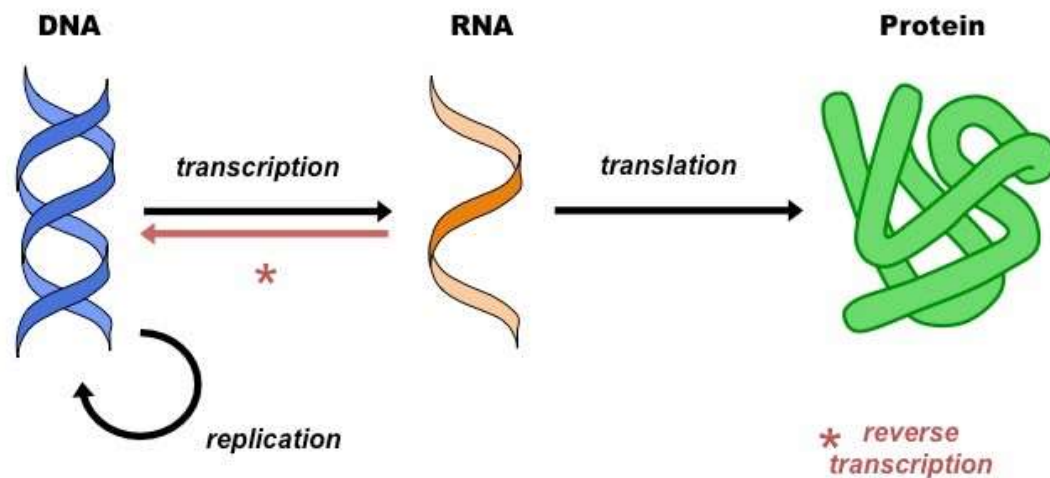




Total RNA Extraction From Rat Blood

RNA Isolation

- RNA (Ribonucleic acid).
- RNA is formed from DNA by a process called **transcription**
- The three most well-known types of RNA: mRNA, rRNA, and tRNA.
- Obtaining pure RNA is an essential step ?
- **Types of RNA isolation?**



Method of RNA Isolation

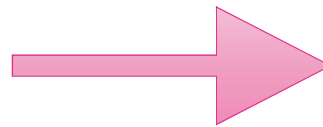
- The basic procedures used in RNA purification are similar to those of DNA, **Why?**
- However, RNA isolation is more difficult than DNA purification, **Why?**





Method of RNA Isolation

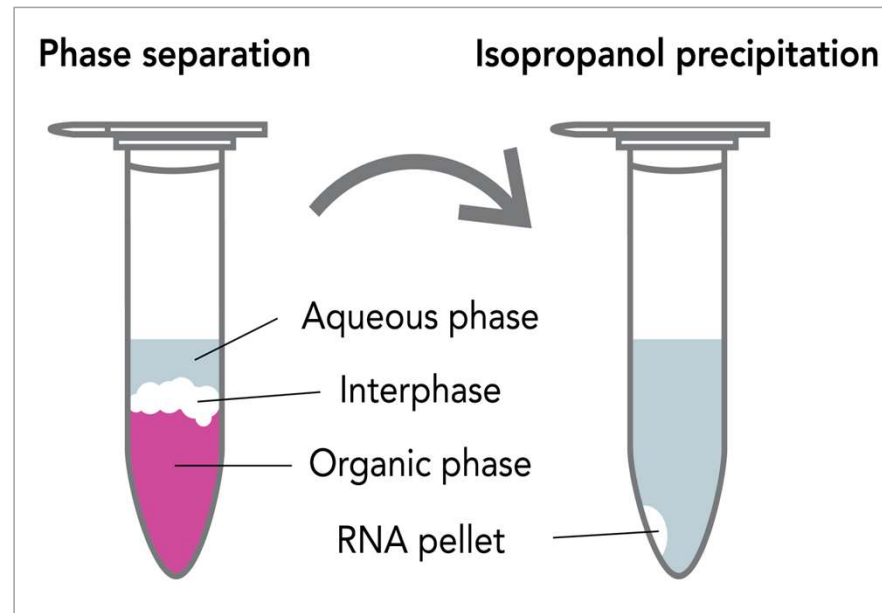
- Organic extraction methods
- Filter- based spin basket formats
- Magnetic particle methods.



