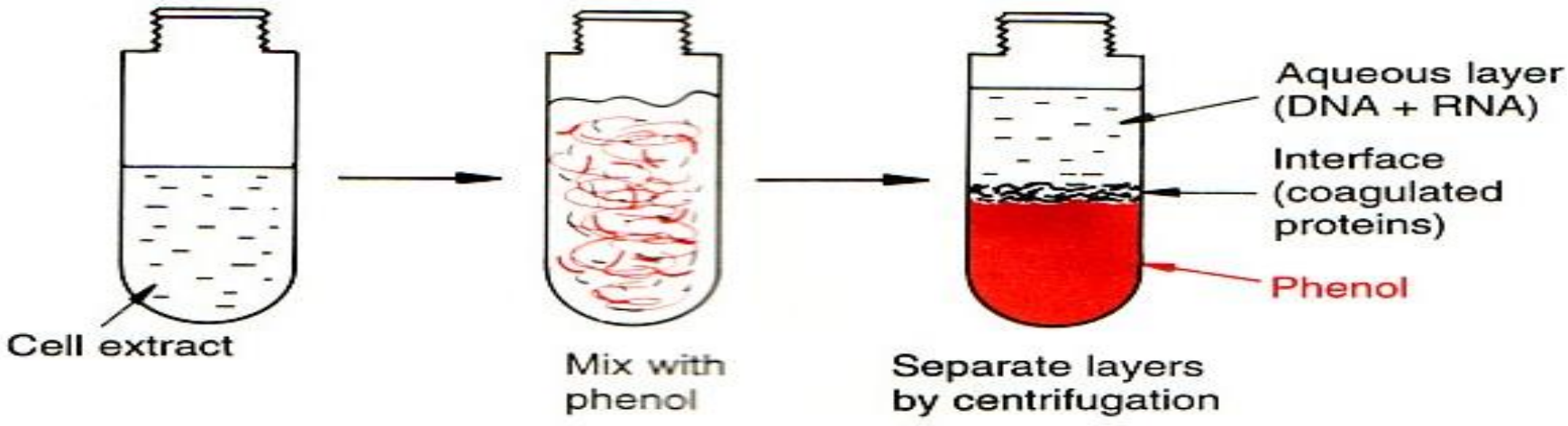
Separation Methods

a) Organic extraction

b) Salting out

a) Separation by Organic Extraction

- *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.
- **Phenol:** **Denatures proteins and solubilizes denatured proteins**



b) Separation by Salting Out

- **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.**

Separation by Salting Out

Salting out method:

- **Cell lysis.**
- **Protein digestion** by proteinase enzyme.
- **Protein precipitation** by high salt concentration.
- **Centrifugation** will remove the precipitated proteins.
- The **supernatant** contains the **DNA.**
- **DNA is then precipitated** by adding **ethanol.**
- **The precipitated DNA** is resuspended in the desired buffer.

Ethanol precipitation:

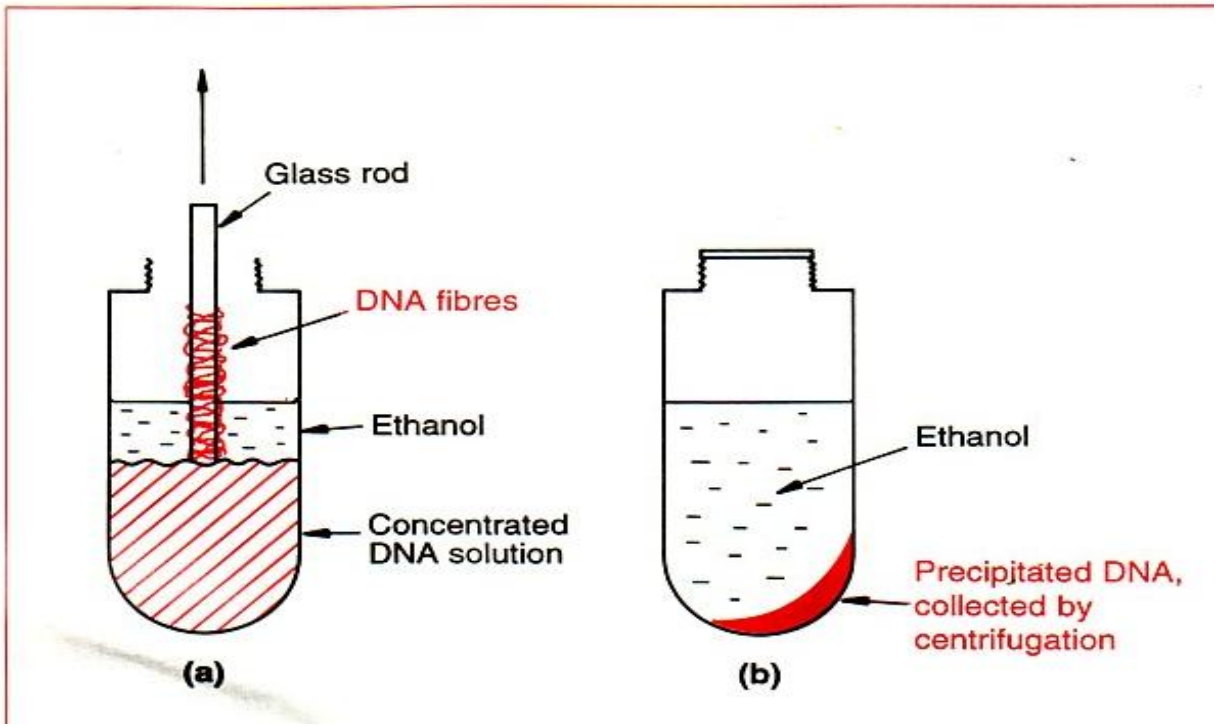
-**Precipitation of DNA:** **Absolute Ethanol is layered on the top of concentrated solution of DNA**

