A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-14; Unit C)

Nucleic Acid Isolation and Purification

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Sources of DNA include

- Blood
- Cultured cells (plant and animal)
- Bacteria
- Biopsies
- Forensic samples i.e. body fluids, hair follicles, bone & teeth roots.

DNA isolation is a routine procedure to collect DNA for subsequent molecular analysis. There are three basic steps in a DNA extraction:

- **Cell disruption**:- This is commonly achieved by grinding or sonicating the sample. Removing membrane lipids by adding a detergent.
- **Isolation of DNA**:- Removing proteins by adding a protease. RNase to digest RNA
- **Precipitating the DNA** :-usually ice-cold ethanol or isopropanol is used. Since DNA is insoluble in these alcohols, it will aggregate together, giving a *pellet* upon centrifugation. This step also removes alcohol soluble salt.

Basic rules

- **Blood** – first lyse (explode) the red blood cells with a gentle detergent such as Triton-X-100.
- **Wash cells** – haemoglobin (and other pigments) inhibits restriction enzymes and TAQ polymerase.
- Work on **ice** to slow down enzymatic processes.
- Wear **gloves** to protect your samples from you!!
- **Autoclave** all solutions and store in fridge (except SDS and organic solvents!)
- Keep all pellets & supernatants until you have the DNA you want.

Getting to the DNA

- Cells – **lyse** all cells in presence of :

- **NaCl** so that DNA is stabilised and remains as a double helix,
- **EDTA** which chelates Mg++ and is a co-factor of DNAse which chews up DNA rapidly.
- **anionic detergent SDS** which disrupts the lipid layers, helps to dissolve membranes & binds positive charges of chromosomal proteins (*histones*) to release the DNA into the solution.
- Include a **protease** *(proteinase K*) to digest the proteins
- incubate the solution at an **elevated temperature** (56^{o}C to inhibit degradation by DNAses) for 4-24 hrs.

Getting rid of the protein

- **Organic solvent extraction** using equal volume phenol:chloroform (24:1)

- Protein at the interface after centrifugation (10000 rpm at 4^{o} c for 10 min.)

Precipitating the DNA

- add 2.5 - 3 volumes **ice-cold 95% ethanol** to the DNA & leave at -20^{o}C overnight.
- Centrifuge sample at 10000 rpm ,10 min., 4^{0}C.
- **Wash** DNA pellet to remove excess salt in 70% EtOH and air-dry.
- **Resuspend** in sterile distilled water(pH7.4)
- Store at 4^{o}C or frozen at -20^{o}C long term.

Quantifying the DNA

- The amount of DNA can be quantified using the formula:

$$\text{DNA concentration } (\mu g/ml) = \frac{OD_{260} \times 100 \text{ (dilution factor)} \times 50\ \mu g/ml}{1000}$$

- Nucleic acids have a peak absorbance in the ultraviolet range at about 260 nm
- 1 A260 O.D. unit for dsDNA = 50 µg/ml
- 1 A260 O.D. unit for ssDNA = 33 µg/ml
- 1 A260 O.D. unit for RNA = 40 µg/ml

