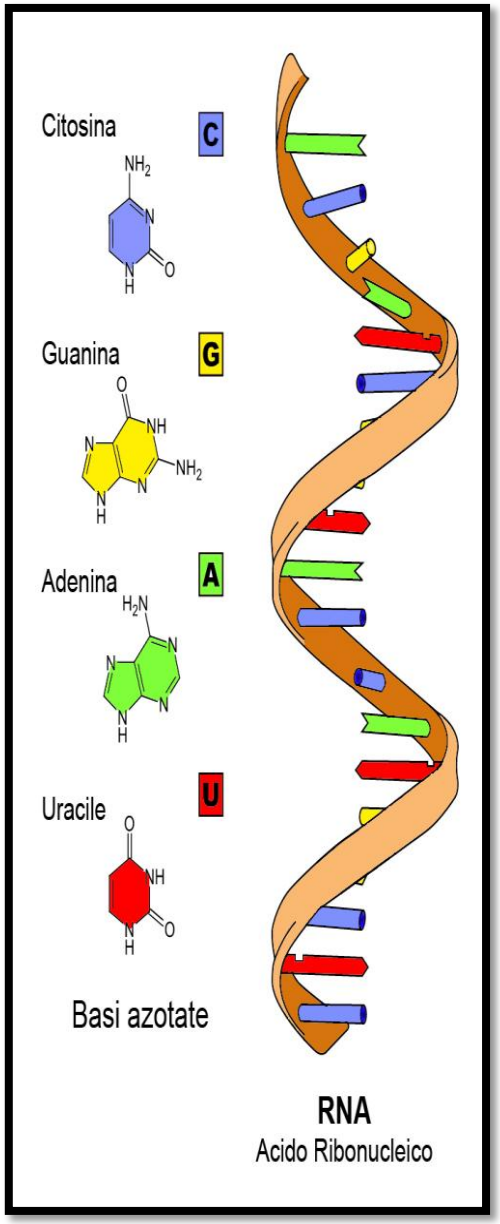
Ribonucleic acid (RNA)

- **Ribonucleic acid** (**RNA**) is more often found in nature as a single-strand folded onto itself. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases guanine, uracil, adenine, and cytosine) that directs synthesis of specific proteins.
- Many viruses encode their genetic information using an RNA genome.

