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Chapter 1 : How do I determine the concentration, yield and purity of a DNA sample?

MicroconÂ® DNA Fast Flow Filter Optimized for the concentration and recovery of genomic DNA with SDS buffer. The low nonspecific binding characteristics of the membrane and the other device components, coupled with its medical-grade o-ring seal, allows the device to accommodate several wash steps with minimal sample loss.

When introduced into a host organism via transformation, a plasmid will be replicated, creating numerous copies of the DNA fragment under study. In this video, a step-by-step generalized procedure is described for how to perform plasmid purification. Plasmid purification includes three basic steps: There are different types of plasmid purification methods available, which are geared toward desired yield, plasmid copy number, and bacterial culture volume. Basic Methods in Cellular and Molecular Biology. Plasmids are circular, extra chromosomal, DNA molecules and in molecular biology they act as carriers, or vectors, for a specific DNA fragment. Bacteria are used to replicate plasmids, so that your DNA of interest is mass-produced. The process by which researchers obtain plasmids from bacteria is called plasmid purification, which will be explained in this video. Obviously, plasmid purification involves purifying plasmids, but what does that mean exactly? To purify plasmids, they must be isolated from the bacterial chromosome, proteins, the bacterial membrane, and bacterial ribosomes. Though many different kits exist for purifying plasmids, the basic principle behind plasmid purification remains the same for all. First, the selected bacterial colony is grown in an appropriate culture media that contains the correct antibiotics. Thanks to an antibiotic resistance gene, encoded by the plasmid, only bacteria containing the plasmid will grow in this media. The bacteria are harvested and lysed under a high pH. The pH is neutralized, salt is added, and then the mixture is spun down to remove debris and genomic DNA. Neutralized cell lysate is added onto a silica column. Plasmid DNA is believed to adhere to the column via a mechanism called anion exchange, where DNA, a strong anion, binds to the negatively charged column via a cation salt bridge. Other material from the lysate, such as proteins, are washed through the column with high salt buffers. Finally, purified plasmid is released from the column when a low salt buffer is added disrupting the salt bridge. For this procedure a lab coat, disposable gloves and protective goggles should be worn. The next day, the bacteria culture is pelleted by centrifugation and the supernatant is removed. The remaining pellet is resuspended in lysis buffer. Once resuspended, bacteria can be transferred to a smaller tube where lysis buffer is added. Lysis buffer has a high pH and contains detergents, such as sodium dodecyl sulfate, which disrupt the bacterial membranes, thereby lysing the bacteria. The solution will turn cloudy with the addition of the lysis buffer and after mixing the solution becomes more clear. The mixture should not be vortexed due to the possibility of shearing, or breaking apart, genomic DNA, which could then contaminate plasmid DNA purification. Then, neutralization buffer is added to neutralize the alkaline conditions and lower the pH. With gentle mixing genomic DNA as well as any proteins bound to it will precipitate, while plasmid DNA will stay in solution. Once again avoid vortexing, so as your plasmids are free of genomic DNA contamination. The supernatant contains the plasmid DNA as well as soluble proteins. This supernatant is placed onto the column by decanting, a fancy name for pouring it off, or pipetting. The pellet can be discarded. Next, high salt washing buffer passes through the column while the DNA remains bound to the silica. Repeated washing steps with high salt buffer removes endonucleases, RNA, proteins, dyes, and low-molecular weight impurities. The flow-through of the wash steps should be discarded. After the final washing step make sure the filter is completely dry without any buffer remaining. The plasmid DNA is still located on the filter. The plasmid DNA is eluted with sterile water or an elution buffer. The DNA is readily available for immediate use in a wide range of applications. There are a number of ways to verify the purity of plasmids after purification. A spectrometer can be used to compare absorbance at different wavelengths to determine the concentration of plasmid DNA. An agarose gel analysis of the purified plasmid can determine if the plasmid is the correct size and there are no contaminants. This step ensures there is no genomic DNA contamination and the plasmid was not modified in bacteria. The plasmid purification procedure can be modified to

accommodate different plasmid sizes, copy number, culture volume, and can include different equipment for the binding, washing, and elution steps. Yield happens to be one of the most prominent distinctions between plasmid preparations and they are often divided into the minipreparation, or miniprep, the midiprep, a maxiprep, and a megaprep depending on the yield desired. In terms of downstream applications, one procedure that commonly follows plasmid purification is transfection, which involves the introduction of plasmid DNA into eukaryotic cells. Often the goal of a transfection experiment is to visualize the structure of cells and tissues with reporter proteins, such as those labeling the neurons in the images you see here. Sometimes multiple plasmids purified by several purification preps can be introduced into the same bacteria, thereby reproducing an entire biosynthetic pathway through the production of a number of enzymes encoded by the plasmids. The end result is the manufacturing of a complex compound, like the antibiotic you see here, by the cell. Purified plasmids may be reintroduced into bacteria, in order generate large amounts of protein encoded by the plasmid. Here you see bacterial cells being homogenized and lysed before a technique called affinity purification can be performed to isolate the target protein. Purified protein is crystallized and its structure then identified. In this video we discussed the basic principles behind this method, its step by step description, and a handful of applications of your plasmid prep. As always, thanks for watching! This article is Free Access.

