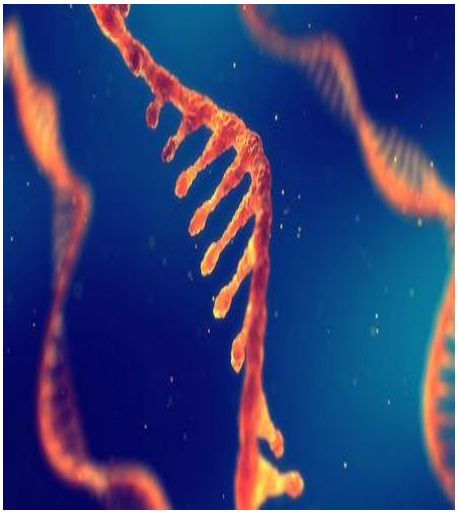

RNA Extraction

Main types of RNA are:

1) mRNA: transfer the genetic information from DNA to protein synthesis place (ribosome) in cytoplasm.

2) tRNA: found in cytoplasm, carrying amino acids for arranging them on the strand of mRNA. There are more than 20 kinds of tRNA in each cell.

3) rRNA: it calls ribosomal RNA, because it builds the ribosomal structure with nucleoproteins, have a role in protein synthesis in the cytoplasm.

To isolate RNA molecules, we have to follow the steps below:

A) Lysis the cell

1- Treat the suspension of *E. coli* with **Diethyl Pyrocarbonate (DEPC), which work as inhibiter to RNAse (exogenous RNAse).** Homogenized and incubate at 4 C.

2- Add (0.4 ml) of 5% SDS to the cell suspension

(SDS acts on Cell wall and membrane to destroy peptidoglycan, also SDS inhibits RNase).

3- Use (2ml) of **lysozyme** (400 µg/ml) containing buffer per (10 ml) of *E. coli*, homogenized and incubated at room temperature (37C) for 5-20 min.

- DNA removed by using **DNase**

B) Purification of RNA

1- Centrifuge the suspension of lysis cells at 3000 rpm for 10 min.

2- Suspend the pellet with equal volume of **Guanidinium thiocyanate** which acts as **strong inhibitors of RNAse** and **denaturation of protein.**

- The contaminant proteins are removed by adding a mixture of (phenol: chloroform: isoamyl alcohol) .
- Transfer the upper layer to another tube and add 1 volume of ice cold absolute isopropanol to precipitate RNA.
- Centrifuge 1000 rpm for 20 min. at 4 C.
- Discard supernatant and wash pellet with 70% ethanol.
- Centrifuge 1000 rpm for 5 min. at 4 C.
- Re-suspend the RNA in TE buffer and frozen.