RNA EXTRACTION

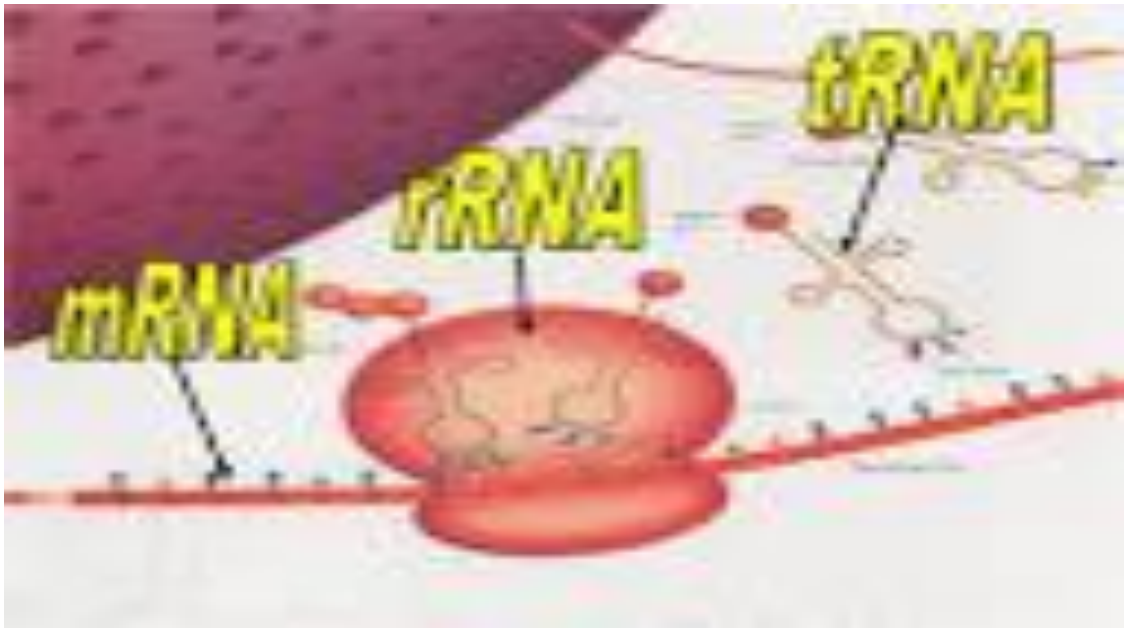
Ribonucleic acid (RNA)

is a polymeric molecule implicated in various biological roles in coding, decoding, regulation, and expression of genes. RNA and DNA are nucleic acids, and, along with proteins and carbohydrates, constitute the three major macromolecules essential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand

Types of RNA

1- Coding RNAs ⇨ mRNA ⇨ 3%

2- Non- coding RNAs ⇨ tRNA, rRNA ⇨ 97%



RNA carries out a broad range of functions:

1- Transfer the genetic code need for the creation of proteins

2-Translating genetic information into the molecular machines and structures of the cell

3- Regulating the activity of genes during development, cellular differentiation, and changing environments.

The goals of RNA extraction

1- Reverse transcription real-time PCR (RT-qPCR).