The GOAL is to
extract pure intact RNA

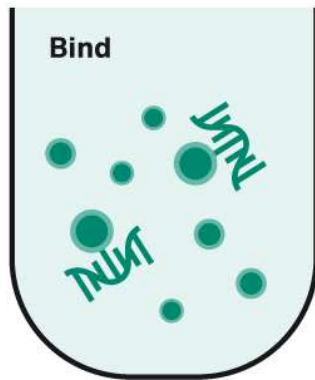
Principles of organic extraction method

Solubility dependence

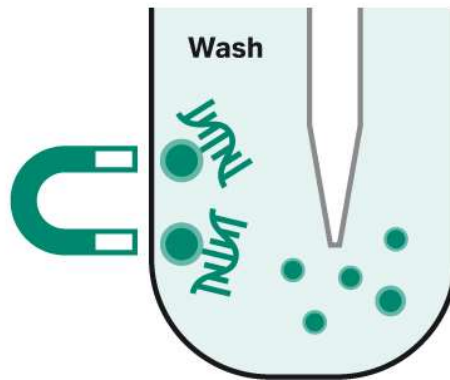


Principles of Magnetic particle method

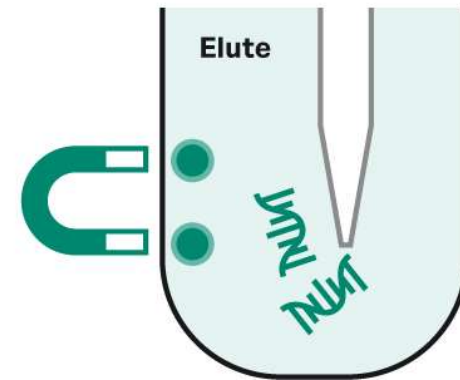
Magnetic particles are added to sample and bind to target molecule