- Fibers of DNA can be withdrawn with a glass rod

- Washing of DNA

- Desalt DNA: Most salts are soluble in 70% ethanol

Figure 3.6 Collecting DNA by ethanol precipitation. (a) Absolute ethanol is layered on top of a concentrated solution of DNA. Fibres of DNA can be withdrawn with a glass rod. (b) For less concentrated solutions ethanol is added (at a ratio of 2.5 volumes of absolute ethanol to 1 volume of DNA solution) and precipitated DNA collected by centrifugation.



2- Use of Commercial DNA purification kits:

- **The common lysis solutions contain**

A. sodium chloride

B. Trimethamine (also known as **tris**) , which is a buffer to retain constant pH

C. Ethylendiaminetetraacetic **(EDTA)** , which binds metal ions

D. Sodium dodecyl sulfate **(SDS)** which is a detergent .

E. An enzyme used in DNA extraction is **protienase K**

3- Heat denaturation

Achieved by boiling samples.

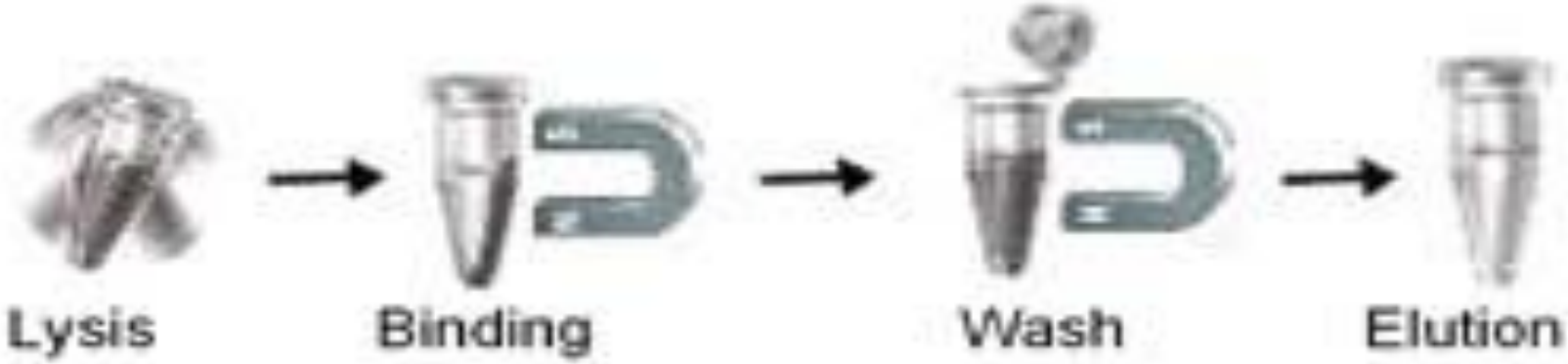
Heating of a sample to 100 c releases DNA into the solution but also **denatures** it by separating the two strand.

Drawbacks: There are remaining inhibitors in the form of degraded proteins and other organic compound or ions .

4- Magnetic beads with DNA binding capacity

- Magnetic beads are **coated with DNA *antibodies*** *or* ***silica*** to bind to DNA.

- Samples are lyses & and then treated with proteinase K.
- The lysates are then applied to the beads.
- Resin is subsequently washed & DNA is eluted of it at 65c
- Magnetic beads are separated from the sample on a magnetic stand.



Summary of DNA extraction :

- There are three basic & two optional steps in a DNA extraction :

1- Cell lysis , to expose the DNA within .

