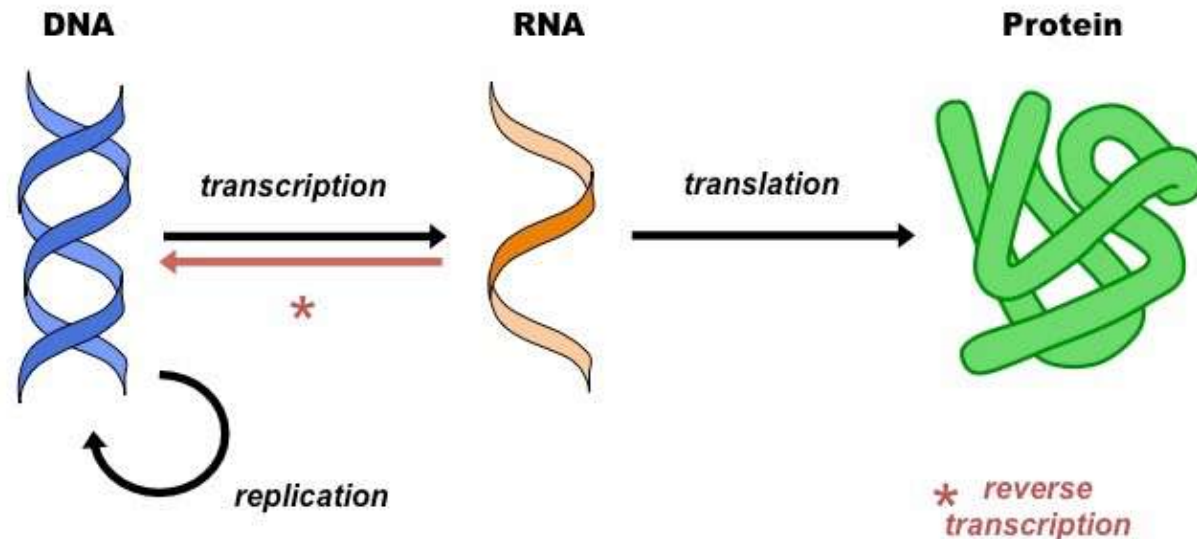




Total RNA Extraction From Tissue

RNA Isolation

- ❖ 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?**



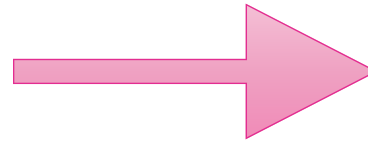
Method of RNA Isolation

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

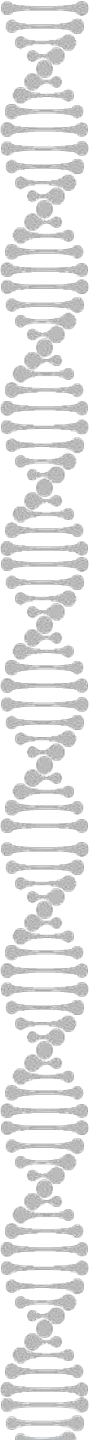


Method of RNA Isolation

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



The GOAL is to..
extract **pure intact RNA**



Practical Part



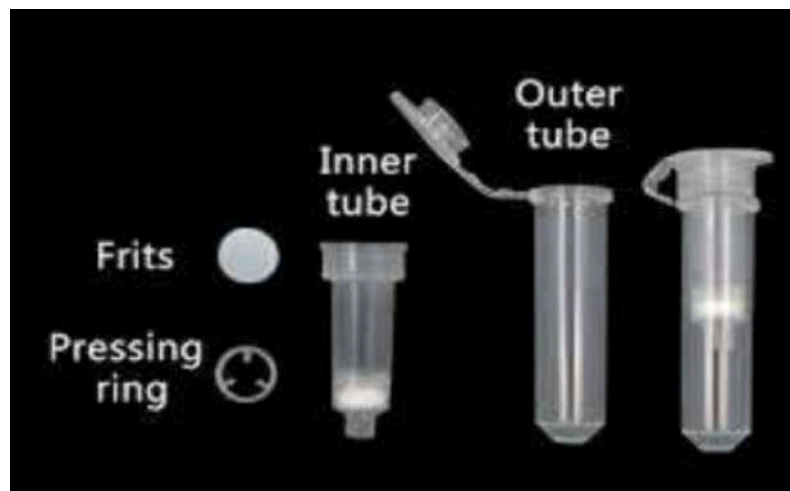
Aim:

- To isolate pure and intact total RNA from Rat Tissue using RNeasy Mini Kit.

