Dna analysis practical write-up

Title: DNA analysis Aim: a) Isolate and Purify Bacterial Chromosomal DNA from a strain of E. coli b) Visualization of restriction fragments by Agarose Gel electrophoresis Objectives: * to isolate and purify bacterial chromosomal DNA from a strain of E. coli * to analyze and identify DNA by use of a spectro-photometer * to use restriction enzymes to cleave DNA into fragments * to visualize the restriction fragments by gel electrophoresis * to compare the different DNA fragments generated by use of molecular markers

Abstract This work describes a lysis method for the isolation and purification of bacterial genomic DNA and visualization of the restriction fragments by agarose gel electrophoresis. It was noted that for one to isolate and purify bacterial chromosomal DNA several steps are taken into consideration. DNA was found to absorb at 260nm wavelength in a UV spectrophotometer. Restriction enzymes were added to cleave DNA which would produce various DNA fragments. DNA can be separated into different sized fragments by gel electrophoresis.

The bacterial DNA was successfully isolated and purified however it could not be observed after running the gel. DNA analysis is a standard practice for defining paternity or maternity, predisposition to disease, embryonichealthand criminal guilty. But in our context, DNA analysis is mainly used for predisposition of diseases in bacteria. Bacteria are pathogenic microorganisms that cause infectious diseases including cholera, syphilis, anthrax and leprosy. The most common fatal bacterial diseases are respiratory infections such as tuberculosis (Barnum S.

R; 1998). Nucleic acids encode information relating to cell structure and function. Cells have the ability to make copies of their DNA and pass this

information to daughter cells. Nucleic acids are polymers of nucleotides. Nucleotides are composed of ribose (a 5` carbon) sugar and either a purine and pyrimidine base at 1` position. The purine bases are adenine (A) and guanine (G) and the pyrimidine bases are cytosine (C), thymine (T) and Uracil (U). Uracil is only found in RNA and thymine is only found in DNA (Wiser M. F; 2002).

Isolation of nucleic acid – three major types of techniques are employed in the isolation of nucleic acids differential solubility, absorption methods or density gradient centrifugation. The choice of method will depend on the type of DNA being isolated and the application. A major goal of nucleic acid isolation is the removal of proteins. The separation of nucleic acids from proteins is generally accomplished due to their different chemical properties. In particular, the highly charged phosphate backbone makes the nucleic acids rather hydrophilic as compared to proteins which are more hydrophobic (Allison L.

A; 2012). Spectrophotometry is a versatile analytical tool. The underlying principle of spectrophotometry is to shine light on a sample and to analyze how the sample affects the light. DNA absorbs light at a wavelength of approximately 260nm (Stryer; 2006). Centrifugation is a process that involves the use of the centrifugal force for the separation of mixtures. Separation is based size, shape and density. It utilizes density difference between the particles/macromolecules and the medium in which these are dispersed (Gupta P. K; 2006).

Dispersed systems are subjected to artificially induced gravitational fields. A buffer is an aqueous solution consisting of a mixture of weak acid and its

conjugate base or weak base and its conjugate acid. Its pH changes very little when a small amount of strong acid or base is added to it and thus it is used to prevent any change in the pH of a solution (Cowan M. K; 2009). Electrophoresis is a diverse technique of separation used to separate and sometimes purify macromolecules especially proteins and nucleic acids that differ in size, charge or conformation by an electric current (Stryer L. 2006). Gel electrophoresis refers to using a gel as an ant convective medium and or sieving medium during electrophoresis. Gel electrophoresis is most commonly used for separation of biological macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein; however, gel electrophoresis can be used for separation of nanoparticles. Materials Used * Luria Broth medium * SET Buffer * TEN Buffer * Choloroform/isoamyl alcohol. 24: 1 mixture * Phenol/ chloroform 1: 1 (Buffer saturated phenol) * Ethanol (95%) stored at -20? * Na Acetate * NaCl: 5M sterilized by autoclaving Sodium dodecylsulphate (SDS) : 26% (w/v) * Bacteria cells * Plastic test tubes * Glass rods * Wide bore pipette * Ice bath * Centrifuge * Ethidium bromide * Agarose * TBE buffer Methodology Each group carried out the following procedures: Used two 50ml sterile plastic tubes, harvested cells by centrifugation for 10 min 4’C. Combined pellets to give approximately 1g wet weight of cells. Washed the pellet, re-suspended it in 20ml Ten buffer by gentle vortexing. Harvested the cells again as described above. Re suspended the cells in 10ml of Set buffer and let them sit on ice for 5min.

