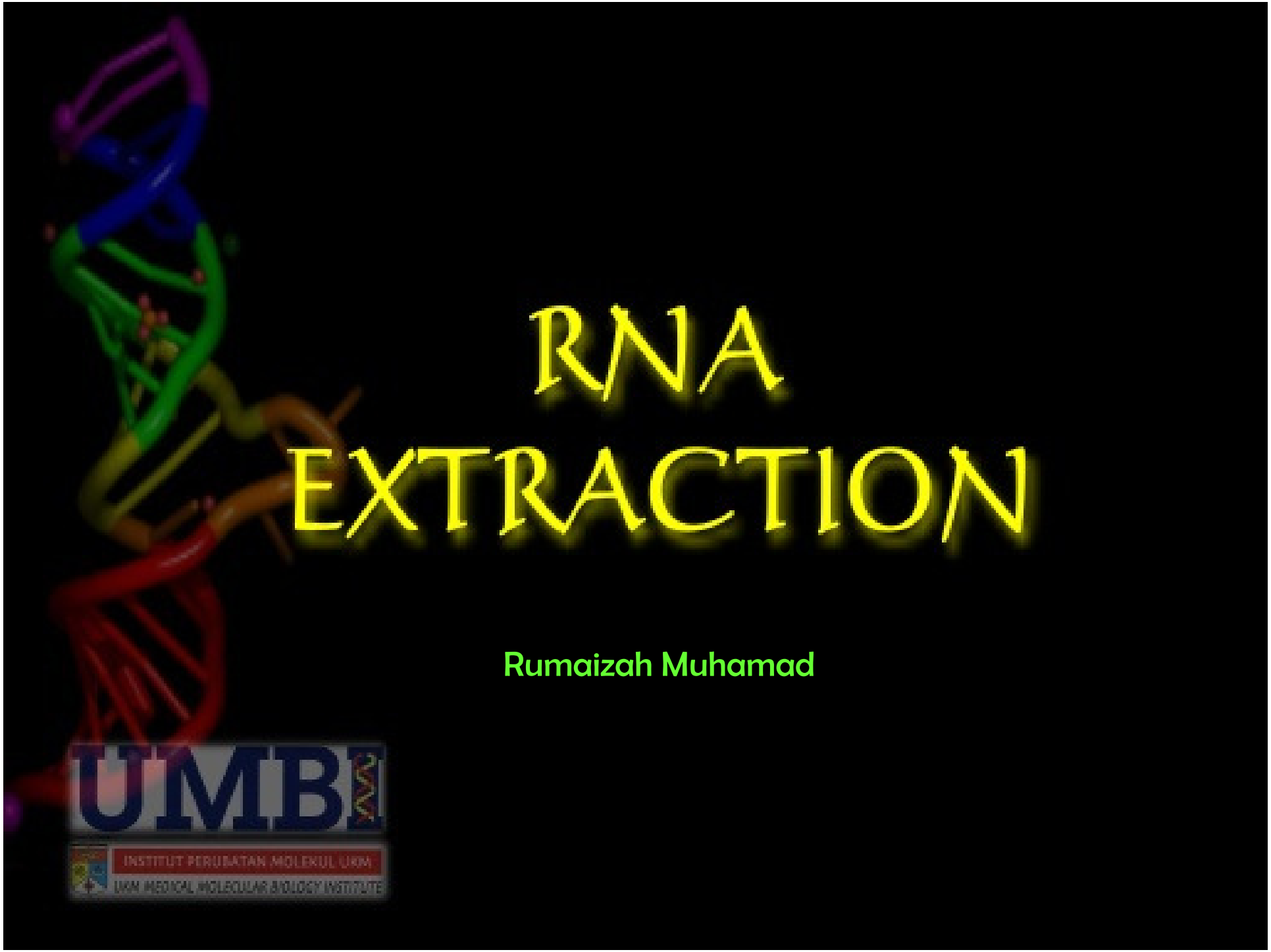
RNA
EXTRACTION
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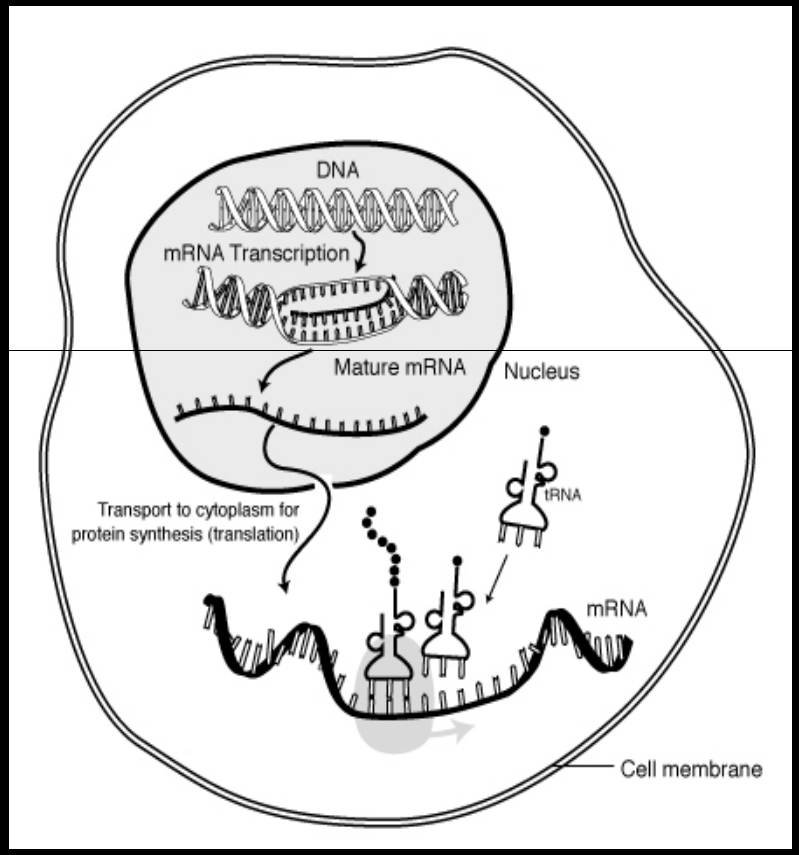
What is RNA?

