Lab :4

By: Bushra aesam

RNA isolation

Ribonucleic acid (RNA) is a biologically important type of molecule that consists of a long chain of nucleotide units. Each nucleotide consists of a nitrogenous base, a ribose sugar, and a pohosphate. RNA is transcribed from DNA by enzymes called RNA polymerases and is generally further processed by other enzymes. RNA is central to protein synthesis. Here, a type of RNA called messenger RNA carries information from DNA to structures called ribosomes. These ribosomes are made from proteins and ribosomal RNAs, which come together to form a molecular machine that can read messenger RNAs and translate the information they carry into proteins. There are many RNAs with other roles – in particular regulating which genes are expressed, but also as the genome of most viruses.

RNA Isolation Strategies:

Efficient methodologies have been empirically derived to accommodate the expedient isolation of RNA, techniques that should be scrutinized and refined continuously. In general, these methods yield cytoplasmic RNA, nuclear RNA, or mixtures of both, commonly known as cellular RNA. Protocols for the isolation of RNA begin with cellular lysis mediated by buffers that typically fall into one of two categories: (1) those consisting of harsh chaotropic agents including one of the guanidinium salts, sodium dodecyl sulfate (SDS), urea, phenol, or chloroform, which disrupt the plasma membrane and subcellular organelles, and which simultaneously inactivate ribonuclease (RNase) and (2) those that gently solubilize the plasma membrane while maintaining nuclear integrity, such as hypotonic Nonidet P-401 (NP-40) lysis buffers.Intact nuclei, other organelles, and cellular debris are then removed from the lysate by differential centrifugation. The reliability of this approach is often dependent on the inclusion of nuclease inhibitors in the lysis buffer and careful attention to the handling andstorage of RNA so purified.

Diethylpyrocarbonate (DEPC).

Is used in the laboratory to inactivate RNase enzymes in water and on laboratory utensils. It does so by the covalent modification of histidine, lysine, cysteine, and tyrosine residues. Water is usually treated with 0.1% v/v DEPC for at least 2 hours at 37 °C and then autoclaved (at least 15 min) to inactivate traces of DEPC. Inactivation of DEPC in this manner yields CO_2 and ethanol. Higher concentrations of DEPC are capable of deactivating larger amounts of RNase, but remaining traces or byproducts will modify purine residues in RNA and may inhibit further biochemical reactions such as in vitro translation.

Comparison between DNA and RNA.

	DNA	RNA
Stability:	Deoxyribose sugar in	Ribose sugar is more reactive

	DNA is less reactive because of C-H bonds. Stable in alkaline conditions. DNA has smaller grooves where the damaging enzyme can attach which makes it harder for the enzyme to attack DNA	because of C-OH (hydroxyl) bonds. Not stable in alkaline conditions. RNA on the other hand has larger grooves which makes it easier to be attacked by enzymes
Pairing of Bases:	A-T(Adenine-Thymine), G-C(Guanine-Cytosine)	A-U(Adenine-Uracil), G-C(Guanine-Cytosine)
Difference:	1.Found in nucleus 2.the genetic material 3. sugar is dexyribose 4. Bases are A,T,C,G	1.Found in nucleus and cytoplasm
Predominant Structure:	Typically a double-stranded molecule with a long chain of nucleotides	A single-stranded molecule in most of its biological roles and has a shorter chain of nucleotides
Types:	Single	1) mRNA (carries DNA message to cytoplasm) 2)tRNA (carries amino acids to mRNA and Ribosomes) 3)rRNA(Ribosomal RNA, workbench for protein synthesis)
Unique Features:	The helix geometry of DNA is of B-Form. DNA is completely protected by the body i.e. the body destroys enzymes that cleave DNA. DNA can be damaged by exposure to Ultra-violet rays	The helix geometry of RNA is of A-Form. RNA strands are continually made, broken down and reused. RNA is more resistant to damage by Ultra-violet rays
Stands for:	Deoxyribonucleic acid	Ribonucleic acid
Definition:	A nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms	A nucleic acid polymer that plays an important role in the process that translates genetic information from deoxyribonucleic acid(DNA) into protein products
Job/Role:	Medium of long-term storage and transmission of genetic information	Acts as a messenger between DNA and the protein synthesis complexes known as ribosomes
Bases & Sugars:	DNA is a long polymer with a deoxyribose and	RNA is a polymer with a ribose and phosphate backbone and four

	phosphate backbone and four different bases: adenine, guanine, cytosine and thymine	different bases: adenine, guanine, cytosine, and uracil