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ISOLATION OF DNA FROM ONION

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ABSTRACT

This has been proven experimentally that DNA is the molecule of heredity. The importance of nucleus which contain DNA will be identifying by the observation, that there exists thread like objective inside the nucleus, called as chromosomes. Gel electrophoresis is a method that separate macromolecules such as nucleic acids or proteins. The electrophoresis term is used to describe the migration of charged particle under the influence of an electric field. Thus, gel electrophoresis refers is the technique in which molecule are forced across a span of gel, motivated by an electric current. On either end of the gel there are activated electrodes that provide the driving force. Therefore, a molecule's properties especially the possession of ionisable groups, determine how rapidly an electric field can move the molecule through a gelatinous medium. One very important application for gel electrophoresis is in DNA Technology. We are now using biotechnology to study the basic processes of life, diagnose illnesses, and develop new treatments for diseases. For example, isolation of DNA from animals, vegetables and microorganisms. Isolated molecule from Onion for enhancing gene expression of a coding sequence, fragment, genetic variant, cassette, vector, cell, plant and seed.

KEY WORDS

DNA, Gel Electrophoresis, Onion

OBJECTIVE

The goal of this exercise is to demonstrate the isolation of DNA from living tis sues.

1. To isolate DNA from onion tissue.
2. To make observations regarding the results of the isolation.
3. To develop a hypothesis from their observations regarding the nature of the molecule.
4. To design an investigation to test their hypothesis.
5. To make research in the field of Pharmaceutical biotechnology.
6.To study DNA (As DNA is the molecule of heredity.)
7. To study Ion exchange chromatography.
8. To study Gel electrophoresis as a technique use for the separation of deoxyribonucleic acid, ribonucleic acid, or protein molecules using an electric current applied to a gel matrix.
9. It is usually performed for analytical purposes, but may be used as a preparative technique prior to use of other methods such as DNA sequencing or Southern blotting.

SCOPE OF THE STUDY OF DNA:

DNA is The Molecule of Heredity-

This has been proven experimentally that DNA is the molecule of heredity. The importance of nucleus which contains DNA will be identifying by the observation, that there exists thread like objective inside the nucleus. This structure will refer to as chromosomes. In DNA Technology, Biotechnology has for thousands of years been used by people who have used yeast to make flour into bread and grapes into wine. We are now using biotechnology to study the basic processes of life, diagnose illnesses, and develop new treatments for diseases. Some of the tools of biotechnology are natural

components of cells. For example, restriction enzymes are made by bacteria to protect themselves from viruses.

They inactivate the viral DNA by cutting it in specific places. DNA ligase is an enzyme that exists in all cells and is responsible for joining together strands of DNA. Restriction enzymes can be use to cut DNA at specific sequences called recognition sites. They then rejoin the cut strands with DNA ligase to new combinations of genes. Recombinant DNA sequences contain genes from two or more organisms. It is possible to determine the genetic difference and the evolutionary relationship among species of plants and animals. Using this technology, it is possible to separate and identify protein molecules that differ by as little as a single amino acid.[1]

INTRODUCTION:

1.DNA:-

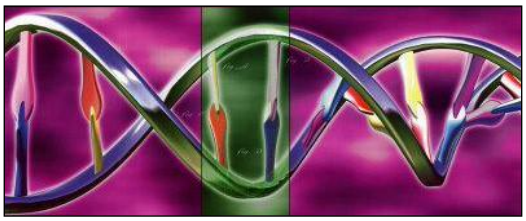

Fig.1. DNA

DNA was isolated by the Swiss physician Friedrich Miescher who, in1869 discovered a microscopic substance in the pus of discarded surgical bandages. As it resided in the nuclei of cells, he called it "nuclein". In 1919 Phoebus Levene identified the base, sugar and phosphate nucleotide unit Levene suggested that DNA consisted of a string of nucleotide units linked together through the phosphate groups. However, Levene thought the chain was short and the bases repeated in a fixed order. In 1937 William Astbury produced the first X-ray diffraction patterns that showed that DNA had a regular structure.In 1928, Frederick Griffith discovered that traits of the "smooth" form of the *Pneumococcus* could be transferred to the "rough" form of the same bacteria by mixing killed "smooth" bacteria with the live "rough" form. This system provided the first clear suggestion that DNA carried genetic information—the Avery-MacLeod-McCarty experiment—when Oswald Avery, along with coworkers Colin MacLeod and Maclyn McCarty, identified DNA as the

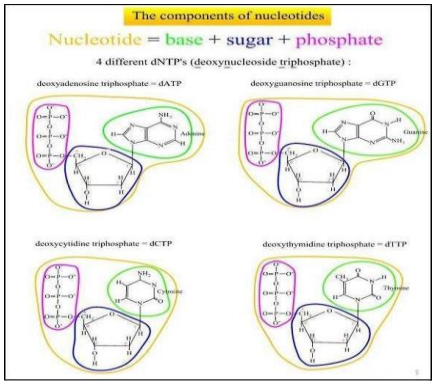

Fig.2. The components of nucleotides

2. How do the nucleotides form a DNA chain?

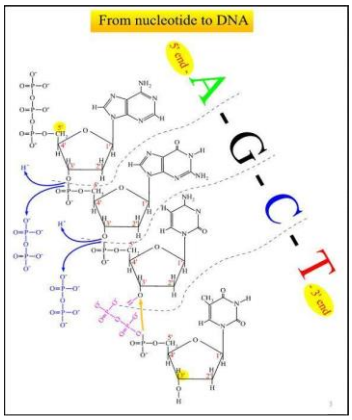

Fig.3. From nucleotide to DNA

DNA is formed by coupling the nucleotides between the phosphate group from a nucleotide (which is positioned on the **5th C-atom** of the sugar molecule) with the hydroxyl on the **3rd C-atom** on the sugar molecule of the previous nucleotide. To accomplish this, a diphosphate molecule is split off (and releases energy). This means that new nucleotides are always added on the 3' side of the chain.