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Chapter 2 : DNA extraction - Wikipedia

Microcon DNA Fast Flow DNA Concentration and Purification 1Overview Microconning a DNA extract is useful when attempting to concentrate it, clean it of lysate and chemical inhibitors or both. The procedure differs slightly depending on which of these results are desired.

Long-term storage of gDNA in water can lead to autolytic degradation. Article DNA yield can be assessed using various methods including absorbance optical density , agarose gel electrophoresis, or use of fluorescent DNA-binding dyes. All three methods are convenient, but have varying requirements in terms of equipment needed, ease of use, and calculations to consider. Absorbance Methods The most common technique to determine DNA yield and purity is measurement of absorbance. Although it could be argued that fluorescence measurement is easier, absorbance measurement is simple, and requires commonly available laboratory equipment. All that is needed for the absorbance method is a spectrophotometer equipped with a UV lamp, UV-transparent cuvettes depending on the instrument and a solution of purified DNA. Absorbance readings are performed at nm A where DNA absorbs light most strongly, and the number generated allows one to estimate the concentration of the solution. DNA concentration is estimated by measuring the absorbance at nm, adjusting the A measurement for turbidity measured by absorbance at nm , multiplying by the dilution factor, and using the relationship that an A of 1. Since RNA also has a great absorbance at nm, and the aromatic amino acids present in protein absorb at nm, both contaminants, if present in the DNA solution, will contribute to the total measurement at nm. Additionally, the presence of guanidine will lead to higher nm absorbance. This means that if the A number is used for calculation of yield, the DNA quantity may be overestimated. To evaluate DNA purity, measure absorbance from nm to nm to detect other possible contaminants. The most common purity calculation is the ratio of the absorbance at nm divided by the reading at nm. A reading of 1. The ratio can be calculated after correcting for turbidity absorbance at nm. A ratio of nm to nm can help evaluate the level of salt carryover in the purified DNA. The lower the ratio, the greater the amount of thiocyanate salt is present, for example. A reading at nm will indicate if there is turbidity in the solution, another indication of possible contamination. Therefore, taking a spectrum of readings from nm to nm is most informative. Fluorescence Methods The widespread availability of fluorometers and fluorescent DNA-binding dyes makes fluorescence measurement another popular option for determining of DNA yield and concentration. Fluorescence methods are more sensitive than absorbance, particularly for low-concentration samples, and the use of DNA-binding dyes allows more specific measurement of DNA than spectrophotometric methods allows. The availability of single-tube and microplate fluorometers gives flexibility for reading samples in PCR tubes, cuvettes or multiwell plates and makes fluorescence measurement a convenient modern alternative to the more traditional absorbance methods. Fluorescence measurements are set at excitation and emission values that vary depending on the dye chosen. The concentration of unknown samples is calculated based on comparison to a standard curve generated from samples of known DNA concentration. Genomic, fragment and plasmid DNA will each require their own standard curves and these standard curves cannot be used interchangeably. Some fluorometers will generate standard curves and calculate the concentration of unknowns for you, eliminating the need for manual calculations. As with absorbance methods, dilution factor must be taken into account when calculating DNA concentration from fluorescence values. Materials required for fluorescence methods are: Depending on the dye selected, size qualifications may apply, and the limit of detection may vary. The usual caveats for handling fluorescent compounds also applyâ€”photobleaching and quenching will affect the signal. To use this method, a horizontal gel electrophoresis tank with an external power supply, analytical-grade agarose, an appropriate running buffer e. A sample of the isolated DNA is loaded into a well of the agarose gel and then exposed to an electric field. The negatively charged DNA backbone migrates toward the anode. The percentage of agarose in the gel will determine what size range of DNA will be resolved with the greatest

clarity. Concentration and yield can be determined after gel electrophoresis is completed by comparing the sample DNA intensity to that of a DNA quantitation standard. Standards used for quantitation should be labeled as such and be the same size as the sample DNA being analyzed. Because ethidium bromide is a known mutagen, precautions need to be taken for its proper use and disposal. Additional Resources The information given here is excerpted from the DNA Purification Chapter of the Promega Protocols and Applications Guide, which contains reference information and protocols on basic molecular biology techniques. Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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Chapter 3 : How to Purify Plasmid DNA | Protocol

The Microcon Â® DNA Fast Flow Filter isis optimized for the concentration and recovery of genomic DNA. The low non-specific binding characteristics of the membrane and the other device components, coupled with its medical-grade o-ring seal, allows the device to accommodate several wash steps with minimal sample loss.

