Practical Biotechnology

Lab 8

Isolation of RNA from Blood

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RNA molecule

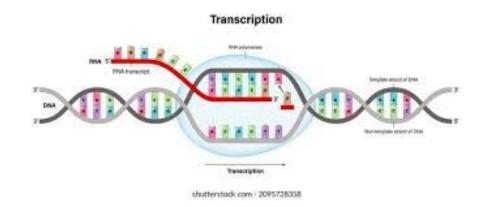
ribonucleic acid, complex compound of high molecular weight that functions in cellular protein synthesis and replaces DNA (deoxyribonucleic acid) as a carrier of genetic codes in some viruses.

RNA consists of ribose nucleotides (nitrogenous bases appended to a ribose sugar) attached by phosphodiester bonds, forming strands of varying lengths.

Synthesis of RNA

Synthesis of RNA is usually catalyzed by an enzyme—RNA polymerase—using DNA as a template, a process known as transcription.

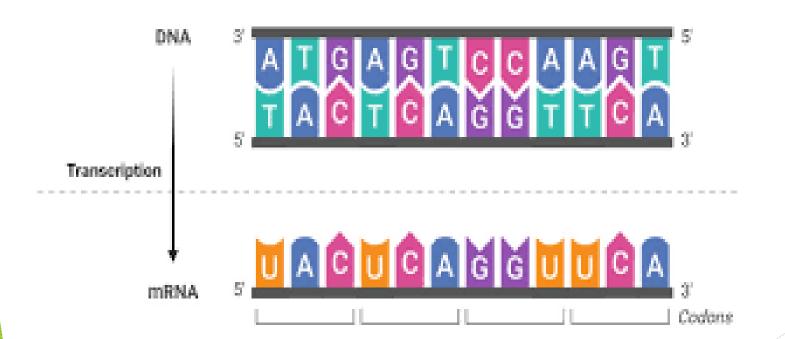
The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the template strand in the 3' to 5' direction, synthesizing a complementary RNA molecule with elongation occurring in the 5' to 3' direction.

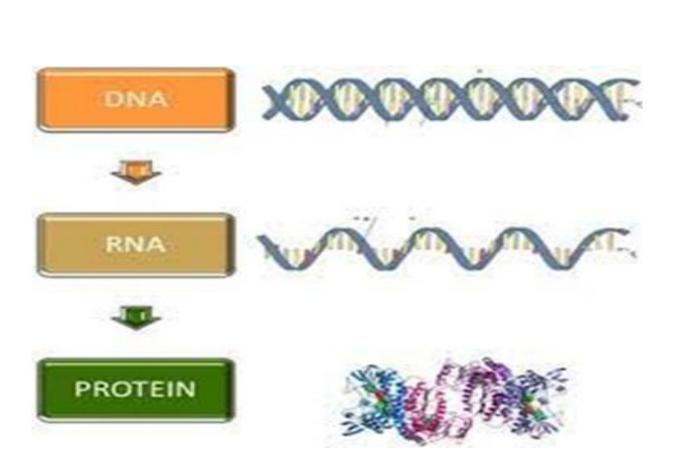


transcription is the process for RNA synthesis from template DNA. At the heart of this activity is the large multisubunit enzyme called RNA polymerase. RNA polymerase is found in all living organisms as well as many viruses. Present in bacteria, archaea, and even eukaryotes.



DNA Transcription (RNA Synthesis)





Types and function

There are three main types of RNA, all of which are involved in protein synthesis:

☐ Messenger RNA (mRNA)

Carries protein-assembly instruction to ribosome.

☐ Ribosomal RNA (rRNA)

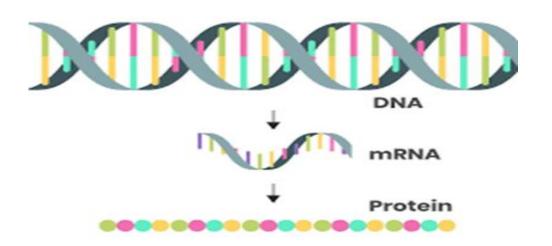
Helps regulate translation at the ribosome ;comprises 60% of ribosome (40% protein)

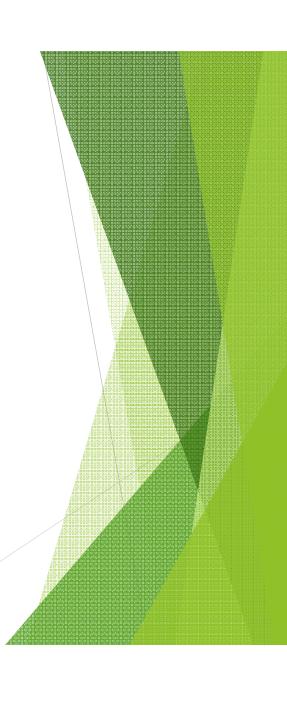
☐ Transfers RNA (tRNA)

Transfers individual amino acids to ribosomes so proteins can be assembled.

In addition to its role in protein synthesis, RNA also performs other functions including:

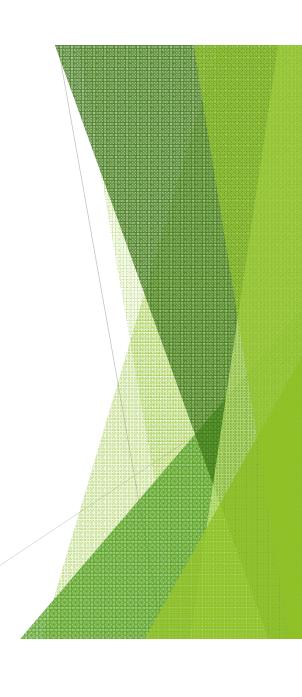
- -Regulation of gene expression.
- -Protein coding.
- -Production of regulatory molecules.
- -Development of gametes by the ncRNA(non-coding RNA).



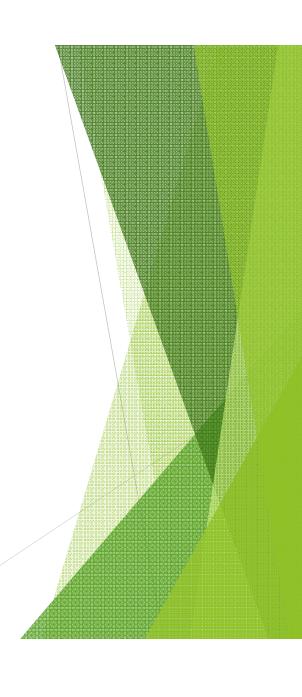


Procedure

- 1- pippte (560 ML) buffer AVL in 1.5 Tube.
- 2- Add (140 ML) sample.
- 3- Mixing for 15 s.
- 4- incubate at room temperature 15-25 C for (10 min).
- 5- Add (560 ML) Ethanol (96-100%).
- 6- Mixing for 15 s.
- 7- Transfer (630 ML)fluid to spin column.
- 8- centrifuge (8000 rpm) for 1 min.
- 9- repeat step 7.



- 10- centrifuge (8000 rpm) for 1 min.
- 11- Add (500 ML) AW1.
- 12- centrifuge (8000 rpm) for 1 min.
- 13- Add (500ML)AW2.
- 14- centrifuge Full speed (14000 rpm) for 3 min.
- 15- centrifuge Full speed (14000 rpm) for 1 min.
- 16- Add (60ML) AVE.
- 17- Waiting 1 min at Room temperature.
- 18- centrifuge (8000 rpm) for 1 min.



Thank you for listening