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



1 Prepare Lysate
• Lyse cells/homogenize tissue

2 Filter Lysate in Filtration Column
• Spin 2 min

3 Bind RNA
• Add ethanol, transfer and spin

4 Wash Column
• Wash and spin 3 times

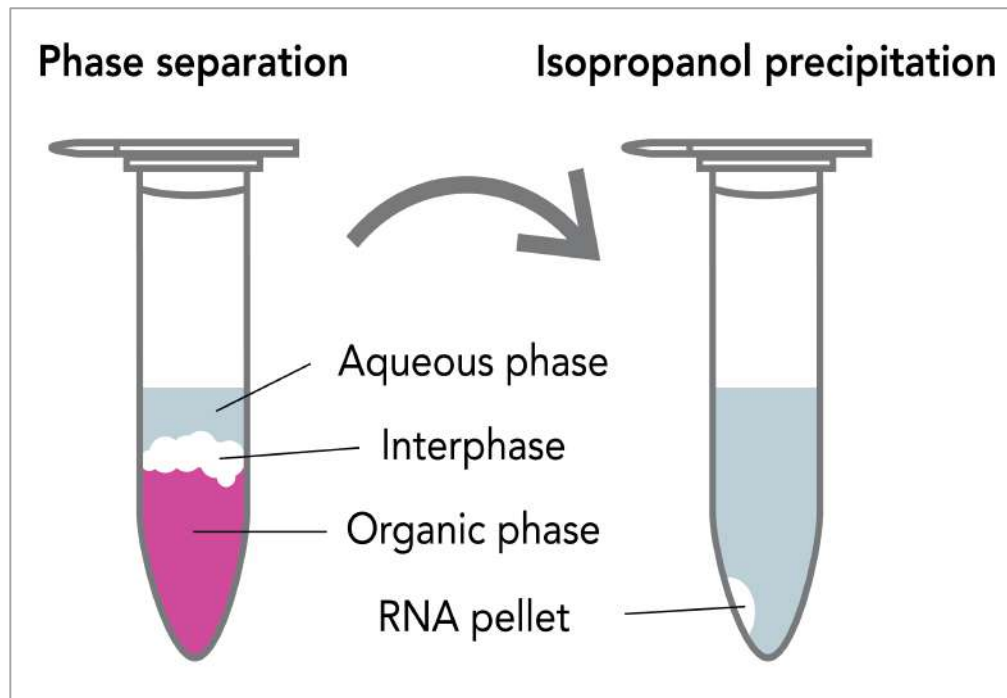
5 Elute Total RNA
• Add elution buffer and spin 1 min

Pure Total RNA

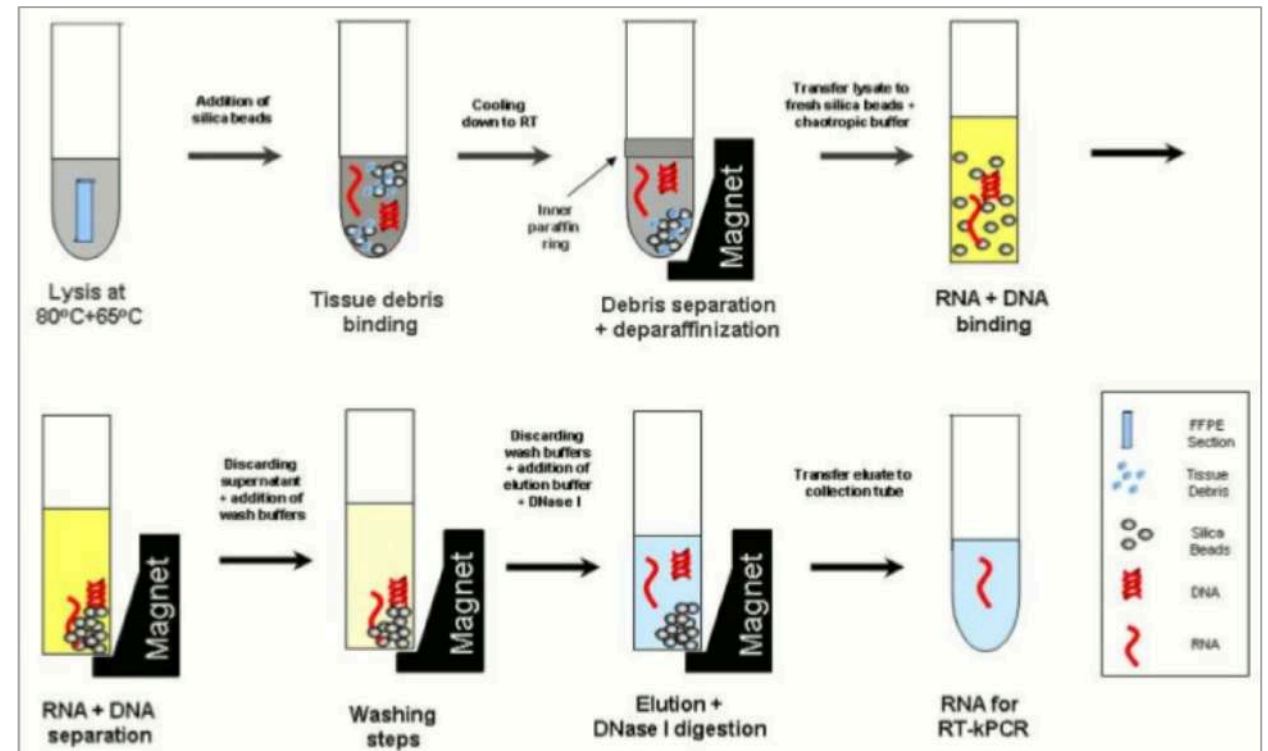


Principles of other methods

Organic extraction method



Magnetic particle method





Results:

- The **concentration, purity, and integrity** of the extracted RNA need to be determined.