- RNA = Ribonucleic acid.
- A type of nucleic acid with only one strand - ribose instead of deoxyribose and using uracil instead of thymine (in DNA).
- Provides the link between the genetic information through protein synthesis (serve as template for protein synthesis).
- Total RNA= rRNA (~85%),
  mRNA (~2%),
  tRNA and other
  molecules (~10 – 15%)



The Purpose of RNA Extraction

- Isolation of intact RNA is essential for many techniques used in gene expression analysis such as:
  - Microarray analysis
  - Northern analysis
  - cDNA library construction
  - RT-PCR

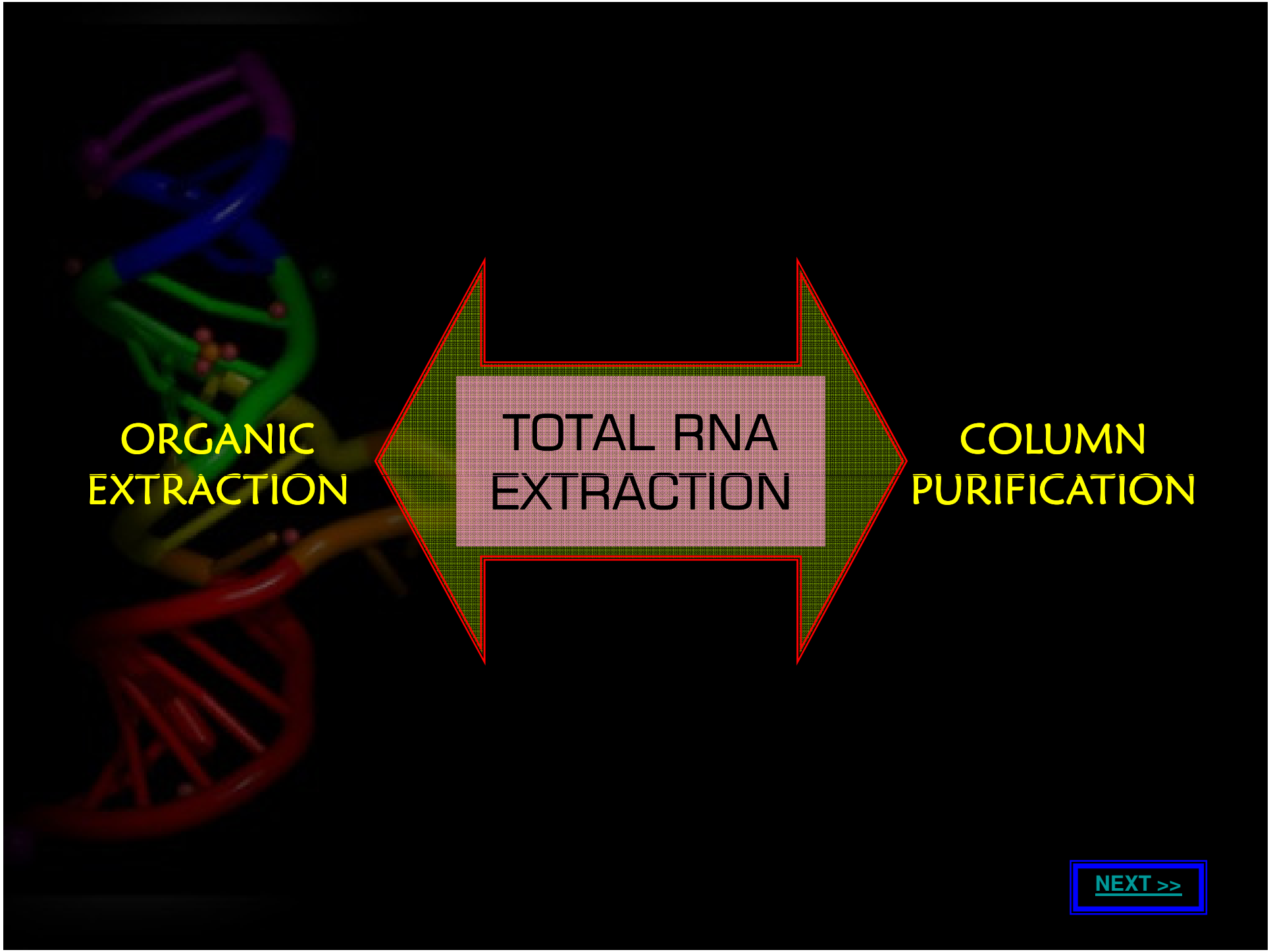
Three ways to handle samples prior to RNA extraction