Structural considerations of dna :-

Structure of DNA According to Chargaff's Rule

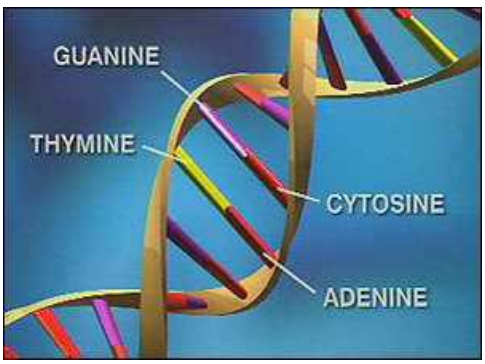

Fig.4. Structure of DNA

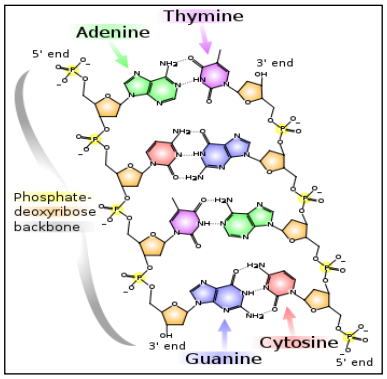

Fig.5. Chemical structure of DNA.

DNA is a long polymer made from repeating units called nucleotides. The DNA chain is 22 to 26 Ångströms wide (2.2 to 2.6 nanometres), and one nucleotide unit is 3.3 Å (0.33 nm) long. Although each individual repeating unit is very small, DNA polymers can be very large molecules containing millions of nucleotides. For instance, the largest human chromosome, chromosome number 1, is approximately 220 million base pairs long. In living organisms, DNA does not usually exist as a single molecule, but instead as a pair of molecules that are held tightly together. These two long strands entwine like vines, in the shape of a double helix. A base linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. If multiple nucleotides are linked together, as in DNA, this polymer is called a polynucleotide.

Fig.6. Double Helix Structure

The backbone of the DNA strand is made from alternating phosphate and sugar residues. The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar. The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings. These asymmetric bonds mean a strand of DNA has a direction. In 1953 James D. Watson and Francis Crick suggested what is now accepted as the first correct double-helix model of DNA structure in the journal *Nature*. Their double-helix, molecular model of DNA was then based on a single X-ray diffraction image. In a double helix the direction of the nucleotides in one strand is opposite to their direction in the other strand: the strands are *antiparallel*. The asymmetric ends of DNA strands are called the 5′ (*five prime*) and 3′ (*three prime*) ends, with the 5' end having a terminal phosphate group and the 3' end a terminal hydroxyl group.

The DNA double helix is stabilized by hydrogen bonds between the bases attached to the two strands. The four bases found in DNA are adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). These four bases are attached to the sugar/phosphate to form the complete nucleotide, as shown for adenosine monophosphate.

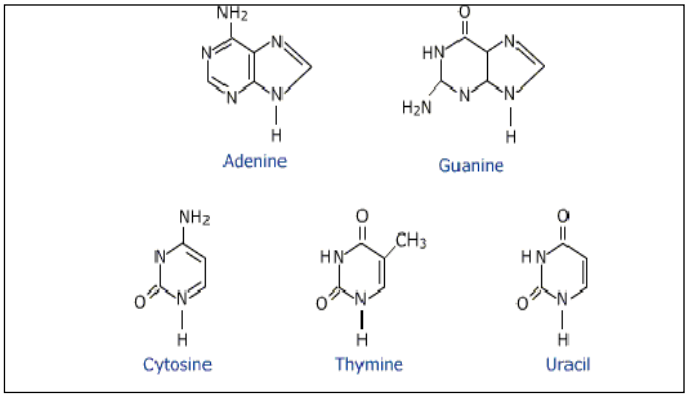

Fig.7. Types of Bases

These bases are classified into two types; adenine and guanine are fused five- and six-membered heterocyclic compounds called purines, while cytosine and thymine are six-membered rings called pyrimidines. A fifth pyrimidine base, called uracil (U), usually takes the place of thymine in RNA and differs from thymine by lacking a methyl group on its ring. [2]

Application:

1.Recombinant DNA technology. [1]

