RNA isolation RADHIKA RIMAL

Principle of RNA isolation

Total RNA is isolated and separated from DNA and protein after extraction with a solution called as Trizol. Trizol is an acidic solution containing guanidiniumthiocyanate (GITC), phenol and chloroform. GITC irreversibly denatures proteins and RNases. This is followed by centrifugation. Under acidic conditions, total RNA remains in the upper aqueous phase, while most of DNA and proteins remain either in the interphase or in the lower organic phase. Total RNA is then recovered by precipitation with isopropanol. RNase enzymes can be inactivated by including diethyl pyrrocarbonate (DEPC).

We isolate messenger RNA because it is a dynamic expression of the genome of an organism. As such, mRNA is central to information flow within a cell.

Following steps are involved in RNA isolation.

Cell lysis and Homogenization-

The first step requires effective cell lysis following homogenization for the complete release of [nucleic acids](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/nucleic-acids). Several methods include chemical treatments such as TRIzol or detergents that disrupt cells to release cellular contents. In addition to chemical methods, enzymatic means may be employed, such as treatment with lysozymes or enzymatic spheroplasting to weaken the cell walls for homogenization To collect the three types of RNA (rRNA, mRNA and tRNA) we have to lyse the cultured cells by using GTC buffer containing Gaunidiniumthiocyanate, sodium citrate and beta-mercaptoethanol. GTC is a chaotropic agent that causes cell lysis and inactives and precipitate RNase and other proteins helping us to obtain intact and native RNA ( RNase is a naturally occurring enzyme that degrades RNA) It works by disrupting the H-bonding between the proteins and breaking the hydrophobic interaction of lipid bilayer because of which all the membrane proteins including the RNase are disrupted. Sodium citrate in GTC buffer is a substitute detergent for protein disruption, while beta-mercaptoethanol is important for removing the disulfide bond between proteins structure.

Sodium Acetate Buffer-

 After lysis of the cell we have the maintain the pH of the solution in acidic range to separate the RNA molecules from other cell components like DNA and Protein .The acidic state of the solution is achieved by adding sodium acetate.

Phenol-Chloroform extraction-

 Many commercial preparations use phenol-chloroform-based extractions to isolate nucleic acids. Phase separation is usually achieved when their constituents are centrifuged to separate aqueous and organic phases. The extracted intracellular components are added to a tube containing Phenol-Chloroform in the ratio 1:1. This solution separates the RNA in upper aqueous layer while protein in the interphase and DNA in lower organic layer after centrifugation. The DNA molecules are negatively charged because of the phosphate backbone but since we maintain acidic pH consisting higher amount of hydrogen ion, it neutralize the negative charge increasing the hydrophobic nature of the DNA as a result of which it stabilises in no polar and denser organic layer. Since RNA is more acidic due to presence of extra -OH group in 2’- Ribose sugar so it remains in the polar aqueous phase. Proteins remains in interphase because of presence of both hydrophobic and hydrophilic amino acids.

Isopropanol Precipitation-

RNA contained in the aqueous phase can be extracted using a variety of methods. One of the commonly used methods is through precipitation with isopropanol. The aqueous layer containing RNA is collected into a separate tube following treatment with isopropanol. The solution is then centrifuged for about 10 minutes which gives us the RNA pellet with some salt solution. The salt solution is removed followed by washing with chilled 70% ethanol for 2-3times. The obtained RNA is then stored with RNA storing buffer in freezer.

 Assessing the quality of extracted RNA-

Several methods are employed to assess the quality of extracted RNA. The easiest approach is to estimate RNA concentration and purity by UV absorbance. On taking OD at 260nm and 280nm, the A260/A280 ratio should be above 1.6.

