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Isolation of genomic DNA

1-What is the DNA?

Deoxyribonucleic acid (DNA) is a molecule that carries most of the genetic instructions used in the development, functioning and reproduction of all known living organisms and many viruses. DNA is a nucleic acid; alongside proteins and carbohydrates, nucleic acids compose the three major macromolecules essential for all known forms of life. Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix. The two DNA strands are known as polynucleotides since they are composed of simpler units called nucleotides.[1] Each nucleotide is composed of a nitrogen-containing nucleobase—either cytosine (C), guanine(G), adenine (A), or thymine (T)—as well as a monosaccharide sugar called deoxyribose and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. According to base pairing rules (A with T, and C with G), hydrogen bonds bind the nitrogenous bases of the two separate polynucleotide strands to make double-stranded DNA. The total amount of related DNA base pairs on Earth is estimated at 5.0 x 1037, and weighs 50 billiontonnes. In comparison, the total mass of the biosphere has been estimated to be as much as 4 TtC (trillion tons of carbon).

DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. Biological information is replicated as the two strands are separated. A significant portion of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences.

The two strands of DNA run in opposite directions to each other and are therefore anti-parallel. Attached to each sugar is one of four types of nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes biological information. Under the genetic code, RNA strands are translated to specify the sequence of amino acids within proteins. These RNA strands are initially created using DNA strands as a template in a process called transcription.

Within cells, DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms (animals, plants, fungi, and protests) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

2- Located of genomic DNA

Eukaryotes DNA genomes, most genomic DNA is located within the nucleus (nuclear DNA) as multiple linear chromosomes of different sizes. Eukaryotic cells additionally contain genomic DNA in the mitochondria

Plants DNA genomes and lower eukaryotes, located in the chloroplasts. This DNA is usually a circular molecule and is present as multiple copies within these organelles.

Bacterial DNA genomes have a single, circular chromosome, located within cytoplas

Genetic information in bacteria is stored in the sequence of DNA in two forms, that is bacterial chromosome and plasmid.

The following are the properties of a bacterial chromosome.

Location: Within nucleoid region , not surrounded by nuclear envelope.

Number: 1 chromosome each cell.

Size: E.coli 4640 kbp.

Component: Single, double stranded, circular DNA. Also contains RNA and proteins that take part in DNA replication, transcription and regulation of gene expression. DNA does not interact with protein histone.

Information: Contain genes essential for cellular functions.

In addition to chromosome, bacterial cells may also contain another genetic element, plasmid.

Features of plasmid are analysed below.

Location: In cytosol of bacterial cells.

Number: From 1 to several.

Size: Much smaller than chromosomes.

Components: Single, double stranded, circular DNA.

Information: Contains drug resistant genes as well as heavy metal resistant genes. Not essential for growth and metabolism

Viral DNA genomes are relatively small and can be single- or double-stranded, linear, or circular. All other organisms have double-stranded DNA genomes.

3- Aims of DNA extracted

With a pure sample of DNA you can test:

***In Eukaryotic (Human cells)** for a variety of reasons as:

1-A newborn for a genetic disease

2- Analysed forensic evidence

3- Study a gene involved in cancer.

4- For PCR

***In Prokaryotic (Bacteria, Viruses,)**

1- Study a gene involved in its characteristic

2- For PCR

4- Main steps to DNA Extraction

1. Acquiring a sample of cells.

2. Burst cells open to release DNA

3. Separate DNA from other cellular components

4. Isolate concentrated DNA

5- Released of DNA from animal tissue

1- Lysis buffer. The cell membrane of the animal tissue is weakened by EDTA

2- Denatures the cellular proteins by SDS

3- Removes all the protein contamination by Phenol Chloroform.

4- Precipitated the high molecular weight genomic DNA in aqueous phase with ethanol or isopropanol

5- Removed the salts by washing with 70% alcohol.

6- Genomic DNA Extraction (Whole Blood)

This kit is designed to isolate the genomic DNA from white blood cells.

1- Lysis solution: Red blood cells are lysed and white blood cells are left intact.

2- The white blood cells and their nuclear membrane are lysed and solubilized by another solution.

3-Then the proteins and other cellular components are removed by phenol- chloroform extraction.

4- Finally the high molecular weight genomic DNA is precipitated by using absolute alcohol.

7- Genomic DNA Extraction (Bacteria)

Genomic DNA extraction involves 3 major steps:

1- Cell lysis

2- Separation of DNA from proteins and other cell debris

3- Precipitation of DNA.

The obtained genomic DNA is resolved by using horizontal agarose gel electrophoresis.

TEACHER'S PRE EXPERIMENT SET UP

1. Heat a water bath or heating block to 50-55°C is required for efficient release of the genomic DNA. A beaker with warm water and a thermometer can also be used.
2. Add 1ml Sterile Water to each vial of bacterial E.C. Cell Pellet. Vortex or vigorously shake until a homogenous mixture, with no lumps, forms. Supply each group with one vial.
3. If extra vials are available, aliquot the reagents for each group as indicated in the following section.
4. Prior to the commencement of the experiment, add 0.5ml Sterile Water to the vial of dry protease to rehydrate. Mix by inverting the vial several times until a white suspension is visible. This solution can be stored frozen for up to 1 week.

MATERIALS FOR EACH GROUP

- 1 vial E.C. Cell Suspension
- 0.8ml DNA Release Buffer
- 80µl Protease

• 0.5ml DNA Salt Solution
• 4ml Precipitation Solution
• 8 2ml Centrifuge Tubes

PROCEDURE

1. Label two 2ml Centrifuge Tubes with your name and transfer 0.2ml E.C Cell Suspension, a suspension of bacteria, to one of your tubes.
2. Add 0.2ml DNA Release Buffer to the tube containing the Bacterial Suspension. Invert the tube several times to slowly mix. The DNA Release Buffer breaks open the bacterial cells releasing the DNA.
3. Add 0.02ml Protease to the tube to digest and remove the cellular material and protein and release the genomic DNA.
4. Close the cap. Briefly mix by inverting the tube 5-6 times and then place in a 50- 55°C water bath or heating block for 1 hour.
5. After 1 hour, add 0.1ml DNA Salt Solution to the tube and mix by inverting the tube several times. The salt solution aids in the precipitation of the DNA.
6. Centrifuge the tube for 5 minutes at 5,000xg to pellet the cell debris. Transfer the supernatant to your other labeled tube.
7. Add 0.8ml Precipitation Solution, close the tube and, whilst watching, slowly invert the tube several times to mix. White DNA strands may appear.

OPTIONAL: The genomic DNA can be visualized on an agarose gel. Follow the steps below to prepare genomic DNA for agarose electrophoresis.
1. OPTIONAL: To pellet the DNA centrifuge the tube at 14,000rpm for 10 minutes. A tight white pellet should be visualized.
2. OPTIONAL: Remove the Precipitation Solution and wash the pellet with 0.5ml 70% ethanol and centrifuge as before. Remove the 70% ethanol and leave the open tube at room temperature for 10-15 minutes to dry. Resuspended in 30µl water and load 10-20µl on a 1% agarose gel to visualize the genomic DNA.