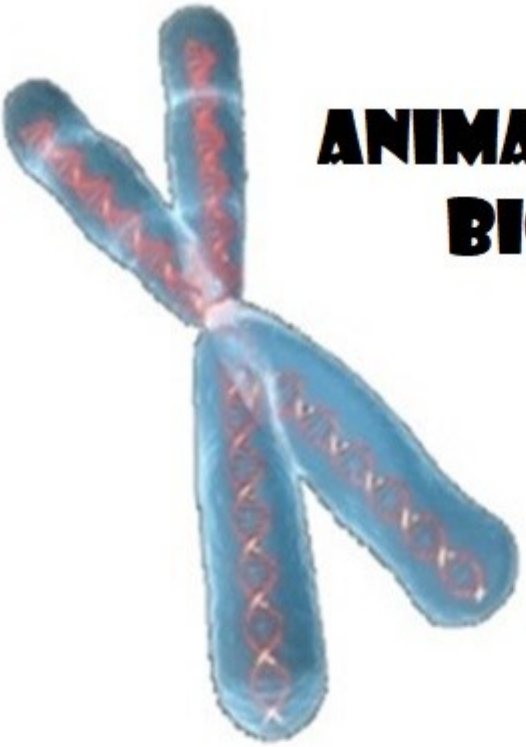
Lab: 3

زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

PRACTICAL



ANIMAL BIOTECHNOLOGY

Subject : RNA EXTRACTION

Department: Animal Resources

Stage : 4

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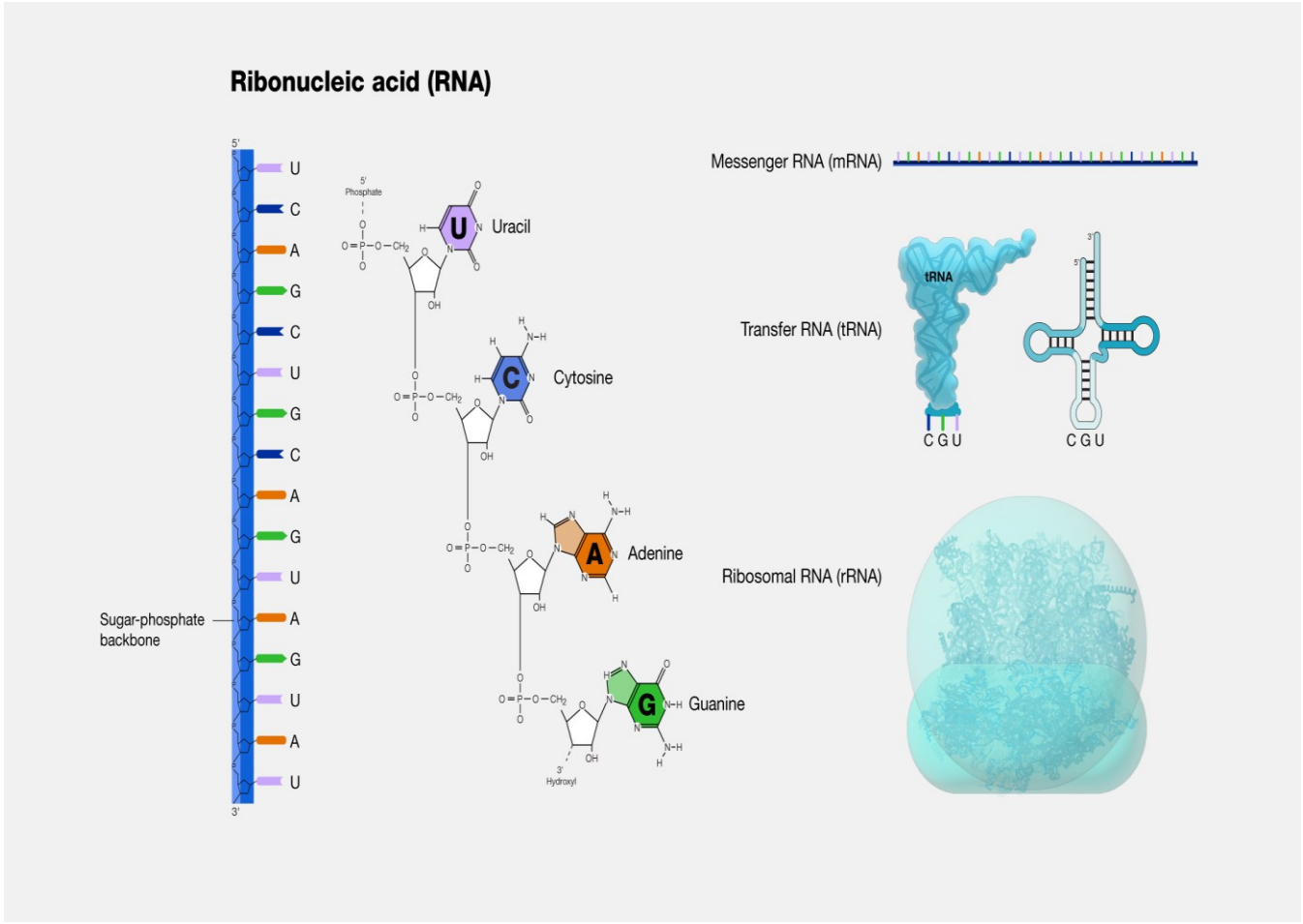
Date : / 2 / 2023