Magnetic particles are captured and remainder of sample is washed away



Target molecule is released from magnetic particles for further analysis





Practical Part



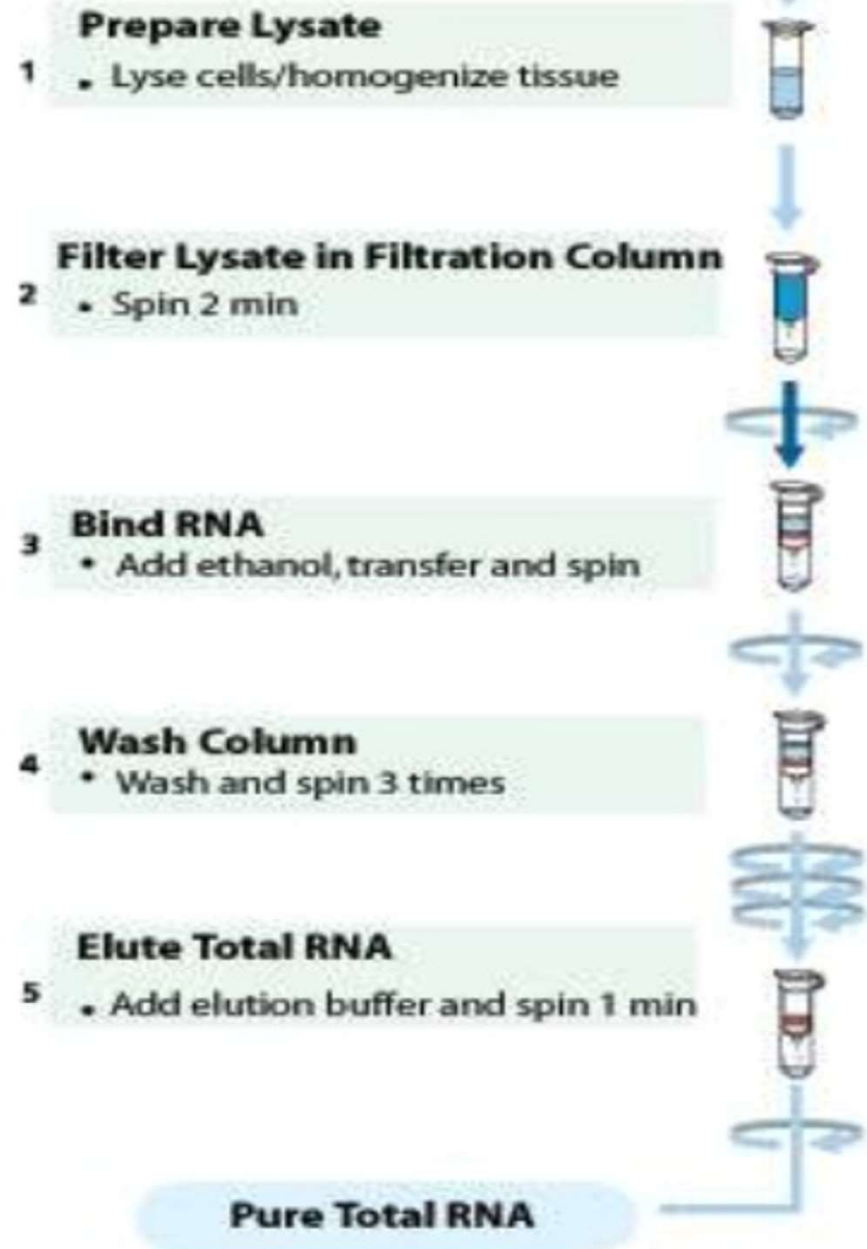
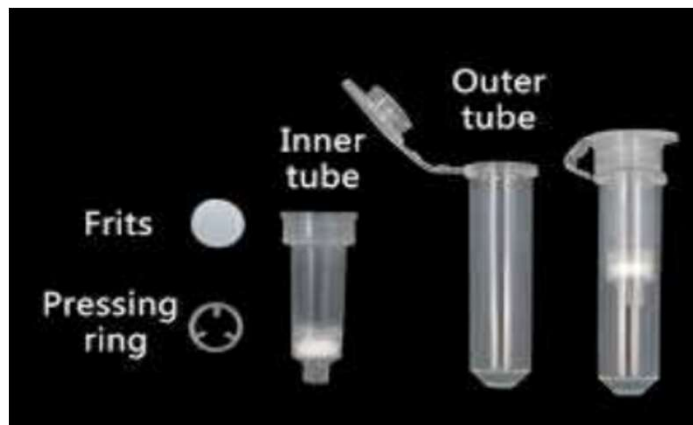
Aim:

- To isolate pure and intact total RNA from Rat Blood using Filter- based spin basket format.

Principle:

- In general, Lysing and denaturing cells to liberate total nucleic acids in the presence of **RNase inhibitory agents** . Additional steps are then required to remove DNA.

Principle of Filter-based, spin basket method





Principle of Gene JET RNA Purification Kit:

- Samples are lysed in Lysis Buffer, which contains guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases.
- The lysate is then mixed with ethanol and loaded on a purification column.
- The chaotropic salt and ethanol cause RNA to bind to the silica membrane while the lysate is spun through the column.
- Subsequently, impurities are effectively removed from the membrane by washing the column with wash buffers.
- Pure RNA is then eluted under low ionic strength conditions with nuclease-free water.



Results:

- The **concentration, purity, and integrity** of the extracted RNA need to be determined.
- The A260/A280 ratio is used to assess RNA purity. An A260/A280 ratio of 1.8–2.1 indicates highly purified RNA.