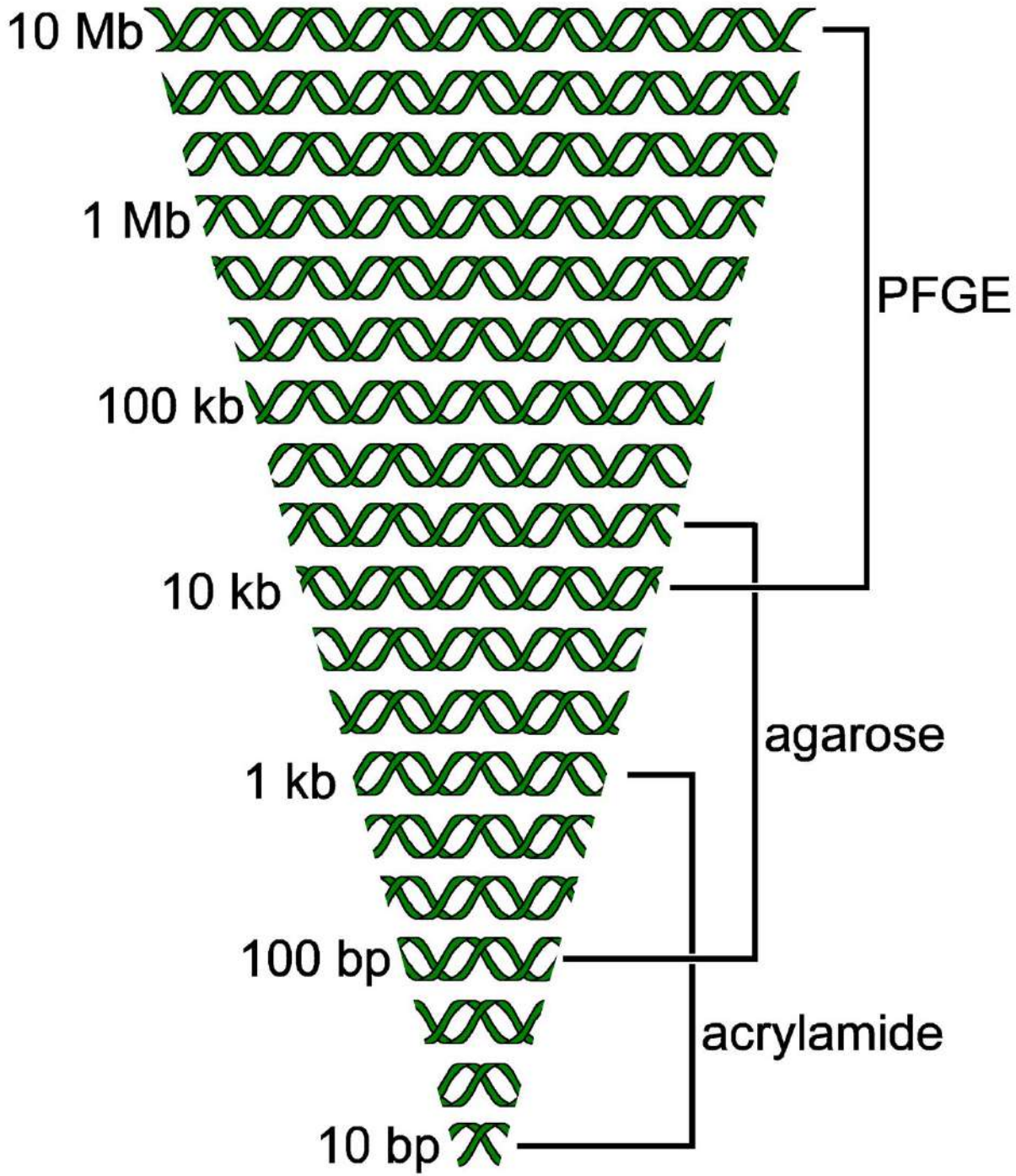
DNA purity

- The purity of the DNA is reflected in the OD260:OD 280 ratio and must be between 1.6 and 2.00.