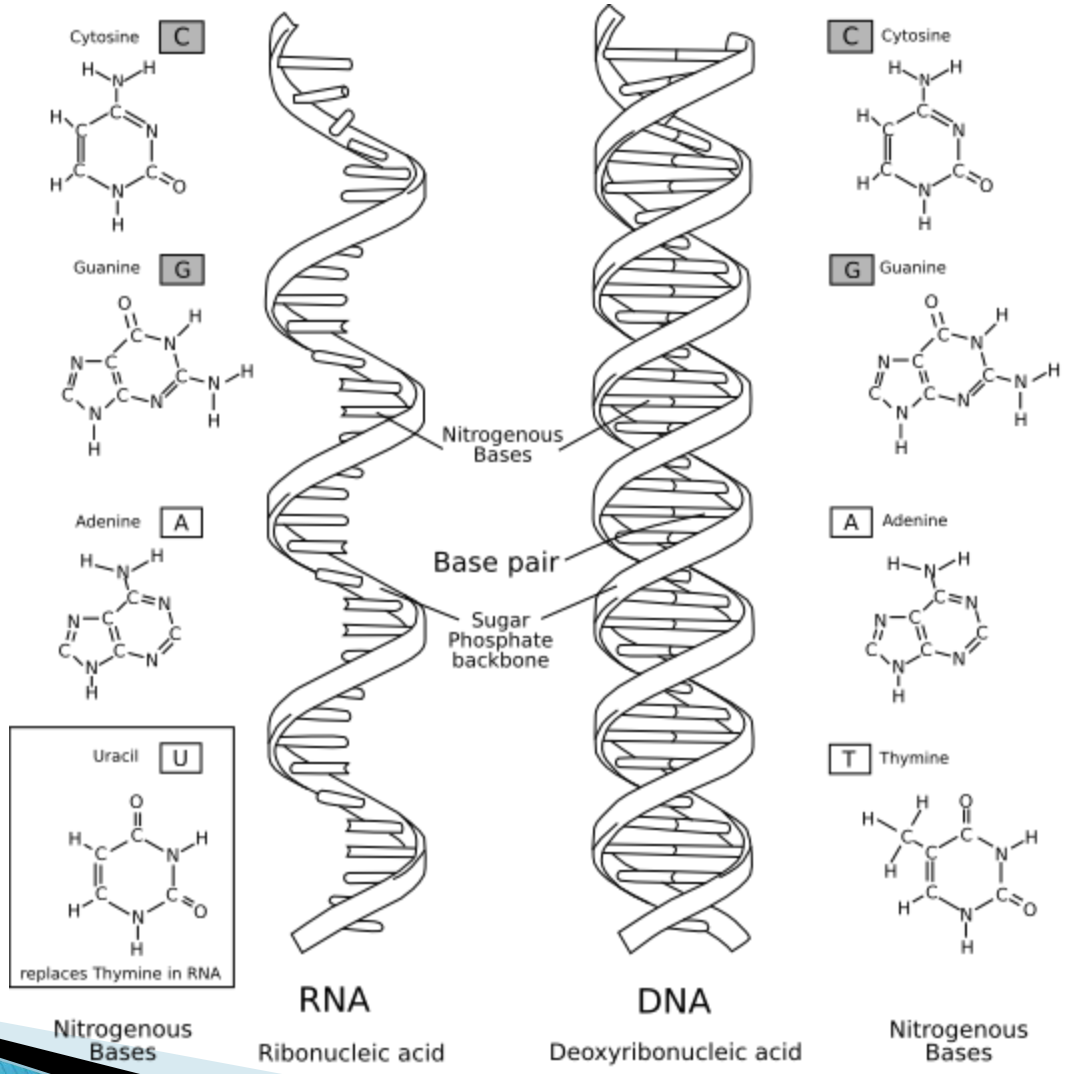
2-Transcriptome analysis using next-generation sequencing.

3- Array analysis.

4- Northern analysis.

5-cDNA library construction.

Structure of RNA



DNA extraction methods cannot be directly applied to RNA as RNA is structurally very different from DNA. RNA is single-stranded, while DNA is mostly double-stranded. It is often difficult to isolate intact RNA. RNases, a group of enzymes that degrade RNA molecules, are abundant in the environment, including on hands and on surfaces and
it is difficult to completely remove/destroy RNases. RNA isolation therefore requires cautious handling of samples and good aseptic techniques. It is important to use only RNase-free solutions during the extraction, as well as RNase-free pipet tips and glassware.

Cell lysis and dissolution

Cell lysis can be achieved using buffers or reagents containing chaotropic agents such as guanidinium isothiocynate, guanidinium chloride, sodium dodecyl sulphate (SDS), sarcosyl, urea, phenol or chloroform. TRIzol or RNAlater or Qiazol can be used to maintain RNA integrity during lysis.

Denaturation of DNA and proteins

DNase can be used to degrade DNA, while proteinase K can be added to digest proteins. Alternatively, repeated organic extraction using phenol and chloroform, or dissolving the sample in buffers containing guanidinium salts, can also be used to remove proteins.

Denaturation and inactivation of Rnases

This can be achieved using any of the chaotropic agents mentioned above, such as phenol and chloroform.