Table No.1: Current status of some recombinant products

Products	Expression system	Clinical indication	Status
Human insulin	*E-coli*	Juvenile-onset diabetes	Already in market
Human somatotropin	*E-coli*	Pituitary dwarftism	
Interferon-α2	*E-coli* and yeast	Hairy cell leukemia and prophylaxis of common cold	
Interferon-γ	*E-coli*	Treatment of cancer and treatment of viral diseases	In final clinical trials
Relaxin	*E-coli*	Facilitates child birth	Animal trials have proven successful and is under human trials
Interleukin-2	*E-coli* and animal cells	Treatment of cancer	In market.
Tissue plasminogen activator	*E-coli* yeast and animal cells	Thrombosis	In market

DIAGNOSTIC AIDS USING rDNA

2. DNA Fingerprinting

a. The first step to making a genetic fingerprint requires getting a sample of DNA. This sample can come from blood, semen, hair or saliva, and may be an extremely small sample. The root from a single strand of hair is enough for researchers to work with. This sample contains white blood cells which are broken open using detergent, and all the useable DNA is separated from the extra cellular material. Next the restriction enzymes are used to cut the DNA into smaller pieces. Restriction enzymes work by cutting the DNA at a specific sequence, which produces either blunt ends or sticky ends, and results in many fragments of different lengths. These fragments are called restriction fragments length polymorphisms, or RFLPs.

b. These RFLPs are then put into an agarose gel. Using gel electrophoresis, the fragments are sorted according to size. When the current of the electric field is turned on, the negative RFLPs will start to move across the gel towards the positive end. The smaller fragments move farther across the gel than the larger ones. Also, alkali is responsible for causing the hydrogen bonds to break, and the DNA to become single-stranded. When the DNA becomes single-stranded, it causes nucleotides to become free, and they will later be used to pair up with probes. The gel is then covered by a piece of nylon and thin paper towels, which are used to absorb moisture from the gel. The DNA fragments get gently transferred from the gel to the surface of the nylon. This process is called blotting.

c. Finally, radioactive probes get washed over the nylon surface. These probes will join to any DNA fragments that share the same composition. The final step to making a genetic fingerprint is to place a photographic film on top of the nylon surface. The probes leave marks on the film wherever they attached to the RFLPs. Dark bands will then show up when the film is developed, which marks the length of the RFLPs that were hybridized. Researchers are then able to read the fingerprint and match it to others. They do this by placing the xray on a light background, and comparing the RFLP lengths in the DNA from the crime scene, to the DNA of the suspect.

When fingerprinting was a fairly new process, during 1984-1990, it could take as long as four to six weeks of lab work to complete and compare DNA fingerprinting evidence. Jeffreys was responsible for coming up with the original DNA fingerprinting technique and in November of 1991, he also was responsible for creating a better test that could obtain results in as little as two days. [3, 4,5,6]

3. Novel proteins generation

In Escherichia coli, the dinB gene is required for the SOS-induced λ untargeted mutagenesis pathway and confers a mutator phenotype to the cell when the gene product is overexpressed. Here, we report that the purified DinB protein is a DNA polymerase. This novel E. coli DNA polymerase (pol IV) is shown to be strictly distributive, devoid of proofreading activity, and prone to elongate bulged (misaligned) primer/template structures. Site-directed mutagenesis experiments of dinB also

demonstrate that the polymerase activity of DinB is required for its in vivo mutagenicity. Along with the sequence homologies previously found within the UmuC-like protein family, these results indicate that the uncovered DNA polymerase activity may be a common feature of all these homologous proteins. [10]

4.Transgenesis

Although several steps in the evolution of transgenic technology had been performed in the 1970´s, transgenesis was not widely recognized before Palmiter introduced the human growth hormone gene into mouse zygotes by pronuclear microinjection and transgenic offspring demonstrated dramatic growth. Since then transgenic techniques have been of immense importance, as they allow new approaches to life science research that general cell culture techniques cannot deliver. The manufacture of large quantities of complex, bioactive proteins like hormones or growth factors for therapeutic purposes (pharming) is only one example of a wide range of different applications for the study of genetic regulation, animal development or disease pathology. For this purpose, foreign DNA is introduced into fertilized oocytes or embryos (blastocysts) of mice, rats and other mammals.

Production of Transgenic Animals

Transgenic animals are frequently created by two different techniques: microinjection of DNA into the pronucleus of zygotes and injection of embryonic stem cells into blastocysts. The procedure is done with the help of a complete microinjection set-containing of a microscope, micromanipulators, microinjectors and micropipettes.

Microinjection of DNA into the pronucleus

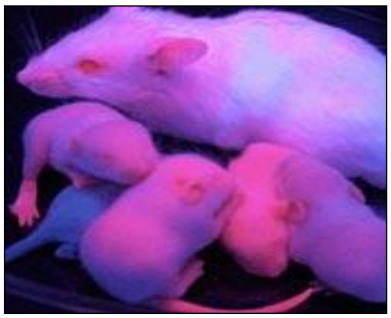

Fig.8.Transgenic animals

The pronuclear microinjection method of producing a transgenic animal is based on the introduction of linear DNA sequences into the chromosomes of fertilized eggs. The foreign DNA must be integrated into the genome prior to the doubling of the genetic material that precedes the first cleavage in order for the animal to be born with a copy of this new information in every cell. For several hours following the entry of the sperm into the oocyte, the male and the female pronuclei can still be seen individually under a normal light microscope and they have not fused yet into a so called zygote. The foreign DNA may be injected into either pronuclei with no difference in results; however, the DNA is typically injected into the male pronucleus because it is slightly larger and closer to the oocyte surface. These oocytes are subsequently transferred into the uterus of pseudopregnant recipient animals. The offspring is screened to confirm a successful integration of the gene of interest for use in further studies.