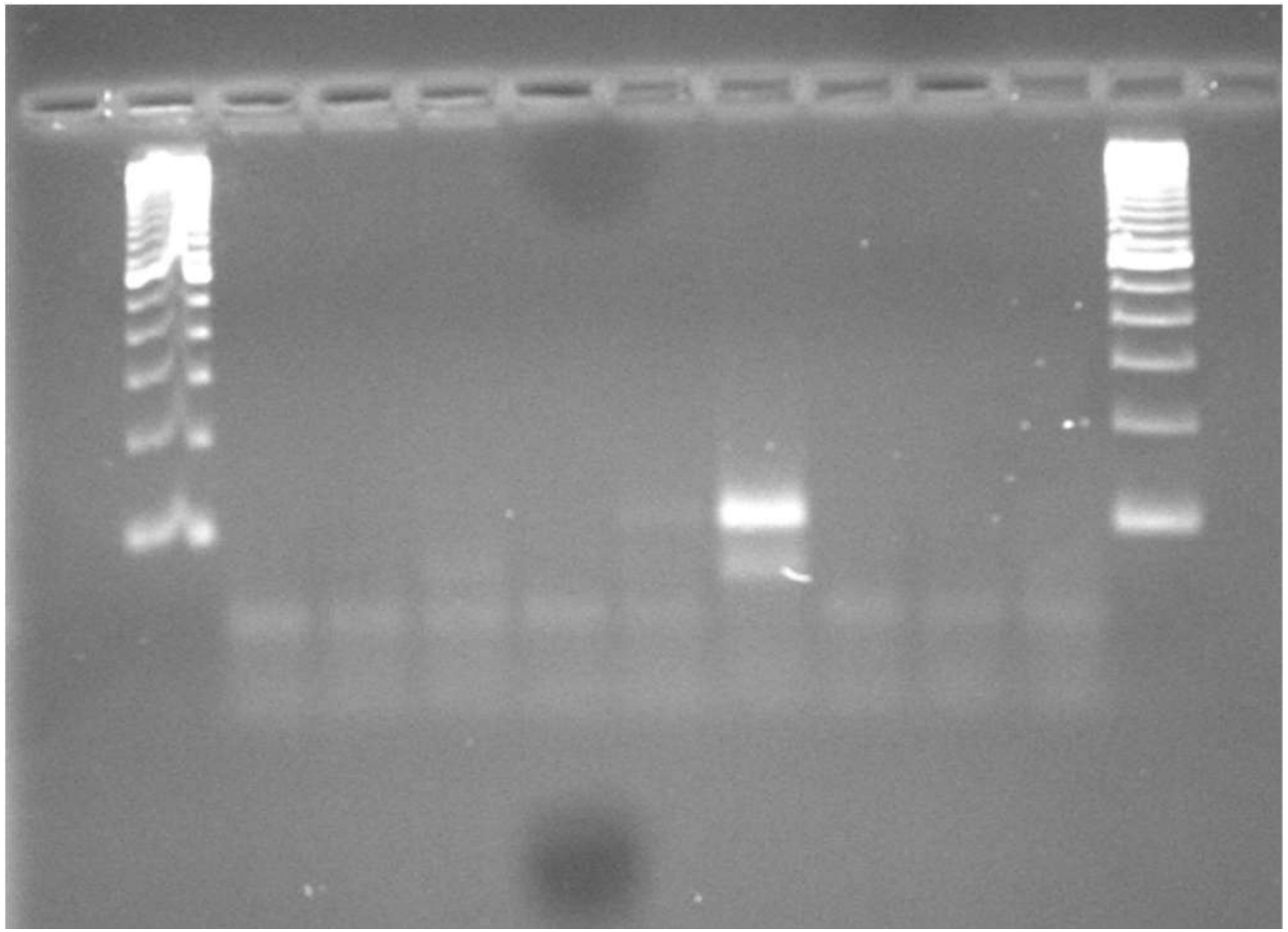
< 1.6 – protein contaminated

> 2.0 – chloroform / phenol contaminated

- Repurify sample.