You can help by adding to it. November There are three basic and two optional steps in a DNA extraction: Breaking the cell membranes open to expose the DNA along with the cytoplasm within cell lysis. Lipids from the cell membrane and the nucleus are broken down with detergents and surfactants. Breaking proteins by adding a protease optional. The solution is treated with concentrated salt solution to make debris such as broken proteins, lipids and RNA to clump together. Centrifugation of the solution, which separates the clumped cellular debris from the DNA. DNA purification from detergents, proteins, salts and reagents used during cell lysis step. The most commonly used procedures are: Ethanol precipitation usually by ice-cold ethanol or isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. Precipitation of DNA is improved by increasing of ionic strength, usually by adding sodium acetate. Phenolâ€”chloroform extraction in which phenol denatures proteins in the sample. After centrifugation of the sample, denaturated proteins stay in the organic phase while aqueous phase containing nucleic acid is mixed with the chloroform that removes phenol residues from solution. Minicolumn purification that relies on the fact that the nucleic acids may bind adsorption to the solid phase silica or other depending on the pH and the salt concentration of the buffer. Cellular and histone proteins bound to the DNA can be removed either by adding a protease or by having precipitated the proteins with sodium or ammonium acetate , or extracted them with a phenol-chloroform mixture prior to the DNA-precipitation. After isolation, the DNA is dissolved in slightly alkaline buffer, usually in the TE buffer , or in ultra-pure water. Method Selection[edit] Some of the most common DNA extraction methods include organic extraction , Chelex extraction , and solid phase extraction. When selecting a DNA extraction method, there are multiple factors to consider, including cost, time, safety, and risk of contamination. Organic extraction involves the addition of and incubation in multiple different chemicals; [5] it often includes a lysis step, a phenol chloroform extraction, an ethanol precipitation , and a washing step. Organic extraction is often used in laboratories because it is cheap, and it yields large quantities of pure DNA. Though it is easy, there are many steps involved, and it takes longer than the other methods. It also involves the unfavorable use of the highly toxic chemical chloroform , and there is an increased risk of contamination due to transferring the DNA between multiple tubes. The unwanted cellular materials bind to the Chelex beads, while the DNA is extracted into the supernatant. The sample containing DNA is added to a column containing a silica gel or silica beads. The DNA binds to the silica, while the rest of the solution can be washed out. This method is very easy and uses only one tube; it also yields high quantities of pure, high-quality DNA, and the procedure can even be automated. Special types[edit] Specific techniques must be chosen for isolation of DNA from some samples. Typical samples with complicated DNA isolation are: The Hirt extraction process gets rid of the high molecular weight nuclear DNA , leaving only low molecular weight mitochondrial DNA and any viral episomes present in the cell. This procedure involves chemical hydrolysis of DNA: Under these conditions, the 2-deoxyribose is converted to w-hydroxylevulinyl aldehyde, which reacts with the compound, diphenylamine, to produce a blue-colored compound. Measuring the intensity of absorbance of the DNA solution at wavelengths nm and nm is used as a measure of DNA purity. DNA can be quantified by cutting the DNA with a restriction enzyme , running it on an agarose gel , staining with ethidium bromide or a different stain and comparing the intensity of the DNA with a DNA marker of known concentration. These procedures allow differentiation of the repeated sequences within the genome. It is these techniques which forensic scientists use for comparison, identification, and analysis.

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Chapter 4 : - NLM Catalog Result

Non-Organic DNA Extraction Procedure 1. Cell Lysis Buffer - lyse cell membrane, nuclei are intact, pellet nuclei. 2. Resuspend nuclei in Protein Lysis Buffer containing a high concentration of Proteinase K. Lyse nuclear membrane and digest protein at 65oC for 2 hours. Temperature helps denature proteins, and Proteinase K auto digests itself 3.

Chapter 5 : DNA Purification and Quantitation

The dual cycle Ethylene Oxide (EtO) treated Microcon Â® DNA Fast Flow PCR grade devices enable centrifugal concentration at a similar rate as the Microcon Â® DNA Fast Flow devices, but the EtO treatment greatly reduces the risk that no uncontrolled contaminating nucleic acids enter the analysis process.