ONION

Fig.9. Onion

Onion is a term used for many plants in the genus *Allium*. *Allium cepa* is also known as the "**garden onion**" or "bulb" onion. It is grown underground by the plant as a vertical shoot that is used for food storage, leading to the possibility of confusion with a tuber, which it is not.

Description

The onion, known scientifically as *Allium cepa*, is, on the surface, a humble brown, white or red, paper-thin skinned bulb; yet, despite its plain looks, has an intense flavor and is a beloved part of the cuisine of almost every region of the world. The word onion comes from the Latin word *unio* for "single," or "one," because the onion plant produces a single bulb, unlike its cousin, the garlic, that produces many small bulbs. The name also describes the onion bulb when cut down the middle; it is a union (also from *unio*) of many separate, concentrically arranged layers.Onions range in size, color and taste depending upon their variety. There are generally two types of large, globe-shaped onions, classified as spring/summer or storage onions. The former class includes those that are grown in warm weather climates and have characteristic mild or sweet tastes.*Allium cepa* is known only in cultivation, but related wild species occur in Central Asia. The most closely related species include *Allium vavilovii* Popov &

Vved. and *Allium asarense* R.M. Fritsch & Matin from Iran.However Zohary and Hopf warn that "there are doubts whether the *vavilovii* collections tested represent genuine wild material or only feral derivatives of the crop."

Varieties

1. Bulb onion – Grown from seed (or onion sets), bulb onions range from the pungent varieties used for dried soups and onion powder to the mild and hearty sweet onions, such as the Vidalia from Georgia or Walla, Walla from Washington that can be sliced and eaten on a sandwich instead of meat.
2. Multiplier onions – May refer to perennial green onions, or to onions raised from bulbs that produce multiple shoots, each of which forms a bulb. The second type is often referred to as a potato onion.
3. Tree onion or Egyptian onion - Produce bulblets in the flower head; a hybrid of *Allium cepa*s.
4.Welsh onion – Sometimes referred to as green onion or spring onion, although these onions may refer to any green onion stalk.

Uses

1. Onions, one of the oldest vegetables, are found in a large number of recipes and preparations spanning almost the totality of the world's cultures.
2. They are now available in fresh, frozen, canned, caramelized, pickled, powdered, chopped, and dehydrated forms.
3. Onions can be used, usually chopped or sliced, in almost every type of food, including cooked foods and fresh salads and as a spicy garnish.
4. They are rarely eaten on their own, but usually act as accompaniment to the main course.
5. Depending on the variety, an onion can be sharp, spicy, tangy and pungent or mild and sweet.
6. Onions pickled in vinegar are eaten as a snack. These are often served as a side serving in fish and chip shops throughout the United Kingdom and Australia, and are referred to simply as "pickled onions".
7. Onions are widely-used in India and Pakistan, and are fundamental in the local cuisine. They are commonly used as a base for curries or made into a paste and eaten as a main course or as a side dish.
8. Tissue from onions is frequently used in science education to demonstrate microscope usage, because they have particularly large cells that are readily observed even at low magnifications.

Aroma attributes: 3-Mercapto-2-methylpentan-1-ol

Potential medicinal use

3-mercapto-2-methylpentan-1-ol in onion was found to have an antioxidant potent that inhibits peroxynitrite induced diseases.

Blood Sugar-Lowering Effects

The higher the intake of onion, the lower the level of glucose found during oral or intravenous glucose tolerance tests. Experimental and clinical evidence suggests that allyl propyl disulfide is responsible for this effect and lowers blood sugar levels by increasing the amount of free insulin available. Allyl propyl disulfide does this by competing with insulin, which is also a disulphide, to occupy the sites in the liver where insulin is inactivated. This result is an increase in the amount of insulin available to user glucose into cells causing a lowering of blood sugar.

In addition, onions are a very good source of chromium, the mineral component in glucose tolerance factor, a molecule that helps cells respond appropriately to insulin. Clinical studies of diabetics have shown that chromium can decrease fasting blood glucose levels, improve glucose tolerance, lower insulin levels, and decrease total cholesterol and triglyceride levels, while increasing good HDL-cholesterol levels. Marginal chromium deficiency is common in the United States, not surprising since chromium levels are depleted by the consumption refined sugars and white flour products as well as the lack of exercise. One cup of raw onion contains over 20% of the Daily Value for this important trace mineral.

Cardiovascular Benefits

The regular consumption of onions has, like garlic, been shown to lower high cholesterol levels and high blood pressure, both of which help prevent atherosclerosis and diabetic heart disease, and reduce the risk of heart attack or stroke. These beneficial effects are likely due to onions' sulfur compounds, its chromium and its vitamin B6, which helps prevent heart disease by lowering high homocysteine levels, another significant risk factor for heart attack and stroke. Onions have been singled out as one of the small number of vegetables and fruits that contributed to the significant reduction in heart disease risk seen in a meta-analysis of seven prospective studies. Of the more than 1,00,000 individuals who participated in these studies, those who diets most frequently included onions, tea, apples and broccoli-the richest sources of flavonoids-gained a 20% reduction in their risk of heart disease.