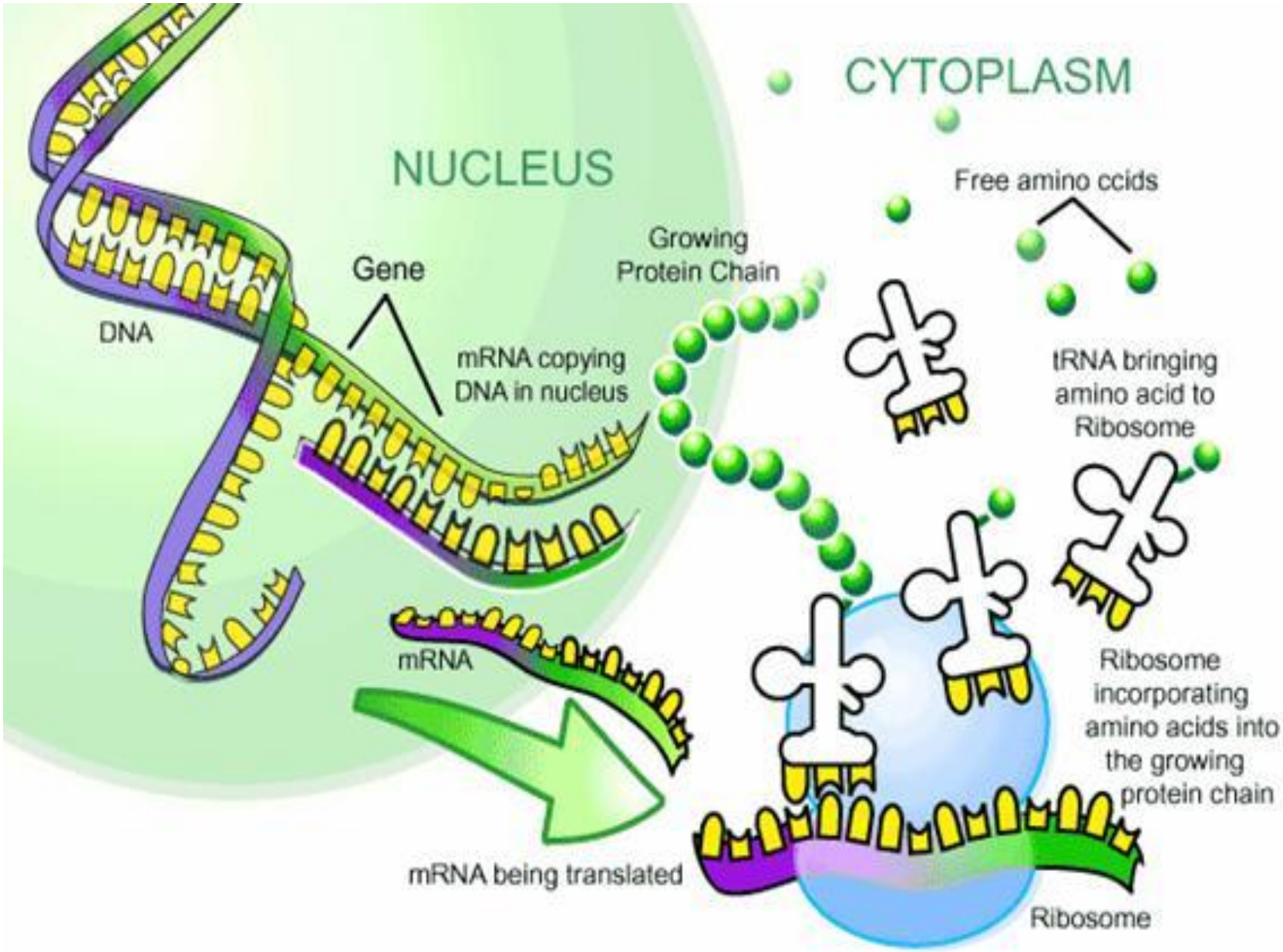


Types of RNA
1. Messenger RNA (mRNA)

- **Messenger RNA** (**mRNA**) is a large family of RNA molecules that convey genetic information from DNA to the ribosome.

- T**ransfer RNA** (**tRNA**) is an adaptor molecule composed of RNA, typically 76 to 90 nucleotides in length, that serves as the physical link between the mRNA and the amino acid sequence of proteins.

- **Ribosomal ribonucleic acid** (**rRNA**) is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms.


CYTOPLASM
NUCLEUS
Free amino ccids
Growing
Protein Chain
Gene
DNA
mRNA copying
DNA in nucleus
tRNA bringing
amino acid to
Ribosome
mRNA
Ribosome
incorporating
amino acids into
the growing
protein chain
mRNA being translated
Ribosome

RNA extraction protocol

- the extraction, purification, and assay of total RNA from human or animal tissue samples.
- This protocol is optimized for the extraction of RNA from small samples of animal tissue and is applicable to both lipid-rich and lipid-poor tissues.

MATERIALS

1. TRIzol® Reagent
2. Ethanol
3. Isopropanol
4. Chloroform
5. Centrifuge Tubes
6. RNAse-free water (DEPC treated)
7. DNAse (Kit)
8. Buffer TE, pbs pH 8.0, RNAse-free water
9. Petri Dish

PBS (7.4)
(Phosphate-Buffered Saline)

- Its a buffer solution commonly used in biological research.
- It is a water-based salt solution containing sodium phosphate, sodium chloride
- The osmolarity and ion concentrations of the solutions match those of the human body (isotonic).

Applications of PBS

1. PBS is isotonic and non-toxic to cells.

2. supports the cell viability by keeping the medium pH and osmolality.

3. Commonly used in biological research

4. Tissue culture and cell washing.

5. Used for molecular biology research

DEPC (Diethylpyrocarbonate) also called diethyl dicarbonate

- The DEPC-treated water (and therefore RNase-free) is used in handling of RNA in the laboratory to reduce the risk of RNA being degraded by RNases.
- DEPC are used for probing the structure of double-stranded DNA.
- The higher concentrations of DEPC are capable of deactivating larger amounts of RNase.

Preliminary Steps
A. Recommendations for RNase-free Technique

RNase contamination will cause experimental failure.
Minimize RNase contamination by adhering to the following
• Always handle RNA in a manner that avoids introduction of RNases.
• Wear disposable gloves and change them frequently to prevent the introduction of RNases from skin surfaces.
• After putting on gloves, avoid touching surfaces that may introduce RNases onto glove surfaces.
• Do not use reagents not supplied in the RNA Isolation Kit. Substitution of reagents or kit components may adversely affect yields or introduce RNases.
• Use only new plasticware that is certified nucleic acid-free.
• Use only new, sterile, RNase-free pipette tips and microcentrifuge tubes.