2- removing membrane lipids by adding a detergents or surfactants .

3- removing proteins by adding a protease .

4- removing RNA by adding an Rnase.

5- precipitating the DNA with alcohol- usually ice cold ethanol. In these alcohols , DNA strand will aggregate together, giving a pellet upon centrifugation . This step also removes alcohol- soluble salt.

DNA Extraction & Purification: Evaluation

- DNA concentration can be determined by measuring the intensity of absorbance with **a spectrophotometers** & comparing to a standard curve of known DNA concentration.
- Measuring the intensity of absorbance of the DNA solution at **wavelength 260nm & 280nm** is used as a measure of **DNA purity**
- **DNA purity: A260/A280 ratio: 1.7 – 1.9**
- **DNA concentration (µg/ml): A260 X 50**
- **DNA yield:**

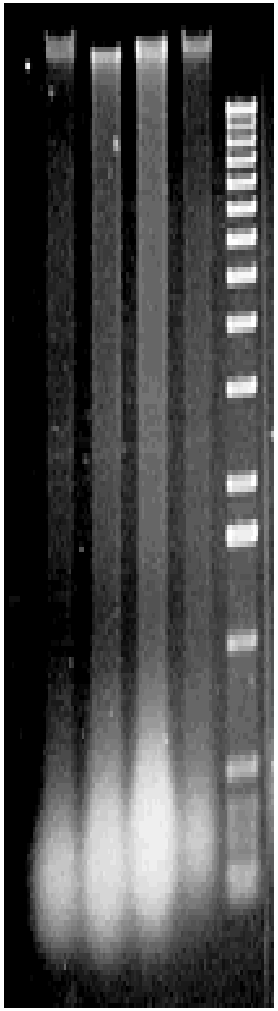
DNA conc. X Total volume of DNA solution

Spectrophotometers

Nanodrop

Qubit® 3.0

Measurement of DNA integrity



Checking for Degradation DNA

- Running your sample through an **agarose gel** is a common method for examining the extent of DNA degradation. Good quality DNA should migrate as a high molecular weight band, with little or no evidence of smearing.
- DNA absorbs UV light at 260 &280 nm & aromatic proteins absorb UV light at 280 nm **Apure sample of DNA has the 260/280 ratio at 1.8 & is relatively free from protein contamination**.
- **A DNA preparation that is contaminated** with protein will have a 260/280 ratio lower than 1.8

Checking the Quality of your DNA by gel

- The product of your DNA extraction will be used in subsequent experiments
- Poor quality DNA will not perform well in PCR
- You will want to assess the quality of your DNA extraction using the following simple protocol:
  - Mix 10 µL of DNA with 10 µL of 2x DNA loading buffer
  - Load this mixture into a 1% agarose gel
  - Analyze results (the following slides provide guidance)

Agarose gel electrophoresis

Principles of nucleic acid separation by agarose gel electrophoresis

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA orRNA. (Kryndushkin et al., 2003).

Nucleic acid molecules are size separated by the aid of an electric field where negatively charged molecules migrate toward anode (positive) pole.

The migration flow is determined solely by the molecular weight where small weight molecules migrate faster than larger ones (Sambrook & Russel 2001).

In addition to size separation, nucleic acid fractionation using agarose gel electrophoresis can be an initial step for further purification of a band of interest.

Agarose gel concentration

Agarose Concentration in Gel (% [w/v])	Range of Separation of Linear DNA Molecules (kb)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0.2-3
2.0	0.1-2

Table 1. The suggested agarose concentrations for separation of different ranges of Linear DNA molecules (Lewis, 2011).

Electrophoresis buffer

Various buffers are used for agarose electrophoresis.

The two most common buffers for nucleic acids are :

1. Tris/Acetate/EDTA (TAE) and
2. Tris/Borate/EDTA (TBE).

DNA fragments migrate with different rates in these two buffers due to differences in ionic strength.

Buffers not only establish an ideal pH, but provide ions to support conductivity.

In general, the ideal buffer should

1. produce less heat,
2. have a long life and
3. a good conductivity.