Active constituents of Onion

Two sets of compounds make up the majority of onion's known active constituents—sulfur compounds, such as allyl propyl disulphide (APDS), and flavonoids, such as quercetin. Each of these groups of compounds has multiple medicinal actions. The sulfur compounds form a strongly scented oil, particularly the compound known as thioproanal-s-oxide or lacrimatory factor. It is responsible for the tearing many people suffer while cutting onions. And onion oil constituents have been repeatedly shown to kill various microbes in the test tube. Studies have not been conducted in humans to determine whether onion is a useful antimicrobial agent.

APDS has been shown to block the breakdown of insulin by the liver and possibly to stimulate insulin production by the pancreas, thus increasing the amount of insulin and reducing sugar levels in the blood. Several uncontrolled human studies and at least one double-blind clinical trial have shown that large amounts of onion can lower blood sugar levels in people with diabetes. It does not reduce blood sugar levels in healthy nondiabetic people. Sulfur compounds in onion oil have also been shown to be anti-inflammatory, both by inhibiting formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). Not all studies have confirmed that these actions occur in humans. The anti-inflammatory effect is strong enough that subcutaneous onion injections and topical onion applications inhibit skin reactions to intensely inflammatory compounds in people with or without eczema, according to the results of one double-blind study. Human studies have not been performed to determine whether onion would be useful in people with asthma or cough, though the anti-inflammatory action cited above suggests it might be. These actions, coupled with an ability to reduce the stickiness of platelets and, overall, to decrease the thickness of the blood, have led to interest in onion as a way to prevent or possibly reduce atherosclerosis.

Human studies have proven mixed as to whether onion is helpful for people with atherosclerosis. Intake of quercetin in the diet, primarily from onion, tea, and apples, has been linked to a decreased risk of having a heart attack. High intake of quercetin and other flavonoids from onion and other foods has been shown to decrease risk of atherosclerosis in an epidemiologic study in the United States, although the result was not considered statistically significant. One open clinical trial showed that a crude onion extract could lower blood pressure in some people with hypertension. On the whole, it is unclear whether or not onion supplements, as opposed to onions eaten as food, have a beneficial effect on heart disease. In a preliminary study of healthy male volunteers, administration of 50 grams of raw or boiled onion prevented the rise in serum cholesterol induced by consumption of a high-fat meal. The evidence on cancer prevention with onion suggests a benefit for some but not necessarily for all types of cancer.

Onions Nutritional value per 100 g (3.5 oz)	
Energy	166 kJ (40 kcal)
Carbohydrates	9.34 g
Sugars	4.24 g
Dietary fiber	1.7 g
Fat	0.1 g
saturated	0.042 g
monounsaturated	0.013 g
polyunsaturated	0.017 g
Protein	1.1 g
Water	89.11 g

TableNo.2. Medicinal properties and health effects

Medicinal Properties

1. Wide-ranging claims have been made for the effectiveness of onions against conditions ranging from the common cold to heart disease, diabetes, osteoporosis, and other diseases.
2. They contain chemical compounds believed to have anti-inflammatory, anticholesterol, anticancer, and antioxidant properties such as quercetin.
3. Some studies have shown that increased consumption of onions reduces the risk of head and neck cancers.
4. In India some sects do not eat onion due to its alleged aphrodisiac properties.
5. In many parts of the world, onions are used to heal blisters and boils.
6. A traditional Maltese remedy for sea urchin wounds is to tie half a baked onion to the afflicted area overnight.
7. In the United States, products that contain onion extract are used in the treatment of topical scars; some studies have found their action to be ineffective, while others found that they may act as an anti-inflammatory or bacteriostatic and can improve collagen organization in rabbits.

8. Onions may be especially beneficial for women, who are at increased risk for osteoporosis as they go through menopause, by destroying osteoclasts so that they do not break down bone.
9. An American chemist has stated that the pleiomeric chemicals in onions have the potential to alleviate or prevent sore throat. However, onion in combination with jaggery has been widely used as a traditional household remedy for sore throat in India.
10. Shallots have the most phenols, six times the amount found in Vidalia onion, the variety with the lowest phenolic content.
11. Western Yellow onions have the most flavonoids, eleven times the amount found in Western White, the variety with the lowest flavonoid content.
12. For all varieties of onions, the more phenols and flavonoids they contain, the more antioxidant and anti-cancer activity they provide. [12,13]

Gel Electrophoresis:

Gel electrophoresis is a method that separates macromolecules (based on size, electrical charge and other physical properties) such as nucleic acids or proteins. The electrophoresis term is used to describe the migration of charged particle under the influence of an electric field. Thus, gel electrophoresis refers is the technique in which molecules are forced across a span of gel, motivated by an electrical current. On either end of the gel there are activated electrodes that provide the driving force. Therefore, a molecule's properties especially the possession of ionisable groups, determine how rapidly an electric field can move the molecule through a gelatinous medium. DNA technology has triggered research advances in almost all fields of biology. Currently hundreds of useful products are produced by genetic engineering. It has become routine to combine genes from different sources, usually different species--in test tubes, and then transfer this recombinant DNA into living cells where it can be replicated and expressed. The most important achievements resulting from recombinant DNA technology have been advances in our basic understanding of eukaryotic molecular biology. For example, only through the use of gene-splicing techniques have the details of eukaryotics gene arrangement and regulation been opened to experimental analysis. Gel Electrophoresis is one of the staple tools in molecular biology and is of critical value in many aspects of genetic manipulation and study. One use is the identification of particular DNA molecules by the band patterns they yield in gel electrophoresis after being cut with various restriction enzymes. Viral DNA, plasmid DNA, and particular segments of chromosomal DNA can all be identified in this way. Another use is the isolation and purification of individual fragments containing interesting genes, which can be recovered from the gel with full biological activity. Gel electrophoresis makes it possible to determine the genetic difference and the evolutionary relationship among species of plants and animals. Using this technology, it is possible to separate and identify protein molecules that differ by as little as a single amino acid.

One very important application for gel electrophoresis is in DNA Technology. We are now using biotechnology to study the basic processes of life, diagnose illnesses, and develop new treatments for diseases. Some of the tools of biotechnology are natural components of cells. For example, Recombinant DNA sequences contain genes from two or more organisms. This technique has allowed researchers to gain the ability to diagnose diseases such as sickle cell anemia, cystic fibrosis, and Huntington's chorea early in the course of the disease. Many researchers are also applying the techniques of biotechnology to find new treatments for genetic diseases. DNA technology has triggered research advances in almost all fields of biology. Currently hundreds of useful products are produced by genetic engineering. It has become routine to combine genes from different sources, usually different species--in test tubes, and then transfer this recombinant DNA into living cells where it can be replicated and expressed.

Gel Electrophoresis is one of the staple tools in molecular biology and is of critical value in many aspects of genetic manipulation and study. One use is the identification of particular DNA molecules by the band patterns they yield in gel electrophoresis after being cut with various restriction enzymes. Viral DNA, plasmid DNA, and particular segments of chromosomal DNA can all be identified in this way. Another use is the isolation and purification of individual fragments containing interesting genes, which can be recovered from the gel with full biological activity. Gel electrophoresis makes it possible to determine the genetic difference and the evolutionary relationship among species of plants and animals. Using this technology, it is possible to separate

and identify protein molecules that differ by as little as a single amino acid. Gel electrophoresis has two types:

1) Agarose gel electrophoresis.

2) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Principle: -Agarose gel electrophoresis is a method used in biochemistry and molecular biology to separate DNA, or RNA molecules by size. This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis). Shorter molecules move faster and migrate farther than longer ones.

Principle: -SDS PAGE uses an anionic detergent (SDS) to denature proteins. the protein molecules become linearized. One SDS molecule binds to 2 amino acids. Due to this, the charge to mass ratio of all the denatured proteins in the mixture becomes constant. These protein molecules move in the gel (towards the anode) on the basis of their molecular weights only & are separated. The charge to mass ratio varies for each protein (in its native or partially denatured form). Estimation of molecular weight would then be complex. Hence, SDS denaturation is used. [14,15]

Application of gel electrophoresis:

It is usually performed for analytical purposes, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization. Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications.

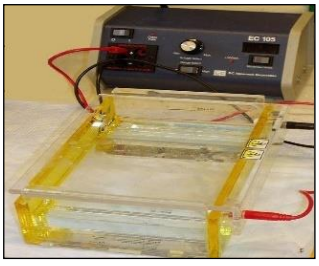

Fig.10. Gel Electrophoresis Apparatus.

MATERIAL AND METHODS [18]:-

1.Materials required: -

- *Blender*
- *Mortar and pestle*
- *Water bath*
- *Ice bucket*
- *Gloves*
- *Erlenmeyer flask*
- *Test tube*
- *Cheesecloth/cofeefiltes*
- *Graduated cylinder*
- *Funnel*
- *Microwave Oven*
- *Distilled Water*
- *parafilm*
- *Glass rod*
- *Pipette*
- *Weigh balance*
- *Onion*
- *Knife*
- *Beakers*
- *95% Isopropanol (min.200ml)*
- *EDTA*

Indicator dye: -

- *Ethidium bromide*
- *Methylene blue*

Buffers: -

- *TAE buffer (tris acetate EDTA buffer)*
- *Agarose gel*
- *DNA Extraction buffer*

2. METHODS: -

a) ISOLATION OF DNA FROM ONION[i] -

1. Carefully slice a small section (about 3-5 grams) of onion tissue from the main body of an onion (not the root tip), and place it in a test tube or small beaker.
2. Using a pipette, add 5-6 ml of DNA Extraction Buffer into the tube or beaker.
3. Mince and grind the tissue with the eraser end of a pencil or other appropriate instrument. The large end of a wooden chopstick works great. A mortar and pestle can also be used to grind the tissue. This releases the cellular contents, including the DNA, from the onion cells.
4. Place a square of cheesecloth (or coffee filter) into a funnel and filter the contents into a clean tube or beaker (squeeze out the excess juice). Measure approximately 5 ml of liquid. Alternatively, use a transfer pipet to remove the liquid portion - try to minimize carry over of onion tissue.