10 Mb
1 Mb
100 kb
10 kb
1 kb
100 bp
10 bp
PFGE
agarose
acrylamide

Agarose Gel
Stained with ethidium bromide (EtBR) to Visualize the DNA



slots where DNA is loaded

1000 bp

700 bp

600 bp

500 bp

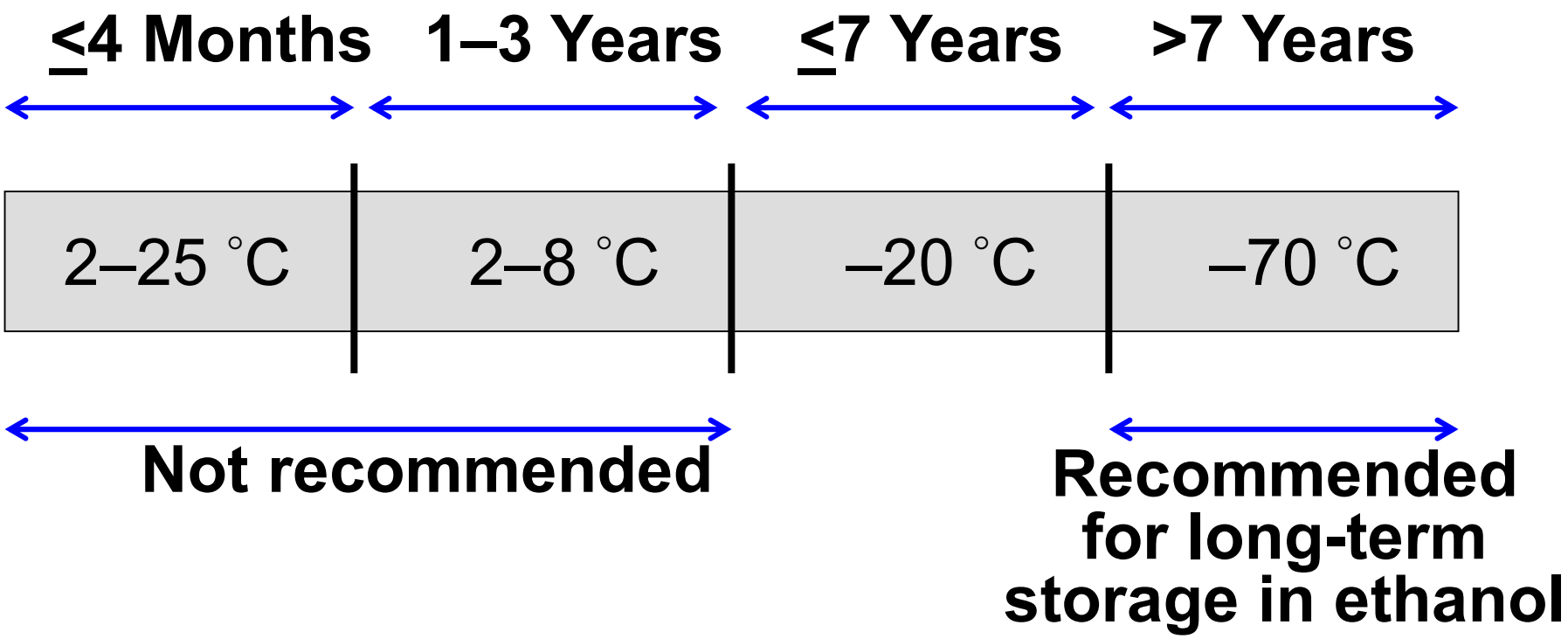
Screening PCR products to test for the presence of specific DNA sequences

molecular weight markers

correct PCR product

molecular weight markers

Nucleic Acid Storage Requirements: Storage of DNA Specimens



Basic Steps in Isolating RNA from Clinical Specimens

Separate WBCs from RBCs, if necessary

↓

Lyse WBCs or other nucleated cells in presence of protein denaturants, RNase inhibitors

↓

Denature/digest proteins

↓

Separate proteins, DNA, and contaminants from RNA

↓

Precipitate RNA if necessary

↓

Resuspend RNA in final buffer

Precautions for Working with RNA in the Clinical Laboratory

- **RNA is not a stable molecule!**
  - It is easily degraded by RNase enzymes.
- Use sterile, disposable plastic ware (tubes, filter tips) marked "**For RNA Use Only**".
- Always wear gloves and work in a hood whenever possible/practical.
- Treat liquids with DEPC, except Tris-based buffers.