Agarose gel running voltage

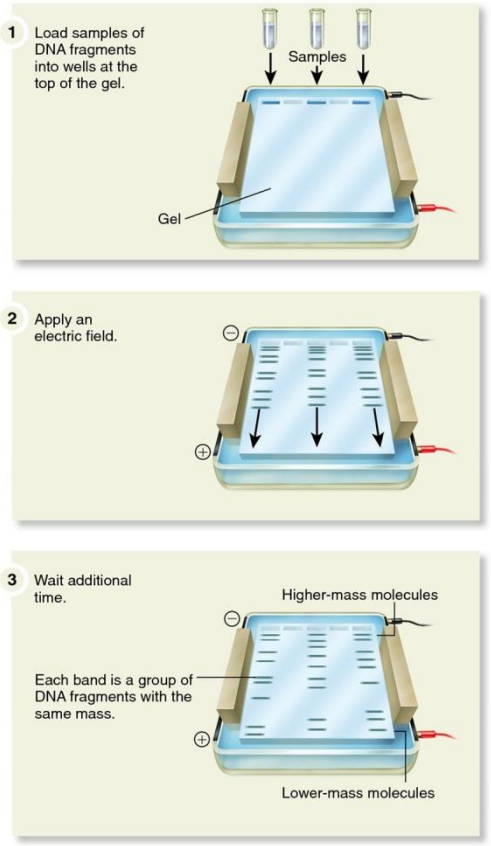
- **Voltage**

Migration of fragments in an agarose gel depends on the difference in electric current.

Different optimal voltages are required for different fragment sizes. For instance, the best

resolution for fragments larger than 2 kb could be obtained by applying no more than 5 volts per cm to the gel

Agarose gel sample loading and visualization



Agarose gel sample visualization

Ethidium bromide is the common dye for nucleic acid visualization.

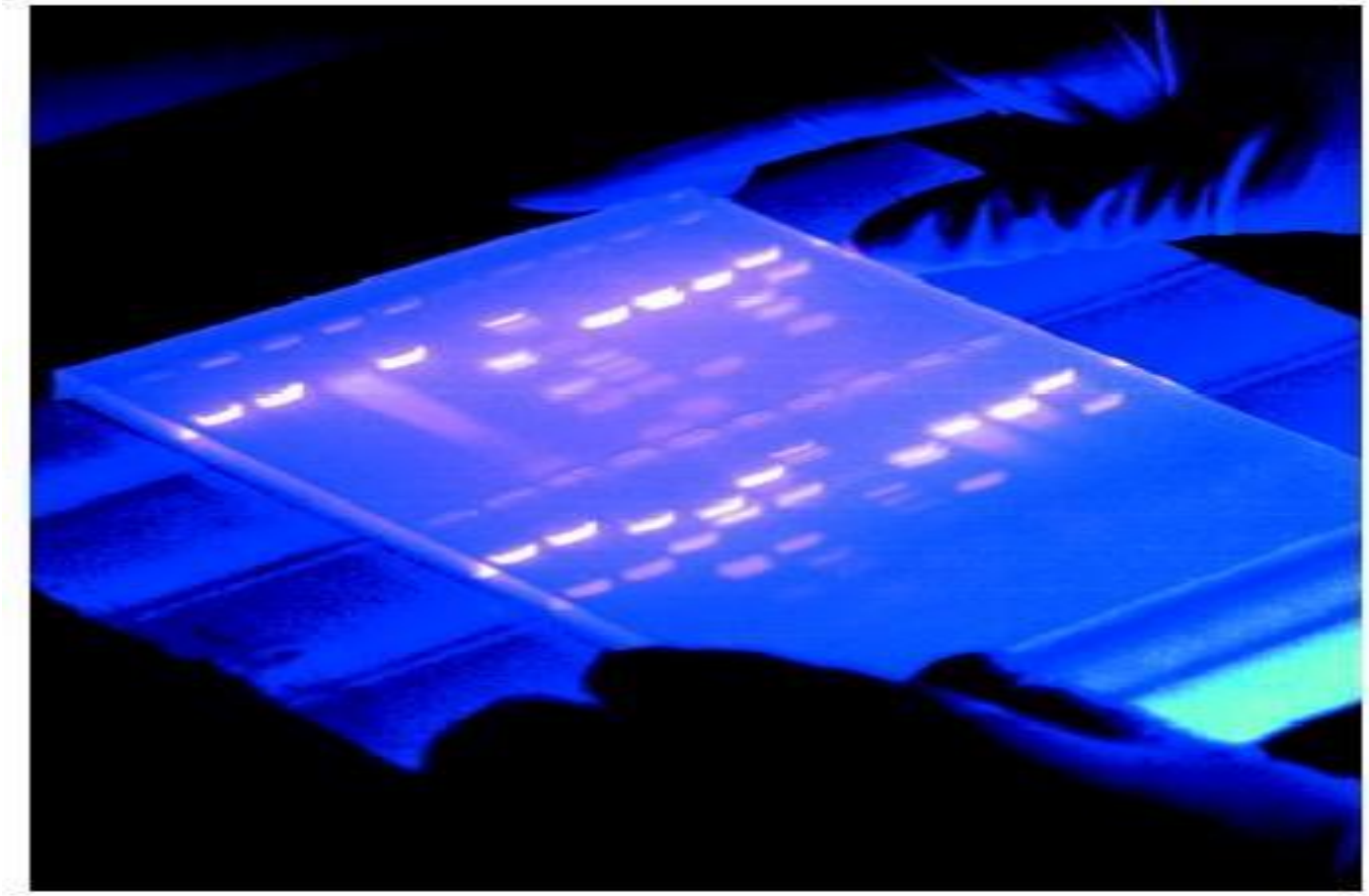
The early protocol that describes the usage of Ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridiniumbromide-) for staining DNA and RNA in agarose gels dates as far back as 1970s (Sharp et al., 1973).