5. Carefully overlay the liquid with 5 ml of **very cold** 95-100% Isopropanol. Alternatively, use 10 ml of **very cold** 70% clear isopropyl rubbing alcohol.
6. Place a glass rod into the test tube or beaker and twirl it at the interface of the two liquids. The DNA will begin to spool(wrap) around the glass rod. A Pasteur pipette which has been heated to melt the end and form a hook also works well for spooling.
7. Gently lift the glass rod out of the solution from time to time and observe the DNA substance attached to it.
8. After spooling for a minute or two, remove the rod from the test tube or beaker to observe the DNA. The DNA will appear as a viscous, gelatinous-like substance adhering to the glass rod. As the DNA adheres to the rod, its initial gelatinous texture will become more compact and fibrous in appearance.
9. Rinse the DNA on the glass rod with 95-100% Isopropanol and allow it to dry for several minutes.
10. Two suggestions for what you can do next with the spooled DNA:

• To facilitate visualization, the DNA on the glass rod can be stained with a methylene blue-based DNA stain. You should also save the test tube or beaker from which you spooled the DNA to stain the residual DNA in solution.
• Alternatively, the DNA can be rehydrated with 1x TE buffer and analyzed on a 0.8% agarose gel along with a standard

b.Staining the Extracted DNA with InstaStain® Methylene Blue

1. To facilitate visualization, the spooled DNA can be stained. * Make sure you do this on a stack of paper towels:

• Using a transfer pipet, place approximately 10 drops of distilled water onto an InstaStain ® Methylene Blue card to liquify the stain.
• Transfer 2-3 drops of the blue liquified stain onto the DNA adhering to the spooling rod.
• Observe the stained DNA. Write a short paragraph describing your observations.

2. Now add 2-3 drops of the liquified blue stain to the tube or beaker from which you spooled the DNA. Observe how the stain reacts with residual DNA in the solution that did not spool onto the rod.
3. Now add 2-3 drops of the liquified stain to a tube or beaker containing only water.
4. Observe and describe the dye in the tube or beaker containing water compared to the solution containing DNA.[18]

c) PREPARE THE DNA FOR AGAROSE GEL ELECTROPHORESIS

Making the gel:

1. Weigh out the required amount (depending on the gel percentage) of agarose into an Erlenmeyer flask.
2. Add the appropriate volume of either 1X TBE or 1X TAE buffer and swirl to mix.
3. Weigh the flask for high percentage gels (3-5%): add an excess amount of distilled water to increase the weight by 10-20%.
4. Boil the mixture in a microwave oven (at middle power) until the agarose melts completely; swirl the flask several times while boiling. To prepare the highest quality agarose gels of any percentage, an additional 3-5 min of boiling after completely melting the agarose is recommended. A significant amount of water evaporates during this procedure and therefore restoring of the initial weight (in step 5) is required to obtain the desired percentage gel.
5. Weigh the flask again and if necessary, add hot distilled water to restore initial weight. For high percentage gels (3-5%): check (by weighing) that the excess 10-20% of water has evaporated and, if needed, continue boiling to remove the said excess, or add hot distilled water to restore the initial weight.
6. Optional: for an intensified gel staining add ethidium bromide to a final concentration of 0.5 µg/ml. Then, mix well and heat the mixture for an additional minute without boiling.
7. Cool the solution to 65-70°C. Pour it carefully on a clean casting plate with an appropriate comb. If bubbles appear, push them carefully away to the sides with a tip.
8. Solidify the gel for about 30 min before using. Low percentage LM agarose gels can be solidified at +4°C.
9. Immerse the gel into the desired electrophoresis buffer and load samples.
10. After electrophoresis the gel can be stained with ethidium bromide, SYBR® Green I or by any other staining technique.
11. Place casting platform with well former sideways in gel stand where you wish to pour the gel (preferably in the 4'C cold room).

12. For a 1.2% gel, add 1.2g high purity, wide range Agarose per 100 ml to be made.
13. Add 100 ml of 0.5X (for small gels) or 200 ml of 0.5X (for large gels) into 250ml bottle used and labeled for "DNA gels".
14. Mix by swirling with the cap on, then loosen cap! and microwave for about 2-3 minutes Stop and mix the solution once or twice during the microwaving.
15. Add 0.5 ug of Ethidium Bromide/ml of gel solution from stock solution (10mg/ml). Thus add 5 ul of stock solution/100ml of gel solution.
16. Pour hot gel into gel cast on a flat surface avoiding bubbles.
17. When gel solidifies, turn gel and tray to proper position and fill gel stand with 0.5X so that it covers gel completely.

d. Loading the Gel:

Mix the reaction volumes on a piece of parafilm as follows:

1. First, load appropriate volumes of ddH2O and Loading Dye onto parafilm

(see mixture volumes below):

2. One sample at a time, add the appropriate volume of sample and mix with one pipet.

3. Then, with the same pipet tip, load the sample with the loading pipet

(one with red tape) into the appropriate well very carefully.

4. Write down what samples are in each well.

Table No. 3: Mixture volumes (for a total of 12 ul)

Standard DNA mixture:		Fragment mixture:	
Standard DNA	2.0 ul	PCR product	5.0 ul
ddH_2O	8.0 ul	ddH2O	5.0 ul
Loading dye	2.0 ul	Loading dye	2.0 ul

e. Running the gel:

Note: DNA and RNA are negatively charging and thus run from positive to negative or from the black to red side if hooked up properly.

