DNA EXTRACTION OF MOSQUITOES AND GEL ELECTROPHORESIS.

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Abstract

In our research we had used three techniques to obtain our DNA results for leaves and mosquitos. These are the following techniques used:

- Plant and mosquito DNA extraction
- Polymerase Chain Reaction
- Gel electrophoresis

We would begin the report by defining what is DNA and the basic information required to know about DNA such as the structure and the DNA extraction. In this research conducted we had arose questions which include:

How can we control the spread of mosquito transmitted disease as well as analyzing DNA extraction from the mosquito with gel electrophoresis?

Our objective set here is to analyse the DNA extraction of mosquito through gel electrophoresis.

In the report a very rapid and efficient protocol of DNA extraction shall also be explained, which is suitable for polymerase chain reaction (PCR) and other molecular biology works. The protocol involves three steps like lysis, solvent extraction and two fold isopropanol precipitations at -20 degree celcius using 1X STE buffer (50mM NaCl, 50mM Tris-Hcl, 100mM EDTA, pH 8.0)

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Research Questions

1. How can we control the spread of mosquito transmitted disease as well as analyzing DNA extraction from the mosquito with gel electrophoresis?
2. Controlling mosquito borne disease is a very important factor to achieve to be able to increase life expectancy and improve the health quality of the human. One of the ways proven to control mosquito borne diseases is to infect and small breed of mosquito with wolbachia and release the species into the wild. More detailed information on how wolbachia works and is impact can read on the following link: https://www.worldmosquitoprogram.org/en/work/wolbachia-method/how-it-works

Introduction

What is DNA?

Deoxyribonucleic Acid (DNA): genetic material of all cellular organisms, gigantic molecule which is used to encode genetic information for all life on Earth. DNA is found in the nucleus of the cell inside the chromosomes. DNA contains instructions that make the protein- the building block of life.

STRUCTURE OF DNA

DNA is a long chain (polymer) made up of small chemical compounds called nucleotides.

Nucleotides are ring shaped structures composed of:

The spread of diseases by mosquitoes around the town and the increasing number of the mosquito transmitted diseases had caused us to analyse the type of mosquito in our area and its morphological features to identify the species and the type of diseases spread by it. We used the globe map observer app to identify the morphological features and the type of species in our area.

Research Methods

Equipment and material

Incubator.
- Centrifuge
- Vortexer
- Freezer (-20 º celcius)
- Nanodrop or Spectrophotometer
- Electrophoresis apparatus

Procedure

1. Wash preserved mosquitoes in sterile distilled water or phosphate buffer saline (PBS) to remove excess alcohol. Fresh mosquitoes can be ground directly.

2.Grind mosquitoes in 1.5 ml eppendorf tube with micropistle in 50-100 µl 1X STE buffer (50mM Nacl, 50mM Tris- HCL,100mM EDTA, pH 8.0) along with 100mM sucrose. Add 1X STE buffer to a total volume of 300-500 µl for a single mosquito and 1 ml for mosquito pool like 4,6,8,10 numbers. Then add 1% SDS, 1% Triton X , 10 µl/ ml RNAse A (20mg/ml), 20 µl/ ml Proteinase K (20mg/ml) and mix it. We used Triton X and Proteinase but did not use RNAse.

3. Lyse for 1 hour 30 minutes at 37º celcius. Gently mix the tube by inverting every 15 minutes.

4. Centrifuge at 12,000g for 10 minutes at 4º celcius .Transfer the supernatant to a fresh tube.

5. Add equal volume of phenol:chloroform(1:1) ,shake the tube well for 5 minutes and centrifuge at 12,000g for 10 minutes at 4º celcius . (This step was skipped to avoid use of Phenol, which is a toxic chemical.)

6.Repeat the above step(5) , then add chloroform:isoamyl alcohol(24:1) and centrifuge at 12,000g for 10 minutes at 4º celcius .

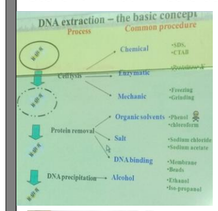
7.Transfer the very clear supernatant to a fresh tube , add two fold volume cold isopropanol and keep it for 1 hour at -20º celcius.

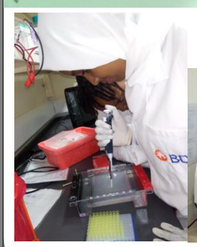
8.Centrifuge at 12,000g for 30 minutes at 4º celcius and then remove the supernatant .