Although the with a lower efficiency compare to the double- stranded DNA, EtBr is also used to stain single- stranded DNA or RNA.

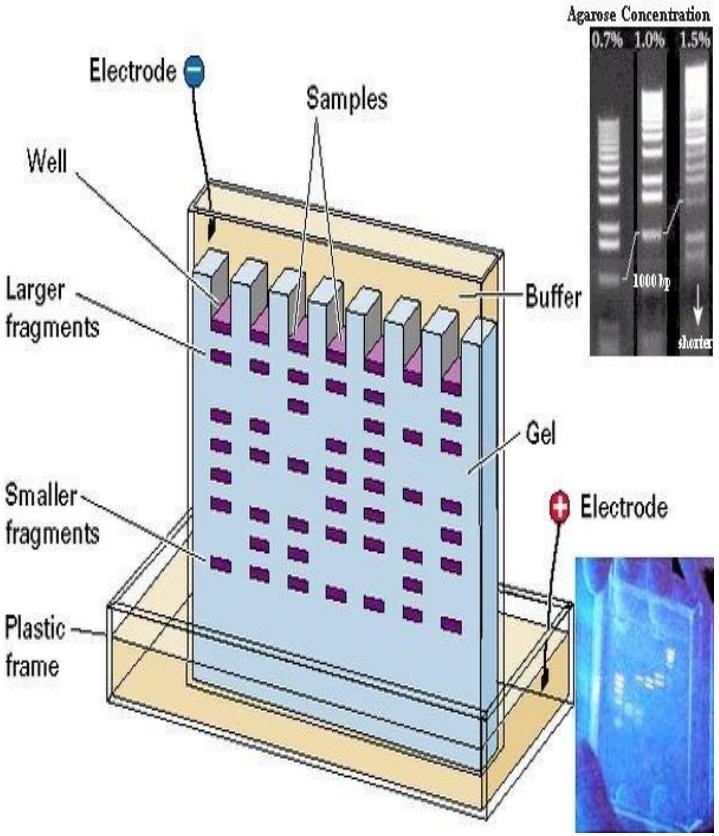
Under UV illumination, the maximum excitation and fluorescence emission of EtBr can be obtained from 500- 590 nm.

Exposing DNA to UV fluorescence should be performed rapidly because
nucleic acids degrade by long exposures and thus, the sharpness of the bands would be negatively affected.