1. Add 3-4 ul of Ethidium Bromide stock solution to the bottom of the gel box on the side nearest you (red, positive side).
2. Place lid over apparatus with black lead on the side with the wells.
3. Run gel at 80V for 1-4 hours depending on the size of the DNA (larger DNA runs more slowly than smaller DNA).

I. Preparing Restriction Digests

1. Obtain microcentrifuge tubes containing DNA Each tube contains 200 ng of DNA.
2. For each DNA sample, set up I digest in a 20 microliter (µl) reaction volume by carefully pipetting the following reagents, in order, to each tube of DNA (touch the pipet tip to a clean spot on the wall of the tube as you dispense each reagent):
3. Mix all reagents by gently flicking the tube between your fingers; then briefly spin each tube in the microcentrifuge for 10 to 15 seconds to settle all components in the tube. Be sure the microcentrifuge is balanced properly!
4. Incubate samples in the 37°C water bath for 1 hour.
5. After the incubation is complete, add 2 µl of 10X gel loading dye [25% (w/v) Ficoll 400 or 25% (v/v) glycerol, 0.1 M EDTA, pH 8.0, 1% (v/v) sodium dodecyl sulfate (SDS), 0.25% xylene cyanol, 0.25% bromphenol blue] to each sample.
6. Spin each sample in the microcentrifuge for approximately 10 seconds to collect all contents at the bottom of the tube.

II. Pouring an Agarose Gel

1. While your samples are digesting, prepare a 1% (w/v) agarose gel in (89 mM Tris base, 89 mM boric acid, 2 mM EDTA). Prepare a sufficient volume for both the gel and gel running buffer.

III. Sample Loading & Electrophoresis

Carefully remove the comb from your gel. Be sure that the gel is oriented with the negative electrode (anode) adjacent to the wells of your gel. **Tip:** Remember the statement “run to red” to remind you that negatively charged DNA molecules will migrate towards the positive, red-colored electrode (cathode). Setting up your gel in the opposite orientation will cause your DNA samples to migrate out of the back of your wells into the buffer. Don't let this happen to you! Submerge your gel until the entire gel is covered by 3 to 4 mm of buffer. Carefully load the entire volume of each sample into different wells of the gel. Be sure to record your loading pattern. Electrophoresis your gel at 100 volts (constant) for approximately 40 minutes or until the bromophenol blue has migrated approximately three-fourths of the length of the gel.

IV. Gel Staining

Because your approach for gel staining may depend on many factors including, availability of an ultraviolet transilluminator, and waste disposal issues, instructions

for gel staining are not described here. Gels can be stained with either methylene blue or ethidium bromide. If gels are to be stained with methylene blue, can follow your own instructions to do this (see Staining with Methylene Blue). However, if gels will be stained with ethidium bromide (a more sensitive but more hazardous detection technique), we suggest that the stain all gels for the class to minimize contact with ethidium bromide (see Staining with Ethidium Bromide). If gels are stained, it should be explained that ethidium bromide acts as an intercalating agent to bind to DNA.

Staining with Ethidium Bromide

To stain gels with ethidium bromide, the gel is submerged in a 1 µg/ml solution of ethidium bromide for 10 minutes with gentle rotation on an orbital shaker, and destained in distilled water for 15 minutes or longer, if necessary. Alternatively, ethidium bromide can be added to each sample prior to electrophoresis to speed up the staining process, although this approach is less desirable because the gel-running buffer will contain ethidium bromide. Handling the buffer then presents a potential hazard to as it can easily be spilled; in addition, the buffer must then be disposed of as an ethidium bromide waste solution. Following destain, gels are placed on an ultraviolet light trans illuminator and a Polaroid™ photograph is taken and used to evaluate results of the experiment. Because ethidium bromide is a known mutagen, should be made aware of proper procedures for handling ethidium bromide. Should also be cautioned about the proper use of an ultraviolet trans illuminator. In many of our courses, gel staining and photography is handled by the instructor while observe.

Staining with Methylene Blue

As an alternative to ethidium bromide staining, the gel can be stained with a non-toxic dye such as methylene blue [0.025% (w/v) solution in distilled water] for 15 to 20 minutes and destained in several rinses of distilled water. The gel can then be air dried overnight on a piece of filter paper and migration distances measured directly from the dried gel, or DNA samples in the wet gel can be visualized by placing the gel on a white light transilluminator. [3,16,17,22]

CONCLUSION:

This exercise provides with a hands-on, informative and fun approach for learning about basic principles of DNA & greatly enjoys the technology involved in the experiments and they are typically intrigued by their ability to use genetic information to solve a mock DNA forensics problem.The experiment often leads to more questions and interest in DNA. Helpful exposed to fundamental techniques in molecular biology such as dna isolation, agarose gel electrophoresis, and calculating the size of DNA fragments based on relative mobility and DNA size standards.This project allows to all at different stages in their education to get actively involved in the entire process rather than working out of a pre-prepared kit. This encourage us to introduce to Isolation of DNA, transgenesis, DNA fingerprinting, basic principles of DNA & DNA related information and its application.

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