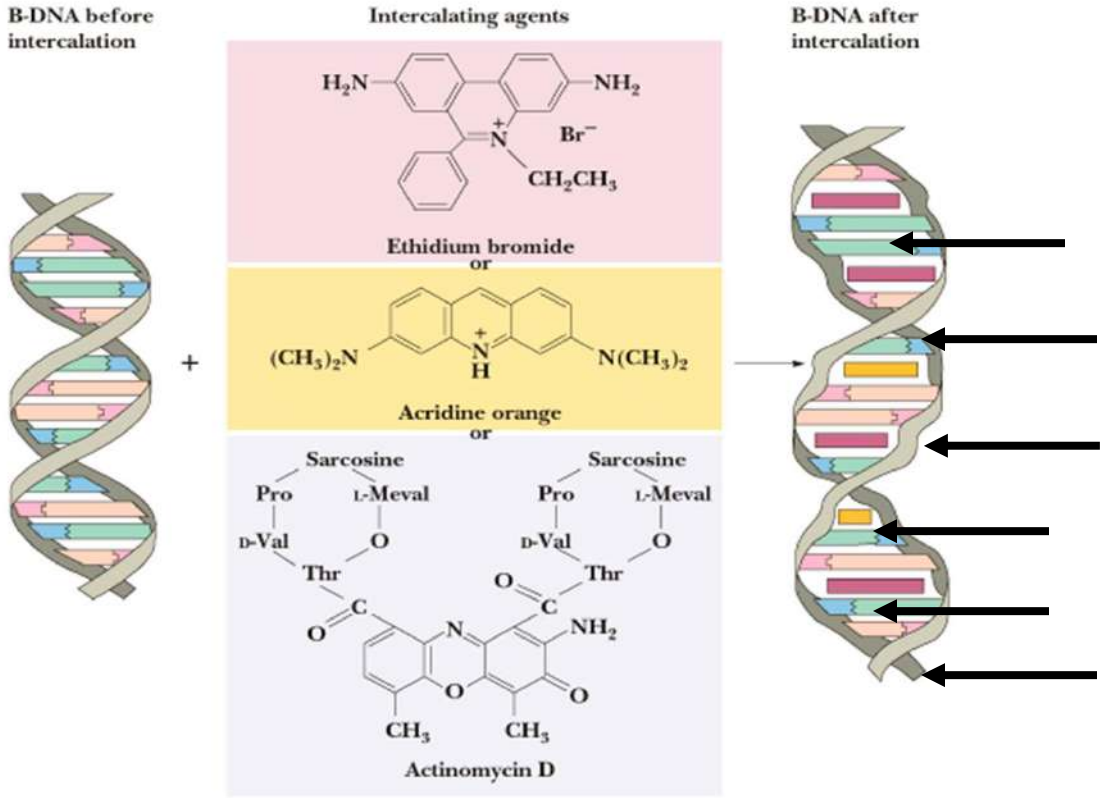
RNA Isolation Methods
Nonorganic Salt Precipitation

- Cell membranes are lysed and proteins are denatured by detergent (such as SDS) in the presence of EDTA or other RNase inhibitors.
- RNA is precipitated with alcohol and rehydrated.
- Advantages:
  - Fast and easy, nontoxic
  - Produces high quality RNA

Intercalating Agents Distort the Double Helix

Several hydrophobic molecules containing flat aromatic and fused heterocyclic rings can insert between the stacked base pairs of DNA. These molecules are called intercalating agents.