Agarose gel sample visualization



Vertical electrophoresis



Gel Electrophoresis

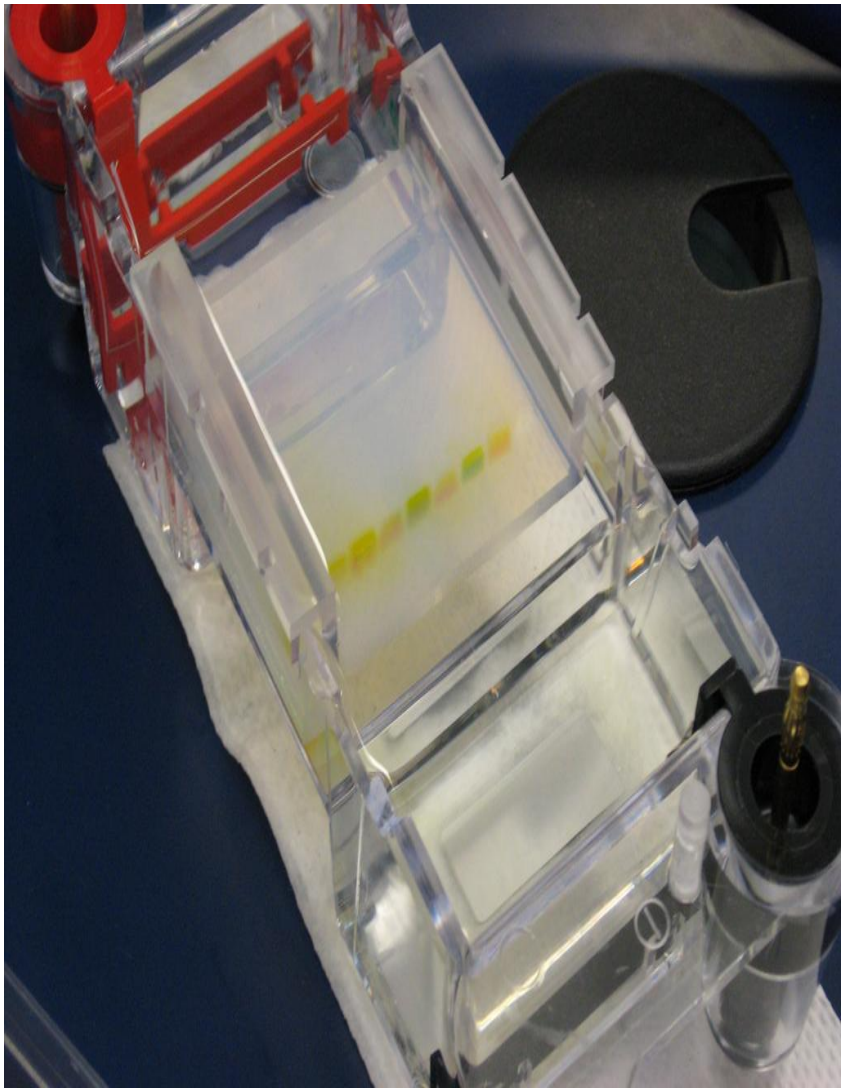
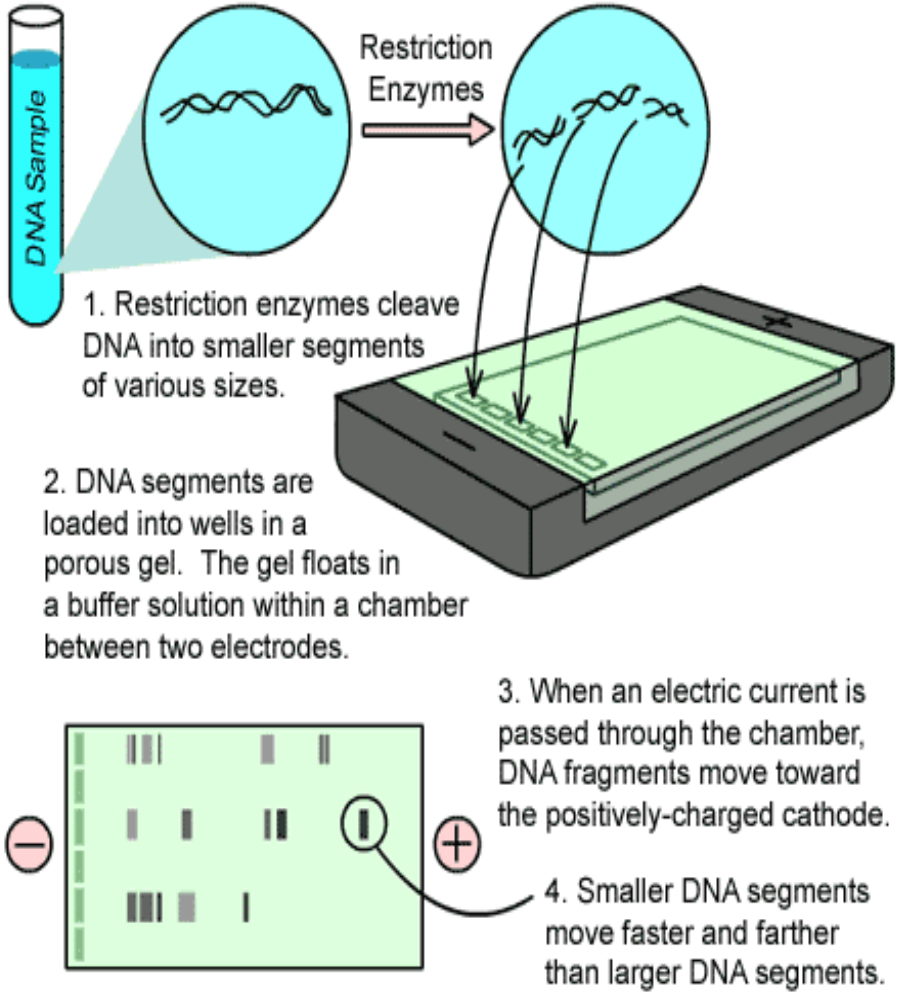
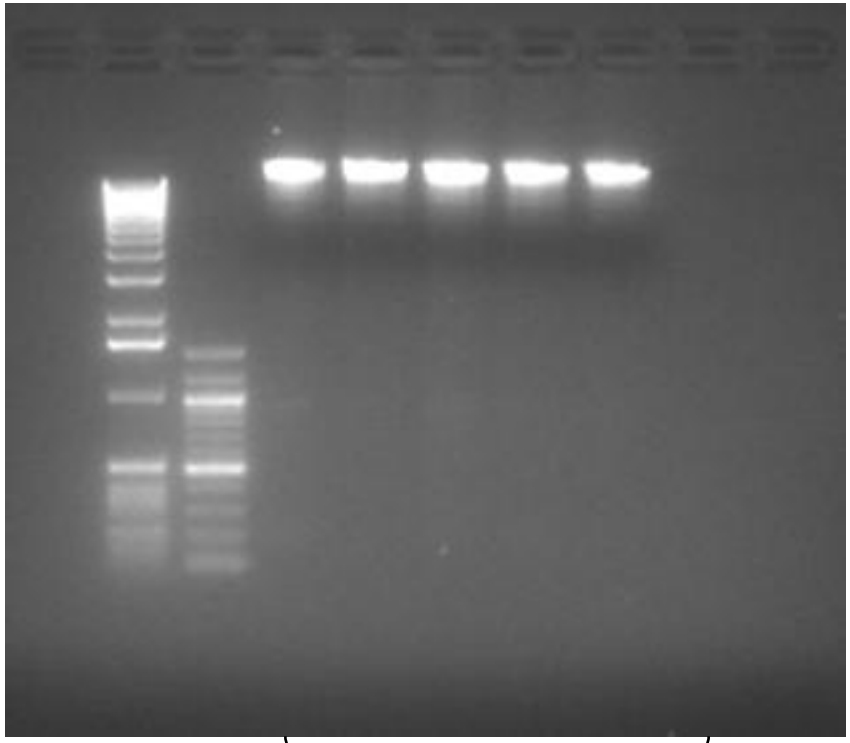