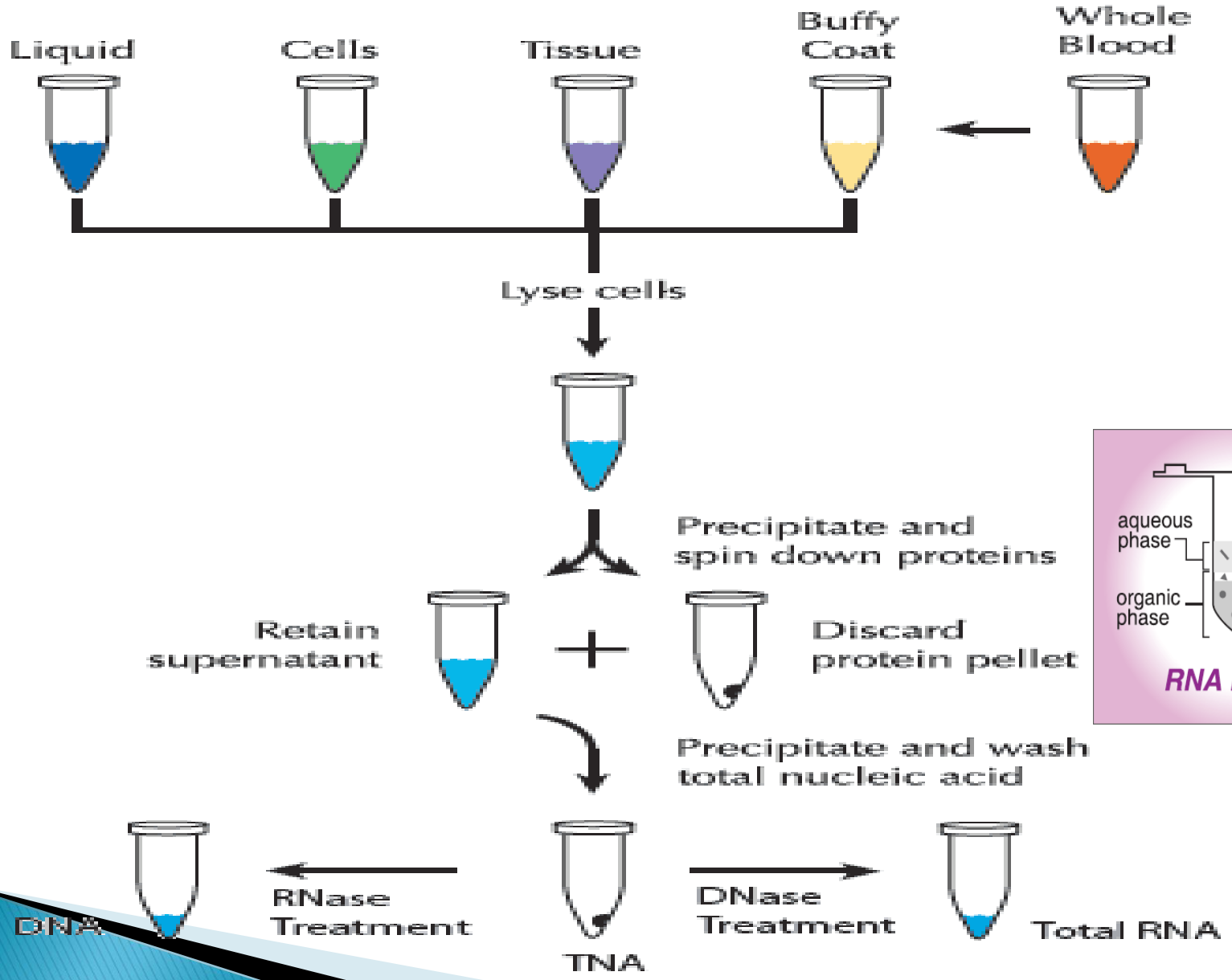
Removal/separation of cellular components

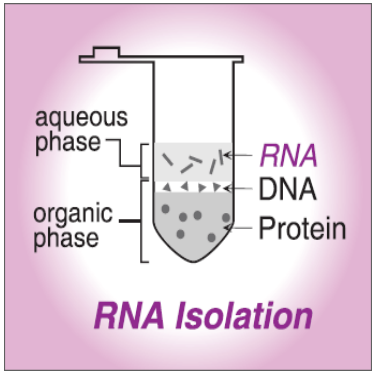
RNA can be separated from other cellular components by adding chloroform and centrifuging the solution. This separates the solution into two phases: organic and aqueous phases. The aqueous phase contains RNA.