Immediately disrupt the fresh samples

Freeze the samples in liquid nitrogen

Store the samples in RNA*later*

(Recommended by Ambion Inc.,)


ORGANIC
EXTRACTION
TOTAL RNA
EXTRACTION
COLUMN
PURIFICATION
NEXT >>

ORGANIC EXTRACTION

HOW IT WORKS??

Organic extraction (acidified phenol and chloroform) removes proteins, lipids, and DNA from the RNA sample. RNA is then recovered by alcohol precipitation.

ADVANTAGES

(i) Can be use for large or small sample sizes

(ii) Can modify extractions to remove high levels of fat and protein from samples.

DISADVANTAGE

(i) Phenol and chloroform are hazardous

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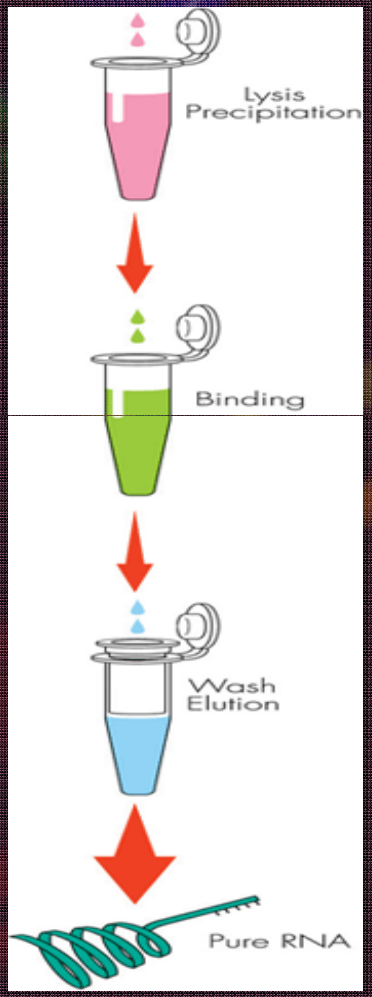
COLUMN PURIFICATION



HOW IT WORKS??

- Glass filters bind the RNA while other cellular components are washed away.
- RNA is eluted in a highly purified form.

ADVANTAGES

(i) Rapid procedure
(ii) No organic solvents required
(iii) No alcohol precipitation needed.

DISADVANTAGE

Not as scalable as organic extraction methods..