Figure S-2: Gel Electrophoresis



Expected Results in a Research Lab

Below is an agarose gel that has 5 genomic DNA samples from various plants. Note that the DNA runs at a very high molecular weight and as a clear, thick band. This DNA was extracted in a research lab under optimal conditions



1 kbp and 100 bp ladders

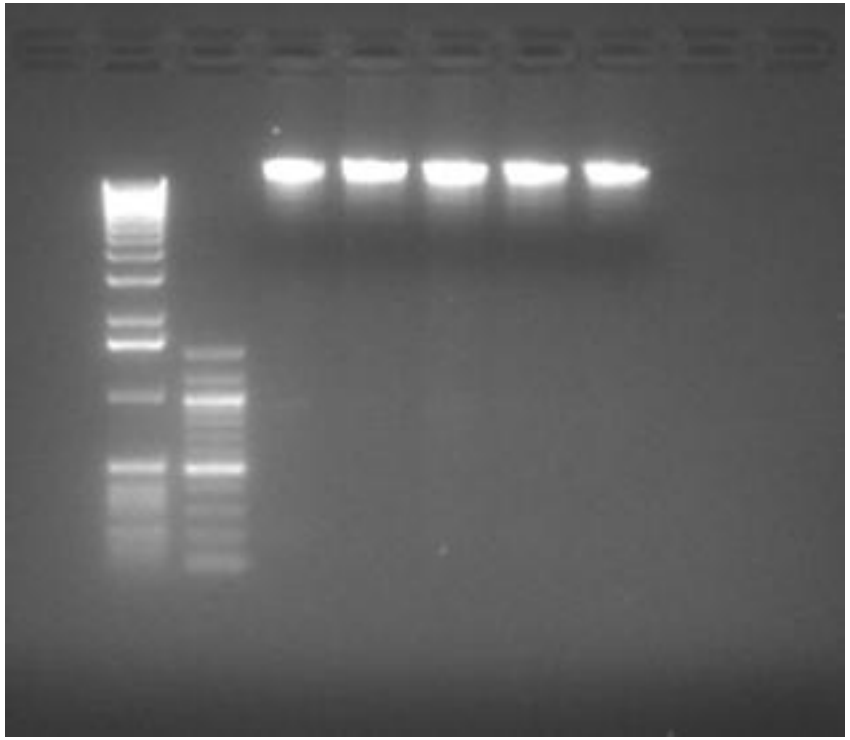
Genomic DNA of 5 species of cereals

Analyzing DNA Samples in a Research Lab

If properly done, genomic extraction should result in bright bands in the very high base pair range of a gel electrophoresis.