Precipitation

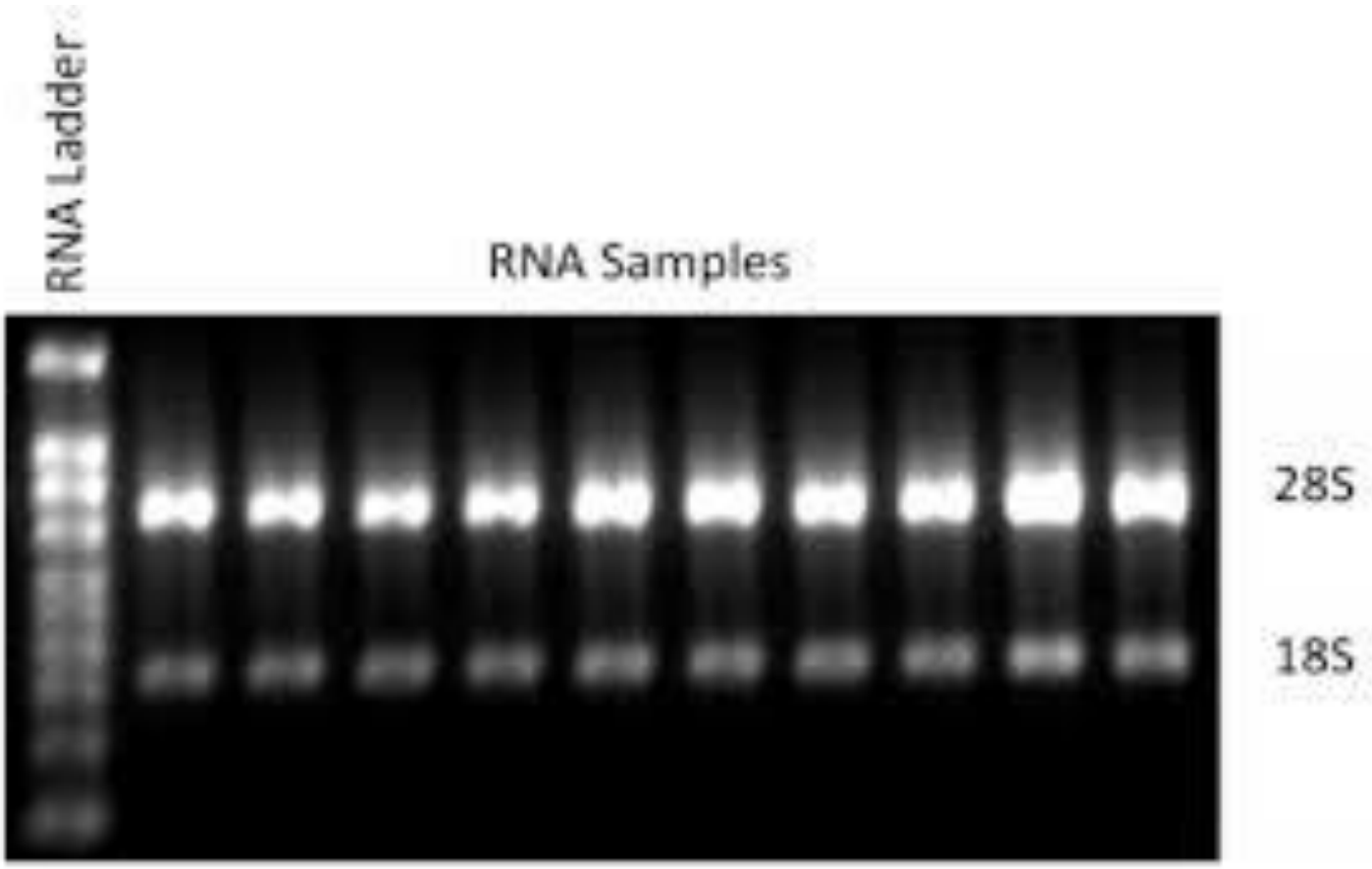
RNA is often recovered from the aqueous phase using isopropyl alcohol. RNA can also be selectively precipitated from DNA through the use of ammonium acetate. Alternatively, lithium chloride can be used to selectively

RNA extraction





RNA Extraction Kit


RNA Ladder
RNA Samples
28S
18S

Isolate RNA with the PureLink™ RNA Mini Kit (qPCR step 2).mp4