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How to prepare AGPG/ TRI reagent Solution

TRI Reagent (a.k.a=Acid Guanidinium Thiocyanate-Phenol-Chloroform) Solution

Starting Solution

- 4M Guanidinium Thiocyanate
- 0.75M Sodium citrate pH 7.0
- 2 M Sodium Acetate pH 4.0
- 10% Sarcosyl (1g/10ml H^2O and filter)
- Phenol (nucleic acid grade, Sigma) (Melt phenol at 65°C, add 0.1% w/v hydroxyquinoline and saturate with H^2O)

Cont. - For 21ml of AGPC (TRI Reagent)

Mix 10ml 4M guanidinium thiocyanate, 352µl 0.75M sodium citrate (pH 7.0) and 528µl 10% sarcosyl.

↓

Add 76µl 14.3 β-mercaptoethanol

(total final volume=10.9ml)

↓

Place 10ml of this mixture in a new tube, add 1ml 2M sodium acetate (pH4.0) and 10ml water-saturated phenol.

↓

This is the final AGPC solution

(This solution is good for at least 2 months at 4°C)

STEPS FOR RNA EXTRACTION

HOMOGENIZATION

1 mL TRI Reagent + 50 – 100 mg tissue/ 5 – 10 x 10^6 cells/ 10 cm^2 culture plate

→ Store for 5 min at room temperature (RT)

PHASE SEPARATION

Homogenate + 0.1 mL chloroform

→ Vortex for 15 seconds, leave at RT for 2 – 3 minutes

← Centrifuge at 12 000 g for 15 min at 2 - 8°C

RNA PRECIPITATION

Aqeous phase + 0.5 mL isopropanol

→ Leave at RT for 10 min

← Centrifuge at 12 000 g for 15 min at 2 - 8°C

RNA WASH

1 mL 75% ethanol for every 1 mL TRI Reagent

→ Vortex
Centrifuge at 7500 g for 5 min at 2 - 8°C

← Air dry 2 – 3 min

RNA SOLUBILIZATION

81 µL RNase-free water

NEXT >>

HOMOGENIZATION

- The first step in RNA extraction is to break down the cells or tissue so that the nucleic acids are released from the cells.
- Homogenization
  - Tissue: mortar and pestle
  - Cells: repetitive pipetting (resuspend)
- RNA extraction methods use a powerful chaotropic salt solution. This 'lysis solution' rapidly disrupts cells without destroying their nucleic acids.
- Total RNA isolation reagent (TRI reagent) as 'lysis solution' combines phenol and guanidine thiocyanate.

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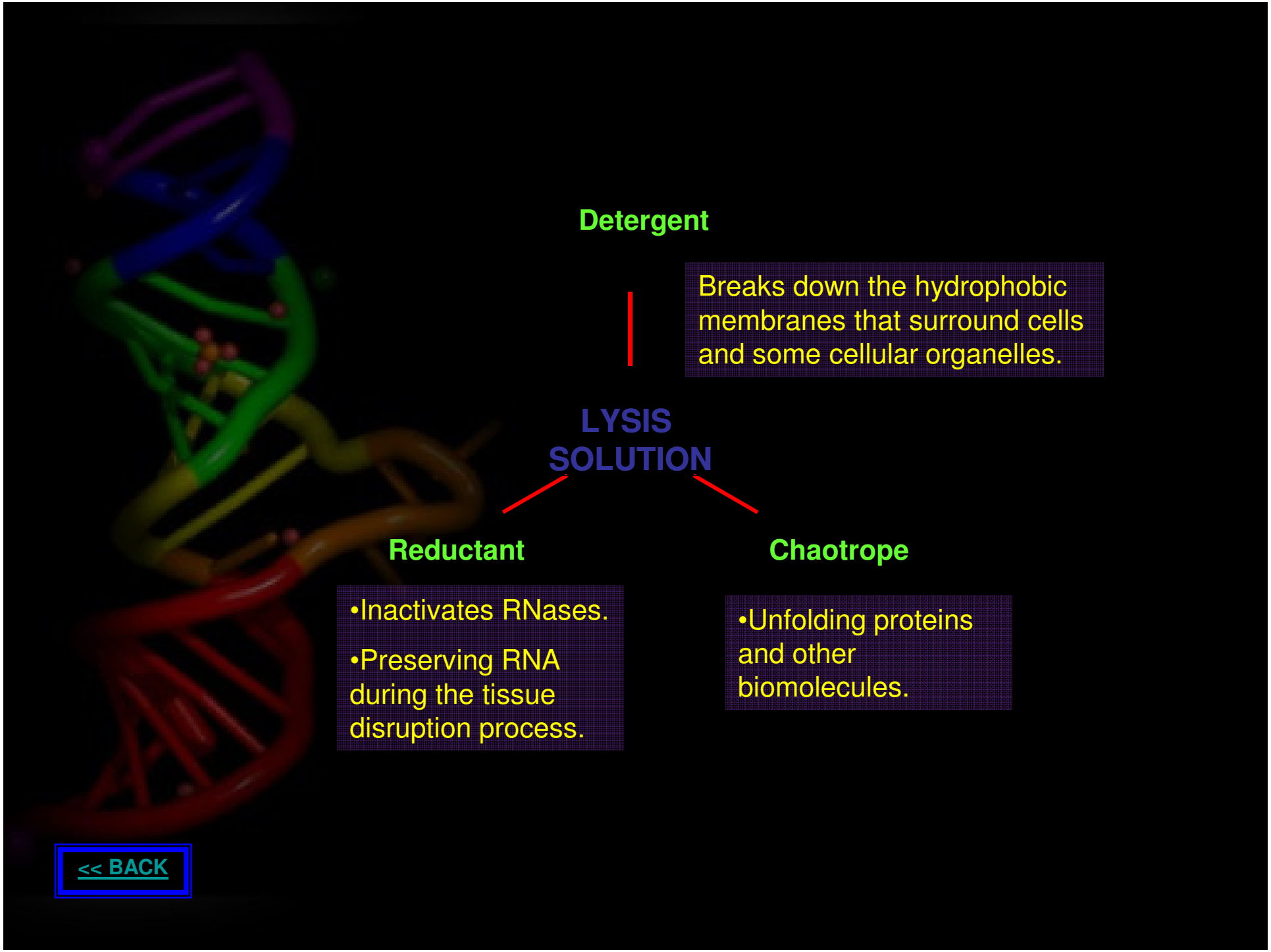
Detergent
Breaks down the hydrophobic membranes that surround cells and some cellular organelles.
LYSIS SOLUTION
Reductant
•Inactivates RNases.
•Preserving RNA during the tissue disruption process.
Chaotrope
•Unfolding proteins and other biomolecules.
<< BACK

