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(RESEARCH ARTICLE)

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In memorial: The Polymerase Chain Reaction (PCR)

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Abstract

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

Kary Mullis was an American Biochemist and Doctor of Chemistry who developed the Polymerase Chain Reaction (PCR) in 1985, considered one of the most important legacies in the history of Humanity, along with the description of DNA (Watson, Crick and Franklin) and the theory of Relativity (Einstein).

Currently, I consider anyone who does not know the PCR developed by Kary Mullis to be illiterate, since it is a molecular technique that came to stay just like COVID19. Unlike the current disease, PCR is a molecular tool that allows a DNA fragment of interest to be amplified to a level of at least one billion copies.

Keywords: PCR; Nobel Prize; Remarkable idea

1 Introduction

This molecular technique is so magnificent that the Swedish Academy of Sciences had no choice but to award Kary Mullis the Nobel Prize in 1993. Dr. Mullis had several detractors, because he was an atypical scientist and in his youth he used LSD and even once mentioned that the idea of PCR was suggested to him by a green raccoon.

He was also in love with surfing, as can be seen in his book dancing naked in the mind field and according to his indications, a small amount of DNA from a problem sample is enough to amplify a gene or part of a gene present in the sample [1, 2, 3]

The product, double-stranded DNA, is easily detected and today is the technique of choice for the detection of pathogens of veterinary and/or human interest.

2 Material and methods

Following his brilliant idea and summarizing, it is necessary to include in a test tube (today Eppendorf tubes, 0.2 mL) the sample (DNA), the four nucleotides (A, T, C, G), a thermostable Polymerase (originally Taq Polymerase), an enzyme cofactor (Mg^{+2}) and the primers of the PCR reaction.

As can be seen, Don Kary's idea was always to emulate the duplication of DNA that occurs in the cell of a living organism, but without the participation of the notorious enzymatic system involved. For Mullis, everything can be done with changes in temperature.

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Thus, to denature double-stranded DNA, apply heat (94°C), then allow primer alignment (X°C) and finally elongation (72°C). So far, a cycle has been completed. The genius of Kary Mullis indicates that the process should be repeated about 30 times.

Thus, the product is amplified a billion times (Figure 1).

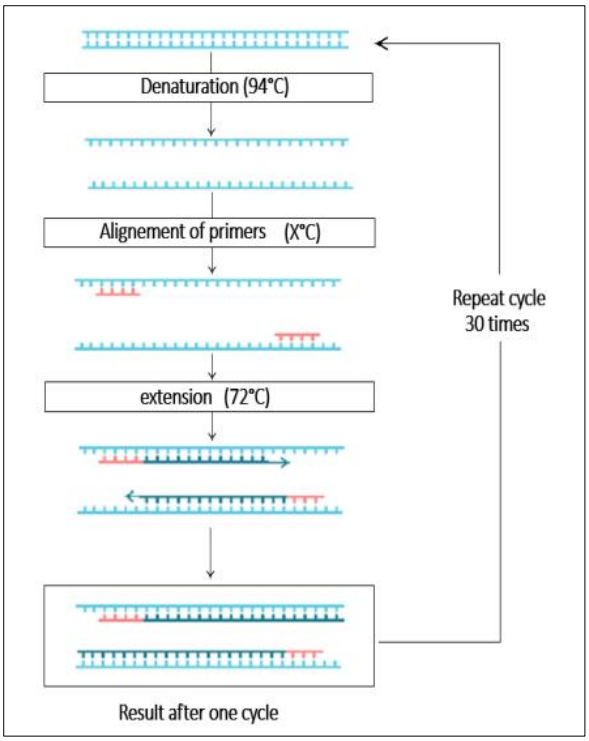

Figure 1 Scheme of a conventional PCR from K. Mullis

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 [4, 5].

Another of the current facilities to carry out a PCR is the existence of a programmable machine to carry out the proposed temperature changes: the thermocycler. Both the temperature and the nucleotide sequence of the primers are specific for the pathogen to be detected. Primers? What's that? A primer is an oligonucleotide, that is, it corresponds to a chemical compound consisting of about 20 nucleotides.

3 Results and discussion

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.

There are variants of this technique, such as nested PCR, which corresponds to two serial PCR reactions, and multiplex PCR, which considers the use of two or more pairs of primers in a single reaction.

Additionally, this technique allows detecting RNA if the conditions are previously granted so that, by means of a reverse transcriptase, DNA can be synthesized from RNA. This technique is called RT-PCR and is mainly used to detect viral pathogens whose genome is RNA, such as canine distemper virus or SARS-CoV-2.

This fantastic idea has allowed around 30 new veterinary doctors to be trained in our country, which have included the detection and identification of pathogens of veterinary interest [6, 7 ,8, 9, 10]. Enough for today.

4 Conclusion

Thanks to the brilliant mind of Kary Mullis, we can now know and apply this knowledge in detection of genes of pathogens that affect humans and other species. His death means another great loss for humanity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflicts of interest in author.

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