9. Wash the pellet with 70% ethanol .

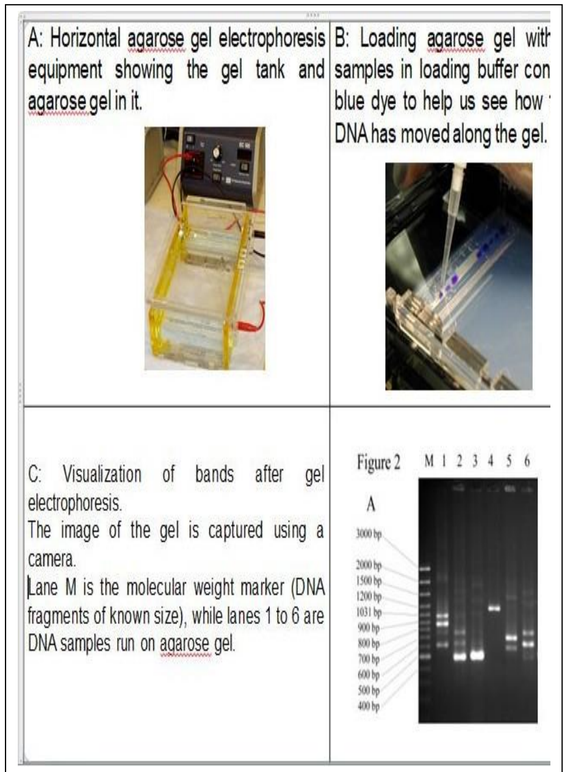
Keep the pellet at 37 º celcius for 10 minutes.

10. Dissolve the dry pellet in nuclease free water or TE buffer (PH. 8.0). Store DNA at 4 º celcius or -20 º celcius.





Results



In the figure 1 above which had two separate gels used for electrophoresis show that the different number of movement of DNA fragments hence show the size of different fragments present in the mosquito DNA samples?

In lane 1 and 2 in gel 2 shows that they have smallest fragments of DNA as they moved the furthest and in lane 15 and 16 in gel 1 also had the smallest fragments of DNA present on their respective wells.

In lane 7, 8,9,10 respectively in gel 1 and gel 2 have the largest DNA fragments hence they did not move further away from their wells.

Discussion

Using the PCR and gel electrophoresis method we can introduce the wolbacia bacteria into mosquito DNA hence reducing the transfer of the viruses. Wolbacia is a safe and natural occurring bacterium which is found in up to 60% of species. However it is not found in aedes aegypti hence transferring zika, chikungunya and dengue viruses.

The first way is introducing wolbacia in the mosquito immune system makes it harder for the viruses to survive in the body hence making it hard for the mosquitoes to be infected with the viruses about.

The second way is by competing wolbacia against the viruses for key molecules like cholesterol. Both the viruses and the wolbacia require cholesterol to survive inside the mosquito.When the wolbacia is present it consume most of the key molecules hence making it harder for the growth of these viruses as result it will be more difficult for the viruses to be transmitted.

Wolbacia does not have a negative effect on the environment, animals and humans as it is a natural occurring bacterium.

Once there has been a successful integration of the wolbacia inside the mosquito gene the specie can be cloned to produce the exact same/identical mosquitoes. Once sufficient amount of cloned wolbacia infected mosquitoes are made, they can be released in small amount to the wild to mate with other female mosquitos that do not have wolbacia present in them. The mating would result in producing offspring that are inherited with the wolbacia bacteria hence reduces transmission of the viruses.

Conclusions

Agarose gel electrophoresis is used to obtain DNA fragments which are greater than 50 base pairs.

The entire procedure of PCR and gel electrophoresis took place in a sterile environment, Pwani University laboratory, to avoid any contamination.

The gel electrophoresis helped in obtaining different mosquito DNA fragments and showed the amount of base pair in a fragment depending on its movement from the negative to the positive side.

This is a genetic engineering process hence can be used for analyzing the fragment, and its content to modify by isolating certain proteins which aid the mosquito to transfer viruses.

Bibliography

- GLOBE observer app
- https://protocolexchange.researchsquare.com/article/nprot-2566/v1
- https://www.worldmosquitoprogram.org/en/work/wolbachia-method/how-it-works
- https://www.worldmosquitoprogram.org/en/learn/faqs#