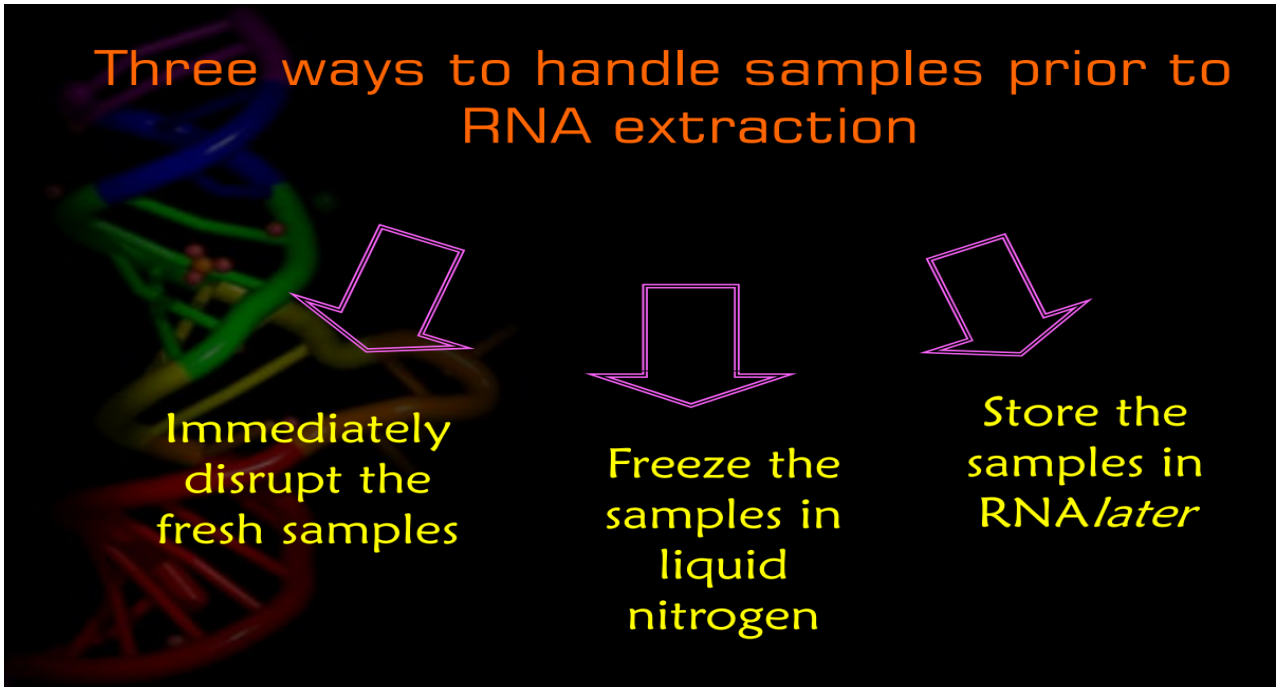
Introduction to RNA

Ribonucleic acid (abbreviated RNA) is a nucleic acid present in all living cells that has structural similarities to DNA. Unlike DNA, however, RNA is most often single-stranded. An RNA molecule has a backbone made of alternating phosphate groups and the sugar ribose, rather than the deoxyribose found in DNA. Attached to each sugar is one of four bases: adenine (A), uracil (U), cytosine (C) or guanine (G). Different types of RNA exist in cells: messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). In addition, some RNAs are involved in regulating gene expression. Certain viruses use RNA as their genomic material.



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-PC



RNA Extraction methods

1. Organic Extraction
2. Column Extraction

ORGANIC EXTRACTION

Organic extraction (acidified phenol and chloroform) removes proteins, lipids, and DNA from the RNA sample. RNA is then recovered by alcohol precipitation. Advantages of this method: (i) Can be used for large or small sample sizes. (ii) Can modify extractions to remove high levels of fat and protein from samples. While Phenol and chloroform are hazardous is disadvantage of this method.

COLUMN PURIFICATION

Glass filters bind the RNA while other cellular components are washed away. RNA is eluted in a highly purified form. Advantages of this method: (i) Rapid procedure (ii) No organic solvents required (iii) No alcohol precipitation needed. While disadvantage is not as scalable as organic extraction methods.

STEPS FOR ORGANIC RNA EXTRACTION

1. HOMOGENIZATION: add 0.5 mL TRI reagent lysis solution (phenol and guanidine thiocyanate), to 200 µL of sample, then store for 5 min at room temperature (RT)
2. PHASE SEPARATION: mix Homogenate with 100 µL chloroform, Vortex for 15 seconds leave at RT for 2 – 3 minutes, then centrifuge at 12000 rpm for 15 min at 2 - 8°C.
3. RNA PRECIPITATION: add 500 µL isopropanol to aqueous phase, Leave at RT for 10 min and Centrifuge at 12000 rpm for 15 min at 2 - 8°C.
4. RNA WASH: add 0.5 mL 75% ethanol, Vortex, Centrifuge at 8000 rpm for 5 min at 2 - 8°C then air dry 2 – 3 min
5. RNA SOLUBILIZATION: add 50 µL RNase-free water.