Experiment 3 &4: Extraction of DNA and RNA
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Experiment 3: Extraction of DNA(Deoxyribonucleic Acid)

Principle:

DNA is extracted by lysing the cells to release the nuclear content, denaturing proteins, and precipitating DNA using cold alcohol. The process involves breaking cellular membranes, removing proteins and other contaminants, and isolating DNA as visible threads.

Theoretical Background:

DNA is the hereditary material composed of nucleotides. Extraction of DNA is essential for genetic analysis, PCR, and sequencing. Common reagents used help in lysing membranes (SDS), inhibiting DNases (EDTA), removing proteins (phenol-chloroform), and precipitating DNA (ethanol).

Reagents and Their Purpose:

Reagent	Purpose / Use
SDS (Sodium Dodecyl Sulfate)	Detergent; breaks cell and nuclear membranes
EDTA	Chelates divalent cations (Mg^{2+}, Ca^{2+}); inhibits DNase activity
NaCl	Maintains ionic strength; helps DNA precipitation
Proteinase K	Digests proteins including nucleases
Phenol-Chloroform	Separates proteins from nucleic acids
Cold Ethanol or Isopropanol	Precipitates DNA by reducing its solubility
TE Buffer (Tris-EDTA)	Stabilizes and stores DNA
Centrifuge	Separates pellet from supernatant during extraction steps

Procedure:

1. Homogenize the biological sample (e.g., cells or tissue) in extraction buffer (Tris, EDTA, SDS, NaCl).
2. Incubate at 60°C for 10 minutes to allow membrane lysis.
3. Add Proteinase K and incubate at 37°C for 15–30 min to digest proteins.
4. Add equal volume of phenol-chloroform and gently invert the tube.
5. Centrifuge at 10,000 rpm for 10 min; collect the upper aqueous phase.

6. Add two volumes of cold ethanol; DNA will precipitate as white filaments.
7. Spool or centrifuge to collect DNA pellet.
8. Wash pellet with 70% ethanol, dry briefly, and dissolve in TE buffer.
9. Store at 4°C or −20°C.

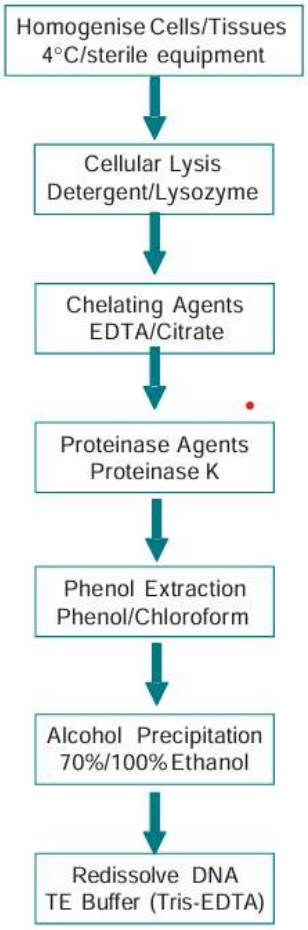

Fig. 1.21 General steps involved in extracting DNA from cells or tissues.

Short Questions:

1. What is the role of SDS in DNA extraction? → To lyse cell membranes.
2. Why is EDTA added? → To chelate Mg^{2+} and inhibit DNases.
3. How does DNA appear after precipitation? → As white, thread-like strands.
4. Why is ethanol used cold? → Cold temperature enhances DNA precipitation.
5. What is the A260/A280 ratio for pure DNA? → Around 1.8.

Multiple-Choice Questions:

1. What is the main function of EDTA in DNA extraction?
   a) Break proteins b) Chelate Mg^{2+} c) Precipitate DNA d) Digest membranes

2. Which reagent lyses the cell membrane?
   a) NaCl b) SDS c) EDTA d) Tris

3. DNA is precipitated using:
   a) Water b) Ethanol c) Acetone d) Sodium hydroxide

4. The protein removal step uses:
   a) SDS b) Phenol-chloroform c) NaCl d) DEPC

5. DNA purity is assessed using:
   a) Gel electrophoresis b) UV absorbance c) pH meter d) Conductivity meter

Common Abbreviations:

- DNA – Deoxyribonucleic Acid
- SDS – Sodium Dodecyl Sulfate
- EDTA – Ethylenediaminetetraacetic Acid
- TE – Tris-EDTA Buffer
- OD – Optical Density.

Reference:

Nelson, D.L. & Cox, M.M. (2021). Lehninger Principles of Biochemistry (8th ed.). W.H. Freeman.

Experiment 4: Extraction of RNA (Ribonucleic Acid)

Principle:
RNA is extracted by disrupting cells in the presence of strong denaturants to inhibit RNases. The guanidinium thiocyanate–phenol–chloroform method separates RNA from DNA and proteins, followed by precipitation with isopropanol or ethanol.

Theoretical Background:
RNA is single-stranded and highly susceptible to enzymatic degradation by RNases. Therefore, RNA extraction requires stringent RNase-free conditions. Guanidinium salts denature proteins and RNases, while DEPC-treated water and β-mercaptoethanol protect RNA integrity.