Added 1000µL of lysozyme and incubated at 37? for 30 min. Divided the cell suspension into two in separate sterile 50ml tubes. Added 5 ml Ten Buffer and 500µl of SDS. Gently mixed the tubes by inverting them until lysis

occurred. To each tube added 1ml 5M NaCl and an equal volume of buffer saturated phenol. The tubes were inverted till the mixture was emulsified. Separated the phases by centrifugation for 10min at 40C. Recovered the upper aqueous phase using a wide bore pipette. When retaining the aqueous phase the pellicle at the interface was avoided. Repeated the extraction until the interface was clear.

Added an equal volume of chloroform and extract residual protein as described above. Transferred the upper aqueous phases from both tubes to a 100ml beaker. Set them on ice and added 1/10th volume 3M Na acetate. Precipitate the DNA by addition of 2 volumes of ice cold 95% ethanol. Mixed thoroughly and allow it to stand for about 5min on ice for the DNA to precipitate. Spooled the DNA out of solution on a glass rod, dipped it into a tube of 95% ethanol and re-suspended in 10ml Ten Buffer. Left to dissolve overnight at 4’C B) Gel electrophoresis The gel was prepared by melting 1. 6g of agarose plus 200ml of 0. x TBE buffer. Swirled the mixture and allowed it to cool to 55?. Added 10? l ethiduim dye Loaded the gel in the following order; 1. Undigested pBSK 2. pBSK + digested with Eco R1 and Xba 1 3. Undigested DNA from a blue colony 4. DNA from a blue colony digested with Eco R1 and Xba 1 5. Undigested DNA from a white colony 6. DNA from a white colony digested with Eco R1 and Xba1 7. Lambda Hind III molecular weight markers After loading the gel it was run at 100 volts for 2 hours. Results We managed to precipitate DNA out of the Bacterial cells. DNA was seen a small white like fragments.

However we could not spool the DNA out of solution using glass rods due to fact that DNA is a fragile compound hence when we twisted / spooled for

DNA we destroyed the DNA strands cutting them into smaller fragments. The following day, analysis of the DNA sample in a spectrophotometer was carried out. It was found that DNA absorbed a specific wavelength of 260nm. This proved the presence of DNA in the sample. Our sample was digested by restriction enzymes and labeled the DNA fragments with an identification dye and ran them on the Gel electrophoresis together with molecular weight markers.

After running the gel no observeable bands of different band fragments were observed. Only the molecular weight markers bands were observed. Discussion The TEN and SET buffer were used to lyse the cells. They are good buffering agent, which solubilizes the DNA, while protecting it from degradation. Eluting and storing the DNA in TBE Buffer is helpful if the EDTA does not affect the downstream applications. EDTA chelates or binds to Mg2+ ions present in purified DNA and can help inhibit possible contaminating nuclease activity (Cowan M. K; 2009).

Balancing of test tubes before centrifugation in order for the centrifugation process to be effective to create centrifugal field that results in maximum separation of cell components. According to Wiser M. F 2002, DNA is very insoluble in ethanol and isopropanol, but both alcohols are very water soluble. Thus, it will dissolve in water to form a solution and cause the DNA in the solution to aggregate and precipitate out. Isopropanol is often better to use because it has greater potency in precipitating the DNA and thus lower concentration is required. This is advantageous because it will take less time for the isopropyl alcohol to evaporate.

