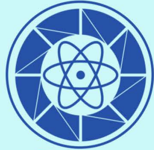

International Journal of Multidisciplinary Research Updates

Journal homepage: https://orionjournals.com/ijmru/

ISSN: 2783-0179 (Online)

(Research Article)

How to detect monkey poxvirus? Hint: Polymerase Chain Reaction (PCR)

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International Journal of Multidisciplinary Research Updates, 2022, 03(02), 017–019

Publication history: Received on 04 July 2022; revised on 09 August 2022; accepted on 11 August 2022

Article DOI: https://doi.org/10.53430/ijmru.2022.3.2.0052

Abstract

A minute of silence. Kary Banks Mullis has passed away (07/08/2019).

However, his legacy remains with us. His legacy has made it possible to detect one of the deadliest viruses since the so-called Spanish flu of 1920, which caused more deaths than the First World War. Kary Mullis invented the Polymerase Chain Reaction (PCR) and that is probably why he was awarded the Nobel Prize in Chemistry in 1993. Today, there are those who wonder if Molecular Biology (and Medicine) is divided into a before and after PCR. Something as important as other important contributions to our Humanity such as those described by James Watson, Rosalind Franklin, Francis Crick, or Albert Einstein.

Yes, monkeypox and its associated agent may be the reason for the use of PCR, and although the minds of great scientists and countries are still focused on COVID19 and the associated pathogen; SARS-CoV-2, would not be unwise to pay more attention to another virus that in humans is said to have been eradicated from the planet since 1980, according to WHO.

Keywords: PCR; Detection; Deoxiribovirus, Ribovirus; Genomes

1 Introduction

"*Viruses are viruses*". André Lwoff (French microbiologist) said when defining them when asked (according to legend). Viruses have a DNA (deoxiribovirus) or RNA (ribovirus) genome. Examples of the first type: herpes virus and smallpox virus. Examples of the second type: Canine distemper virus and SARS-CoV-2. Associated diseases is not the same as the causative pathogen (1). Any pathogen that has a DNA or RNA genome can be the reason for the use of the North American biochemist's invention. Although in the case of SARS-CoV-2, a prior DNA synthesis reaction must be carried out from the RNA of the virus, the following stages follow the scheme proposed by K. Mullis to the letter.

Both scientists were awarded the Nobel Prize: Lwoff in 1965 and Mullis in 1993. [2]. The former spectacularly defined viruses with "viruses are viruses", since there is no other pathogen that generates progeny ("offspring") as do viruses, because they do not follow the typical binary fission. The second scientist has delighted us with a huge idea to amplify a gene from a pathogen of interest, be it from animals or humans. "*Viruses are viruses*" also points to what was just mentioned, viruses are very similar, whether they affect animals or humans.

2 Material and methods

The monkeypox virus genome is double-stranded DNA approximately 198,000 nucleotide bases in size. The sequence can be known if you enter the Genbank® database [3]. If the genome is double-stranded DNA, it can be the perfect substrate for the reaction proposed by K. Mullis [4]. But first you must choose some of your own genes, which is

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conserved or generates some protein of the virus of interest, for example one that has been used to make a vaccine. Let us remember that the generation of an immune response in the patient is due to the exposure of some "piece" of the virus to the immune system of the affected organism, a viral protein generally.

The Genbank database will give us the sequence of the gene that encodes the chosen protein. Thus, once the sequence is known, it is possible with a computer program to generate specific primers to amplify the chosen gene or gene fragment whose size is previously known and which is amplified millions of times. Subsequently, depending on the Mullis modality chosen (conventional or real-time), they will be able to verify the presence of new DNA fragments. In conventional modality the detection of positive samples can be performed by nucleic acid electrophoresis that is, by the migration of biomolecules (DNA fragments) in an electric field and subsequent "revealing" of the gel by means of a chemical agent that binds to the DNA and also “fluorescent” when hit by ultraviolet light [5, 6].

3 Results and discussion

The interest fragments can be sequenced and thus obtain the percentage of nucleotide identity (PNI) of the fragment obtained in our laboratory in reference or comparison with the official Genbank ® data.

The characteristics of an optimal primer will be described in another text, but it should be mentioned that its sequence must be complementary to that of the sample DNA.

In the case of smallpox, since it is a virus with a DNA genome, the fragments obtained after PCR can directly follow the aforementioned scheme: PCR, electrophoresis and sequencing to obtain the PNI. By having the PNI, it will be possible to elucidate whether it corresponds to the sequence of the original virus or to any of its variants, as it can currently be defined with respect to SARS-CoV-2, with the difference that when dealing with an RNA virus (SARS-CoV- 2) the previous stage of reverse transcription must be incorporated, for which the reaction is called RT-PCR.

It is important to define the detection target, that is, the gene to be detected and I repeat: it must be one's own gene, one that is conserved and that hopefully is involved in the generation of a protein used in the generation of antibodies through a vaccine.

We have carried out this strategy in a third world country and if we could, so can you [7, 8, 9, 10].

Figure 1. Agarosa 2% Gel electrophoresis of PCR samples. (Lines 1-6 positives samples; line 7: Molecular Marker)

4 Conclusion

Thus, the detection of genomes can be performed on any pathogen that interests us. K. Mullis' idea is great and Lwoff's way of defining viruses is phenomenal.

Compliance with ethical standards

Acknowledgments

I thank the FAVET students (for achieving part of their dreams with us) and to Dr. Aron Mosnaim from the Wolf Foundation, Illinois, USA (since 2020).

Disclosure of conflict of interest

No conflicts of interest in author.

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