PHASE SEPARATION

- Homogenate (from homogenization step) must be store for 5 min at RT to
  - permit the complete dissociation of nucleoprotein complexes.
- Chloroform used to:
  - Separate solution in aqueous phase, interphase and organic phase
  - RNA in aqueous phase, DNA (interphase) and protein (organic phase)
- RNA, DNA and protein separation are based on density centrifugation.
- Centrifugation in COLD temperature (2 - 8°C). If perform at high temperature - a residual amount of DNA may mix in the aqueous phase.

Before separation

After separation

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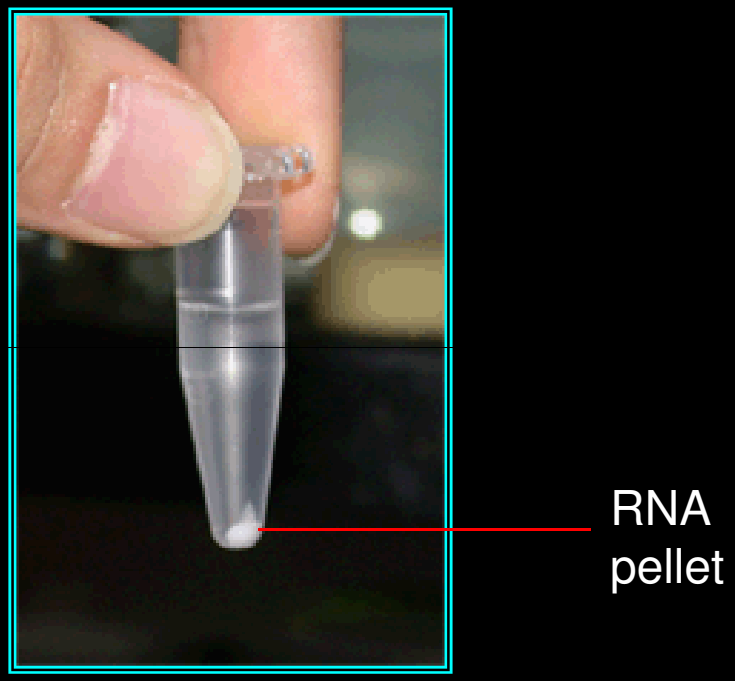
Cont. – PHASE SEPARATION

- Why use chloroform??
  - Chloroform is organic solvent. So, lysed cell components that are hydrophobic will be trapped in these solvent (eg: membrane lipids, hydrophobic polypeptide sequences (protein) or polysaccharides etc.)