Salts such assodium chlorideand ammonium acetate remove histone and non-histone chromosomal proteins bound to the DNA. As soon as 95% ethanol was added after sodium acetate for DNA precipitation, the whole solution turned cloudy with a lot of white precipitate, precipitating down. According to Allison L. A, 2012; sodium acetate which is negatively charged and low pH was used which contributes to charging positively the DNA. A combination of this plus high salt molarity enhances formation of aggregates of DNA and facilitates the pelleting procedure. Chloroform isoamyl-alcohol is a type of detergent.

It binds to protein and lipids of cell membrane and dissolves them. By this it disrupted the bonds that hold the cell membrane together and cause it to breakdown. It then forms complexes with these lipids and proteins, causing them to precipitate out of solution (Besty T and Keogh J; 2005). This reduced chance of contaminated DNA being obtained hence making it possible for us to be able to precipitate DNA only. Alcohol (95%ethanol) is used to precipitate DNA. SDS which stands for 'sodium dodecyl sulfate' is a strong anionic detergent that can solubilize the proteins and lipids that form the membranes.

This will helped the cell membranes and nuclear envelopes to break down and expose the chromosomes that contain the DNA. In addition to removing the membrane barriers, SDS helped release the DNA from histones and other DNA binding proteins by denaturing them (Barnum S. R; 1998). Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. When exposed to ultraviolet light, it will

fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA (Wiser M.

F; 2012). Molecular weight size is a set of standards that are used to identify the approximate size of a molecule run on a gel. These markers were composed of nucleic acids of different sizes. A few reasons you may not see bands on the gel after electrophoresis: When preparing the gel for electrophoresis TBE buffer was used. This was done so that the temperature can be maintained and lubricate the electrolyte. Loading dye was added this helped weigh down the DNA so that it can sink into the bottom wells and not float in the buffer solution. According to Gupta P.

K, 2006; loading dye moves quickly than the actual DNA parts so it is an indicator to when to turn off the power on the electrophoresis chamber. The dye also makes the DNA visible to the naked eye, giving it a purplish color and making it easier to work with. After Gel electrophoresis no bands of DNA were observed. This according Allison L. A (2012) might have been as a result of any of the following * DNA concentration might have been too low. * DNA sample is contaminated with RNA and Protein * DNA bands are too small and have run out of the gel The buffer system in which the gel is suspended is not doing its job correctly. The buffer might have to be made fresh. * The electrophoresis apparatus is not in the correct orientation (electrodes not connected to the right poles). The major drawback in the experiment was that our fellow colleagues were not able to isolate and purify their DNA. Also when working with DNA temperature regulations were not sometimes adhered to, it was sometimes left on the surface tables for long

periods esp. when the samples were being analyzed in the spectrophotometer.

Recommendations With proper teamwork and co-ordination among my fellow classmates much larger quantities of DNA could have been isolated and purified. The DNA should not be kept at room conditions for a long time. Conclusion The experiment was partly a success managed to isolate and purify DNA, analyzed it using a spectrophotometer. However bands of DNA could not be visualized after running the gel. References 1. Allison L. A. (2012). Fundamental Molecular Biology, 2nd edition. Denvers. John Wiley and Sons Inc. 2. Barnum Susan.

R, (1998), Biotechnology: An introduction, New Delhi, Vikas Publishing House. 3. Besty Tom and Keogh Jim, (2005), Microbiology demystified, New York; MacGraw-Hill. 4. Cowan Majorie Kelly, (2009), Microbiology: A Systems Approach, 3rd edition; New York; MacGraw-Hill. 5. Gupta, P. K. (2006). Elements of Biotechnology, Meerut. Rastogi Publications. 6. Stryer L, Berg J. M and John Tymozcko. (2006). Biochemistry. 5th edition. California. W. H Freeman and Company. 7. Wiser, M. F. (2002). Methods in cell biology. Berlin. Springer Verlog CHINHOYI UNIVERSITY OFTECHNOLOGY

Name Tanyaradzwa R Ngara Reg Number C1110934J Course Recombinant DNA Technology Module Code CUBT 203 Program Biotechnology Level 2: 1 Lecturer Dr Mlambo Practical Write-up DNA analysis