B. Recommendations for Storing RNA

- Begin the RNA Isolation Kit protocol immediately

following acquisition of cells by The Extraction Buffer stabilizes RNA Cell extracts resulting from completion of Part I of the protocol may be stored at –80°C.

- Following protocol completion, use isolated RNA immediately for amplification, or store at –80°C for up to 6 months.

C. Additional Lab Equipment and Materials Required

Ensure that you have ready access to the following laboratory equipment and materials before you begin. These items are not included in the RNA Isolation Kit:

Equipment & Materials

1. Microcentrifuge (Eppendorf) tupes and 2 ml tubes
2. Micro pipettor
3. Incubation oven
4. Nuclease-free pipette tips

1. Reagents (optional)

2. DNase, RNase-free water

D. DNase Treatment of Samples

In order to prevent these artifacts as well as genomic DNA contamination, we strongly recommend Dnase treatment. In addition, if performing reverse transcription or amplification using RNA amplification Kits after RNA isolation.

**Dnase Treatment (Using specific Kit)

1. Add 8ul of 10X DNase I Buffer.
2. Add 2ul of DNase I Enzyme.
3. Vortex, quick spin and incubate at 42 C for 25 minutes.

Stored of RNA

1. Tissue Harvest stored immediately
2. Snap Freezing in Liquid Nitrogen:
3. Keep the tissue frozen until the homogenization procedure is ready to be performed.
4. into RNA Later. (The volume of RNA Later should be at least ten times the volume of tissue)
5. Store the tissue (until homogenization) according to the following:

- Initially - overnight at 2-8 C, Then – indefinitely < -20 C
- up to four weeks at 2-8 C,
- up to 7 days at 2-8 C,
- up to 1 day at 37 C.
- For cultures of cells, pellet out of growth media, wash 3 X PBS, and resuspend in RNA Later. (Do Not Freeze!)

Ptotocol

1. Homogenization

1. **For tissues that are snap frozen or slightly in excess, the homogenization of the tissue should be done by mortar and pestle (cooled to temp in a liquid nitrogen bath).**
2. **At the same time, transfer at least 1mL TRIZOL / 100mg tissue to be homogenized into a falcon tube**
3. **Transfer the tissue to the pestle and grind until a layer of very fine dust is all that is left.**
4. **Use an RNase free spatula to transfer the dust to the TRIZOL solution. Be sure to get as much dust as possible. Vortex mixture thoroughly.**
5. **The homogenization is performed in the presence of the 1mL TRIZOL / 100mg tissue until the tissue is completely dissolved in solution.**