RNA PRECIPITATION

- Isopropanol: Precipitate RNA from the aqueous phase.
- RNA precipitate (often visible before centrifugation) forms a gel-like or white-pellet on the side and bottom of the tube.



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RNA WASH & SOLUBILIZATION

- 75% ethanol:
  - Wash RNA pellet away from any salt residual.
- RNase free water:
  - Clean RNA resuspended (RNase free water) to ensure stability and long term storage.

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RNA QUALITY CONTROL

- Can be assessed by spectrophotometer (eg: NanoDrop)
- Ratio of the absorbance at 260 and 280 nm is used to assess the RNA purity.
- Value 1.8-2.0 indicates that the RNA is pure.
- If <1.8 : protein contamination

  > 2.0: solvent contamination

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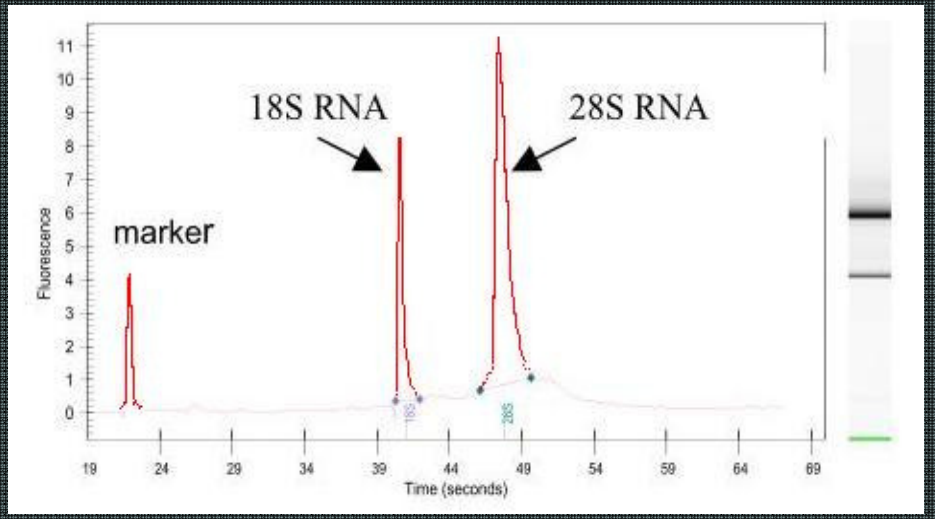
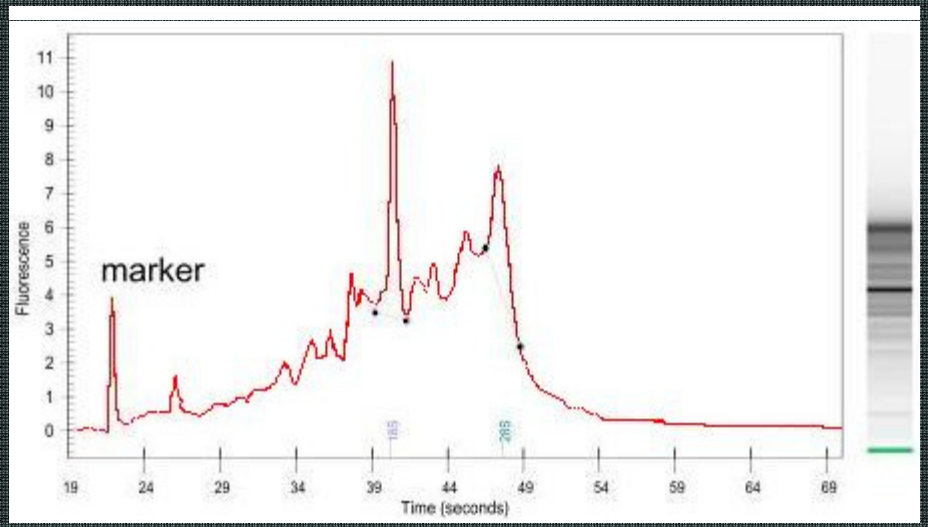
- The quality of RNA can be astimated by Agilent Bioanalyzer.
- The integrity of total RNA samples can be determined by RNA integrity number.



High quality total RNA



Partially degraded total RNA

The isolated RNA quality check by Agilent Bioanalyzer System

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Troubleshooting

- If you get bad purity of RNA:
  - Eliminating DNA contamination with DNase treatment
    - To remove residual genomic DNA and obtain highly pure RNA.
  - Purify with column purification

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Thank you ...