Reagents and Their Purpose:

Reagent	Purpose / Use
GITC (Guanidinium Thiocyanate)	Denatures proteins and RNases; lyses cells
β-Mercaptoethanol	Reduces disulfide bonds in RNases
Phenol-Chloroform	Separates RNA from DNA and proteins
Isopropanol	Precipitates RNA by reducing solubility
70% Ethanol	Washes RNA pellet and removes salts
DEPC-treated Water	RNase-free water for dissolving RNA
Centrifuge	Separates aqueous and organic phases and pellet
Ice or Cold Conditions	Maintains RNA stability throughout the procedure

Procedure:

1. Homogenize tissue or cells in lysis buffer containing GITC and β-mercaptoethanol.
2. Incubate briefly on ice for cell lysis.
3. Add equal volume of phenol-chloroform, mix by inversion.
4. Centrifuge at 12,000 rpm for 10–15 min at 4°C.
5. Transfer the upper aqueous phase to a new RNase-free tube.
6. Add 0.5–1 volume of isopropanol to precipitate RNA.
7. Incubate on ice for 10–15 min, then centrifuge to pellet RNA.
8. Wash pellet with 70% ethanol, air dry, and dissolve in DEPC-treated water.
9. Store RNA at −80°C.

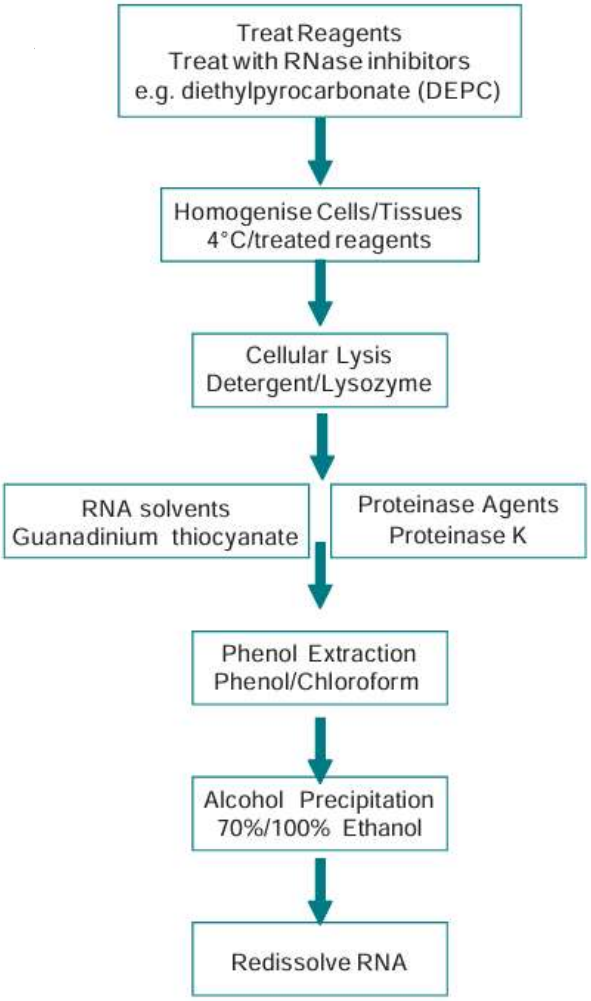

Fig. 1.22 General steps involved in extracting RNA from cells or tissues.

Short Questions:

1. Why is RNA extraction more sensitive than DNA? → Because of RNase contamination.
2. What is the role of GITC? → Denatures RNases and proteins.
3. Why add β-mercaptoethanol? → To break RNase disulfide bonds.
4. Why is isopropanol used instead of ethanol sometimes? → Precipitates RNA more efficiently.
5. Ideal A260/A280 ratio for RNA purity? → Around 2.0.

✓ Multiple-Choice Questions:

1. DEPC-treated water is used to:
   a) Inhibit DNases b) Wash protein c) Prevent RNase activity d) Precipitate RNA

2. Which chemical inactivates RNases during RNA extraction?
   a) SDS b) β-mercaptoethanol c) NaCl d) Tris

3. The reagent used to denature proteins in RNA extraction is:
   a) NaOH b) GITC c) Glucose d) Tris

4. Which temperature is ideal for RNA handling?
   a) 37°C b) Room temp c) On ice d) 60°C

5. A260/A280 ratio for pure RNA should be:
   a) 1.6 b) 1.8 c) 2.0 d) 2.2

Common Abbreviations:

- RNA – Ribonucleic Acid
- GITC – Guanidinium Thiocyanate
- DEPC – Diethylpyrocarbonate
- RNase – Ribonuclease
- OD – Optical Density

Reference:

- Nelson, D.L. & Cox, M.M. (2021). Lehninger Principles of Biochemistry (8th ed.).
- Watson et al. (2018). Molecular Biology of the Gene (7th ed.).
- Harper's Illustrated Biochemistry (32nd ed., 2023).