2. Separation Phase

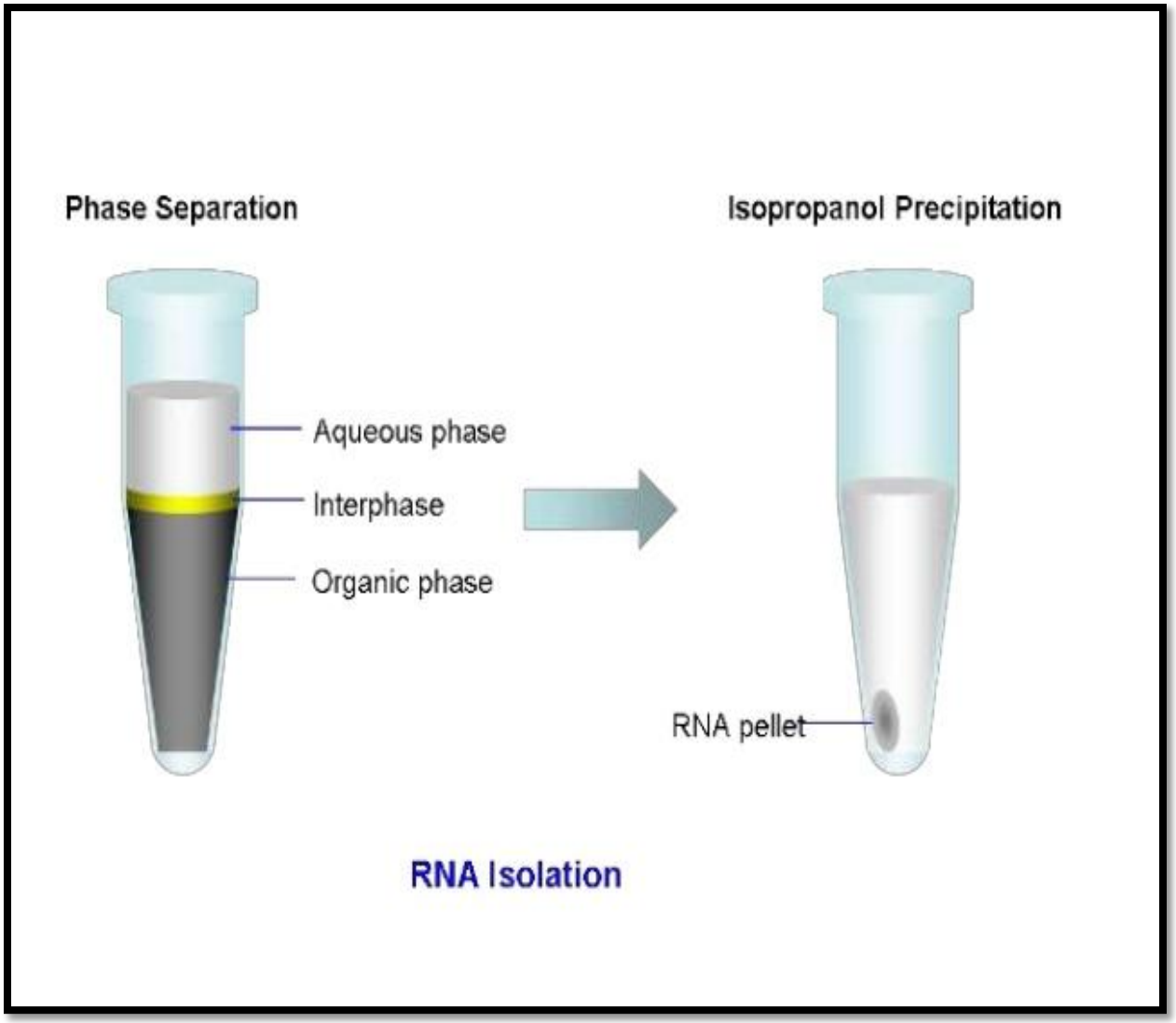
1- Add 200ul chloroform / 1mL TRIZOL (originally used), vortex for 15 seconds, and leave at room temp for 2-3 minutes.

2- Centrifuge samples at 12,000g for 15 minutes at 2-8 C.

3. RNA Precipitation

1. Following centrifugation, there will be three phases visible within the tube. Transfer the aqueous phase (top) to a fresh tube, being careful not to contaminate the solution with the other phases.
2. Contamination will be obvious by the presence of any flakes or unclear liquid.
3. Add 500ul isopropanol / 1mL TRIZOL (originally used) to the new tube and incubate at room temp for 10 minutes.
4. Centrifuge samples at 12,000g for 10 minutes at 2-8 C.

RNA Precipitation



4. RNA Wash and Resuspension

1. To remove the supernatant, Wash RNA pellet with 80% EtOH / 1ml TRIZOL (originally used) and vortex.
2. Centrifuge samples at 7,500g for 5 minutes at 2-8 C.
3. Remove supernatant. Allow remaining EtOH to air dry for 2-3 minutes.
4. Transfer tubes to 70 C heat block and let sit for 2-3 minutes.

5. Redissolving RNA

1- The RNA pellet was dried

2- RNA was dissolved in RNase- free water(or 0.5%SDS solution) through pipette tip for few time.

3- incubating for 10 minutes at 60 °C.

2. SDS
(Sodium Dodecyl Sulfate)

- The SDS is an organic compound used in many cleaning and hygiene products. Derived from inexpensive coconut and palm oils.
- The SDS is a detergent that is known to denature proteins.
- It is used in gel electrophoresis for the determination of protein molecular weight.
- Used in nucleic acid extraction procedures for the disruption of cell walls and dissociation of nucleic acid of protein complexes.

7- Quantification and Quality of RNA

- 1- Quantify each sample using Nanodrop.
- 2- Run 5ul of each sample on Agarose gel.

Troubleshooting

- Low RNA yield
- RNA degraded
- Inhibition of downstream enzymatic reactions
- Low A260/A280 ratio