Applying molecular biology techniques to implement biology essay

Science, Biology

Introduction

The term bioremediation eventually refers to the branch of biotechnology that implements the use of microorganisms in order to perform degradation for contaminations in the environment that pose environmental and human risks (Pritchard 1991). Basically, it is the act of breaking down the undesirable substances and treating waste using certain microorganisms like bacteria (Wilson & Kevin 1993). Current researches have proven that the addition of matched microbe strains to the medium affects the resident microbe ability to break down contaminants (Jeffrey 1995). Those processes are widely used in biotechnology and so many investigations are undertaken by researchers in this area to develop bioremediation (DeLong & Pace 2001). For instance, Pseudomonas putida is one of the organisms investigated by researchers and it is a gram-negative soil bacterium capable of degrading naphthalene which is found in contaminated soils as a product of petroleum refining (Lovley 2003). In addition, Dechloromonas aromatica is capable of degrading aromatic and perchlorate compounds. Eventually, it is a soil bacteria genus. Another organism is under investigations currently is Deinococcus radiodurans which is a radiation-resistant extremophile bacterium. It is also used in the bioremediation of solvents and heavy metals by using genetic engineering. It could degrade ionic mercury and toluene in radioactive mixed waste environments (Lovley 2003). This scientific project aims to study molecular biology techniques used in analysing and examining the DNA from La Trobe moat and examine the DNA collected. Also, it investigates the implementation of bioremediation in different applications.

Materials & Methods

Restriction digest: visualise fragment present and test for polymorphisms.

Sequence analysis: Analyse the DNA sequence to identify bacterial species present.

Sequencing preparation: Observe sampled DNA for sequence.

Spectrophotometry: evaluate the quantity and quality of DNA extracted.

DNA Extraction-miniprep: Extract the copied DNA from the bacteria.

Inoculation: Inoculate liquid media with a bacterial colony to create copies.

Transformation: Insert the ligated plasmid into competent bacteria.

DNA Ligation: It must be plasmid in order to insert it into bacteria by transform.

Electrophoresis 1: Use gel electrophoresis to visualise PCR sample.

Extract DNA: Extract DNA from the bacteria.

PCR: Obtain 16S rDNA targets from a mixture of bacterial DNA.

Collect bacteria: Get samples from moat sediment.

Electrophoresis 2: Separate DNA fragments from restriction digest to confirm plasmid type.

Results

T: GEN2MHG 2013Prac 2Tuesday gel photosyulliatues. jpg

Pair Sample (Musab & Chin Lee)Fig. 1 Gel ElectrophoresisThe experiment worked perfectly because the pipette volume was supplied exactly as given. The pipette volume must have a volume of 50 µl. It is a mixture of 1. 25 µl sense primer, 1. 25 µl antisense primer, 1 µl DNA template, 21. 1 µl H2O and 25 µl of MangoMix Tag PCR Buffer. Since the mixture is supplied as given with a total volume of 50 µl, therefore, this part of the experiment had succeeded. The absorbance reading for A260 was 0. 311 while A280 had a value of 0. 320. In this experiment, the dilution factor was 20. The concentration of DNA is calculated using the equation (Genetics department, 2013).

DNA conic in ng/µl = A260 reading x 50 ng/µl constant X dilution factor Eq. 1

Substituting in Eq. 1 the result obtained was 311 ng/µl. The Purity of sampled DNA can be calculated using the following equation (Genetics department, 2013).

Quality = A260: A280Eq. 2

The result from Eq. 2 was 0. 972 purity. Pair Sample (Musab & Chin Lee)T: GEN2MHG 2013Prac 5yulliatues. jpg

Fig. 2Restriction Digest Gel

The experiment in this part worked perfectly because the steps of the experiment were followed sharply. The observed results are reasonable

which can be used to find the other data. The E-value of this sample is 0. 0. The maximum score is 2489 while the maximum identity is %100. Moreover, the accession number is NR_075009. 1. The genus and species of the organism is Geobacter sulfurreducens PCA. Geobacter KN400 capacity was enhanced and investigated for extracellular electron transfer and electricity production (Butler et al. 2012).

Discussion

The purpose of this report is to analyse, collect and examine the DNA from La Trobe moat using molecular biology techniques. In addition, it shows the areas where the bioremediation can be used. Different steps were taken to achieve the final results. Analysing the results above showed that the DNA sample belongs to the organism Geobacter sulfurreducens PCA. The purity and concentration of the sampled DNA are within the acceptable range because the experiment was done accurately step by step. Since each product was supplied as given, the observed values and data were acceptable and gave expected results. In fact, DNA fragmentation is implemented to make sure that all DNA fragments have the same size. Also, it is done to ensure that each fragment has the same sequence position (Gregory et al. 2006; Jeffrey 1995). Furthermore, to minimize the amount of contamination as much as possible, primers are chosen so that almost the entire 16S rRNA gene should be amplified. This would result in less contamination sensitive compared to a smaller part of the gene (Diaz 2008). Therefore, that contamination is present but less sensitive in this case and can be distinguished due to 16S amplification. The blue-white screen is

basically a screening technique that is used to instantly detect the recombinant bacteria in vector-based molecular cloning experiments. The DNA sample is ligated into a vector which is transformed into pCR2. 1-TOPO bacteria competent cell which makes the process (Gregory et al. 2006). X-gel is used while the competent is grown. Finally, white colonies DNA indicate that the vector contains recombinant plasmids, where blue colonies indicate non-recombinant plasmids (Diaz 2008). Geobacter is used in various applications due to its unusual electron transfer and environmental restorative capabilities. Geobacter is rod shaped with flagella. They have capabilities that make them very practical in bioremediation as they are an anaerobic respiration bacterial species. It was found to be the first organism with the ability to oxidize organic compounds and metals. The oxidation includes iron, petroleum and radioactive metals compounds into environmentally benign carbon dioxide (Reguera et al. 2005). Researchers are still investigating Geobacter applications and researches are underway and on-going. In addition to oxidation, it is used for clean-up underground petroleum spills and also for the precipitation of uranium out of groundwater. Since Geobacter has the ability to produce electricity, it could be act and hence used as a natural battery by using biomass or converting animal or human waste to electricity which is a form of a renewable energy. Nanotechnologists do researches to create microbial nanowires to use them in microelectronic circuits (Reguera et al. 2005). In Conclusion, The project aimed to analyse the organisms and DNA from La Trobe moat using molecular biology techniques and also the use of bioremediation in different applications. The sequence obtained in the project was examined and

described in details referring to related articles with researchers investigations on those organisms. In the future, investigating more samples will enhance learning outcomes and will let students find out more about different organisms.