Sizes of Genomic DNA for various Species in kbp

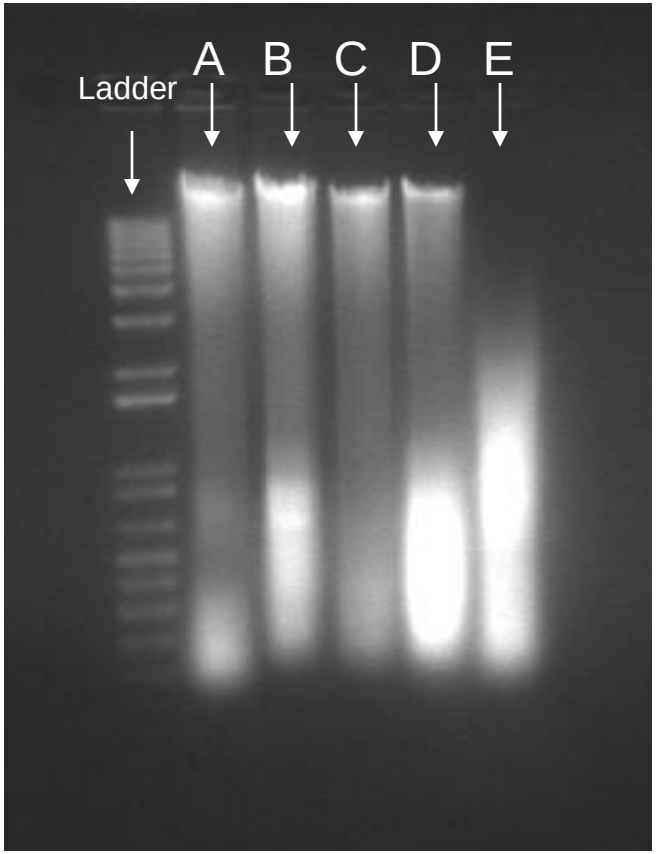
E. Coli	4,640,000bp
Yeast	12,100,000bp
Fruit Fly	140,000,000bp
Human	3,000,000,000bp
Pea	4,800,000,000bp
Wheat	17,000,000,000bp



The genomic fragments run at ~12kbp because they are sheared during extraction

Expected Results in a Classroom Lab

- This is expected. Even though this genomic DNA preparation is not perfect, it is suitable for use as a PCR template
- Lane A: Barley
  Lane B: Corn
- Lane C: Oat
- Lane D: Rice
- Lane E: Wheat



- Note that the DNA has sheared (particularly for wheat) – broken up into numerous fragments and is not a clean single band at the top – these are the mid-ranged sized fragments (1000-10,000bp size range)
- The bright bands at the 100 - 1000 bp range are RNA, which also gets extracted using this protocol

Analyzing DNA samples

in a Classroom Lab

Analysis of samples:

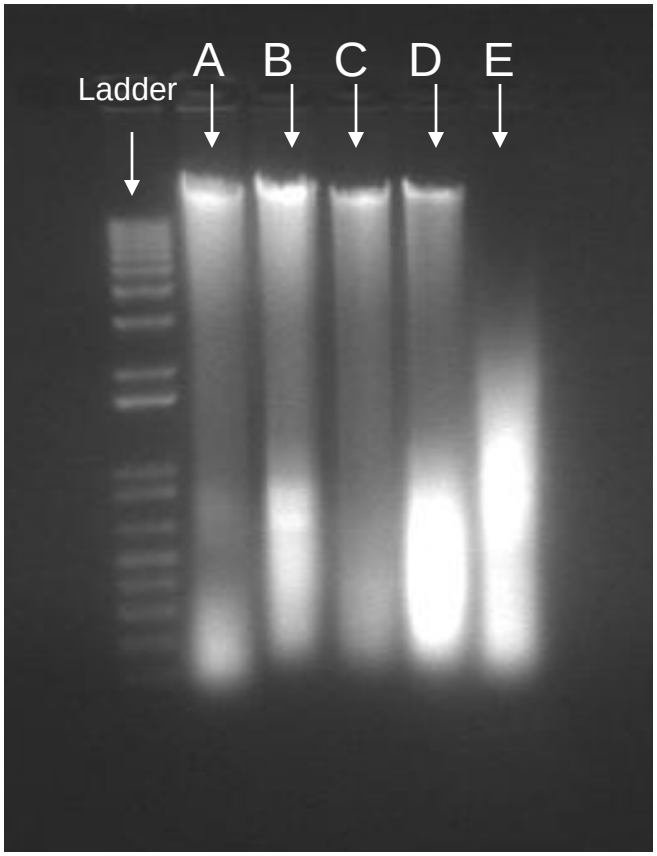
Barley (A): This sample is fine

Corn (B): This sample is fine

Oat (C) : This sample is fine

Rice (D) : This sample is fine

Wheat (E): This sample has severe degradation, can work for PCR but should re-extract



THANK YOU