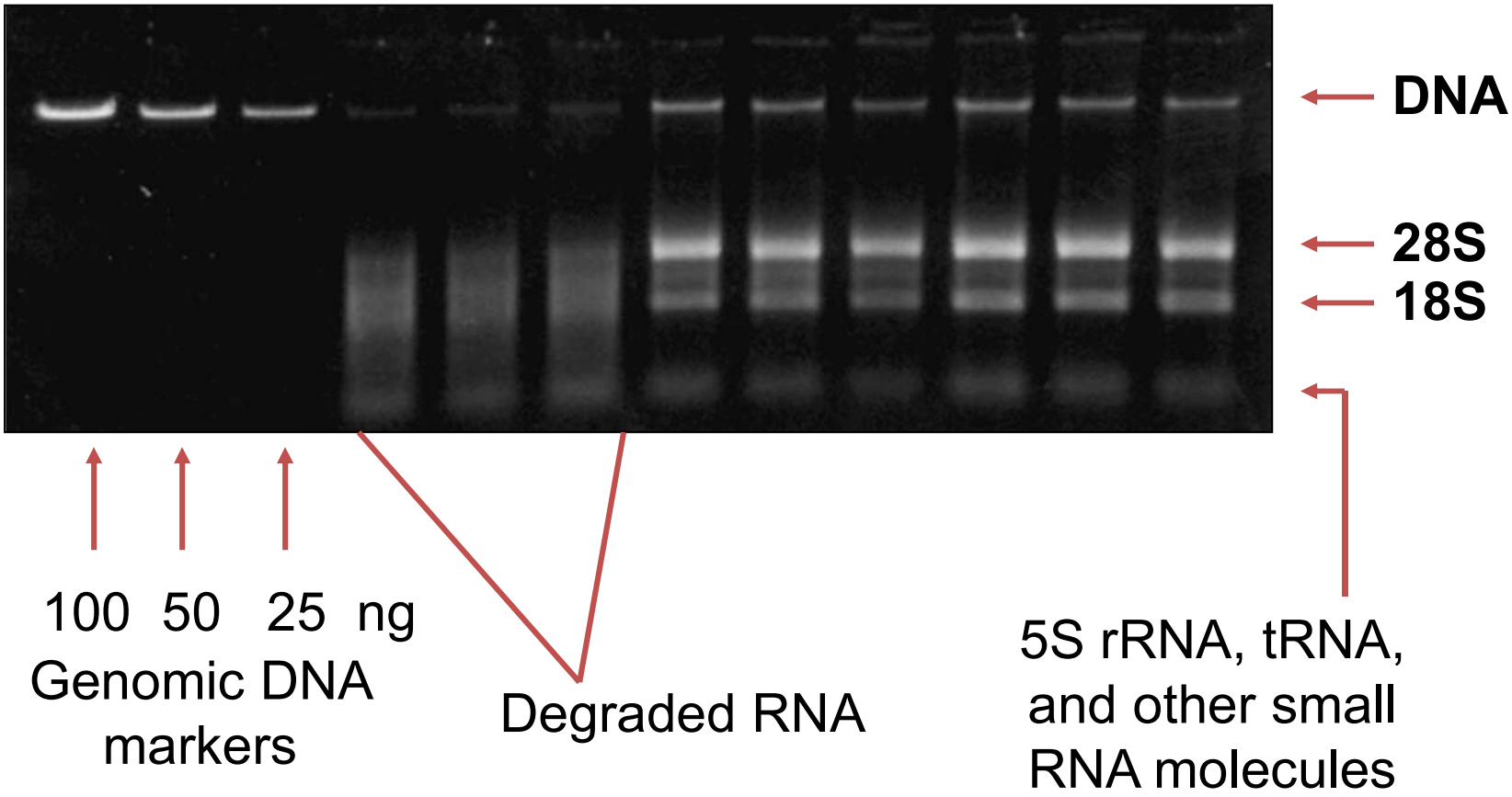
Intercalating agents are potential Cancer-inducing reagents.



Quality from Agarose Gel Electrophoresis

- Genomic DNA:
  - 0.6% to 1% gel, 2.5-3.0 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, 2.5-3.0 µg/ml ethidium bromide in gel and/or in running buffer
  - Electrophorese at 80–100 volts, 20–40 minutes.

Cultured Cell RNA



Storage Conditions

- Store DNA in TE buffer at 4 °C for weeks or at –20 °C to –80 °C for long term.
- Store RNA in RNase-free ultra pure water at –70 °C.

Summary

- Sample for DNA and RNA extraction
- Lysis of cells at elevated temperature + detergent + enzyme in salt buffer
- Removal of cellular proteins
- Precipitation of nucleic acids with ethanol
- Quantitation and purity measurement of DNA

Acknowledgement and Suggested Readings:
1. Biotechnology Procedures and Experiments Handbook; S. Harisha; Infinity science Press LLC
2. Molecular cloning, A laboratory Manual; Sambrook and Russell; Third Edition; Cold Spring Press

Thanks