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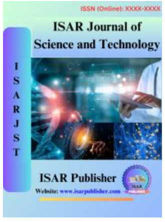

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The legacy of Kary Mullis

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Abstract: Many envy or ignore it; others, including myself, consider it ahead of its time. Molecular medicine is defined as something that comes before and after the polymerase chain reaction (PCR). Today, there are undoubtedly various modifications or variants of PCR: nested PCR, multiplex PCR, and even a pre-reaction has been added, giving rise to RT-PCR.

His legacy is indelible and lasting; it was not for nothing that he received the news of his Nobel Prize in 1993 while surfing in California.

This admirable technique has been used in several attempts to scrutinize the genome of any pathogen, whether DNA or RNA. Without going any further, SARS-CoV-2 variants can be identified by applying this technique in conjunction with nucleotide sequencing.

In the time we have left, and if another pathogen of human or veterinary interest were to emerge (as will happen), we already have a powerful technique to try to understand its genome, its possible variants, and be in a better position to confront it.

Keywords: Molecular medicine, Polymerase chain reaction (PCR), RT-PCR, Genome sequencing, SARS-CoV-2 variants.

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Background

Shaping or understanding a genome has been a long and tedious task that has long preoccupied some scientists (1, 2, 3). This has required compiling several investigations that may have been initially unrelated. Without going any further, the discovery of an enzyme capable of synthesizing DNA from RNA revolutionized the scientific community in 1970 when Temin and Baltimore reported on it (4, 5)

Sanger and his colleagues, for their part, were able to identify the genome of a virus for the first time in 1982 thanks to their famous sequencing method (6, 7)

Thus, the list of scientists who have contributed to the development of the dizzying discipline of virology continues.

However, at the level of Watson and Crick who pointed out the content and shape of DNA as part of the human chromosome (8, 9) we can include Kary Banks Mullis, an American biochemist who in 1993 shared the Nobel Prize in Chemistry with Michael Smith (10)

Kary Mullis attended the Georgia Institute of Technology in Atlanta, where she earned a bachelor's degree in chemistry. He moved with her family to Berkeley to continue her studies at the University of California, where she earned her PhD in biochemistry under the guidance of J. B. Neilands. In 1981, at a time when biotechnology was a rapidly developing field, she was in California studying polymerases for Cetus Corporation (11).

Controversial? Perhaps because of several quotes he uttered during his lifetime. A genius? Without a doubt.

Materials and Methods

What is his legacy? He had the brilliant idea of successfully simulating the process of cell replication—that is, duplicating its genome—in the laboratory. He achieved this by modifying the temperature of a tube containing DNA of known origin, appropriate primers, nucleotides, and a thermostable enzyme (Taq polymerase) in the presence of Mg^{+2} (12).

Sounds easy, right? Well, today it is, since there is a reagent called Master Mix, which contains the nucleotides, Taq Polymerase, and Mg^{+2}, in appropriate concentrations. In Kary Mullis's time, each of the ingredients in Master Mix had to be added separately to the reaction tube (A, T, C, G, primers, and Mg^{+2}). This greatly facilitated the unsuccessful reaction due to contamination (13)

It's worth mentioning that today we have a device that replaces the thermoregulated baths that Kary Mullis and colleagues probably used to make the temperature changes involved: the thermocycler, and the process is repeated at least 30 times (14).

What temperature changes? Well, following the instructions for a conventional Polymerase Chain Reaction, the process begins by heating the reaction tube containing all the reagents to 94-95°C, which separates the DNA if it is double-stranded. The temperature is then lowered to the annealing temperature of the primers used, facilitating their union with the template DNA, if any. Finally, the temperature is raised to 72°C, which generally coincides with the temperature at which a T*aq* Polymerase forms a double strand of DNA (15)

Why repeat at least 30 times? Easy, because by repeating the process at least 30 times, the initial material is amplified 2^{30} times, or 1073 million times one DNA fragment of interest, allowing it to be visualized. Amazing, huh? But true!

Currently, there are several variants to the above, and perhaps the most ingenious of all is to add a previous step using Baltimore, Muzitani and Temin's discovery: a reverse transcriptase, ideal for amplifying the genome of those pathogens whose genome is RNA, such as influenza, SARS-CoV-2, or canine distemper viruses (16, 17, 18)

Discussion

Implementing a PCR protocol following Kary Mullis's guidelines and/or involving the prior incorporation of a reverse transcription (RT-PCR) step in the case of an RNA virus currently poses no challenge.

Which variant should be used? This will depend on the objective. For example, performing a nested PCR requires the use of at least four primers, preferably with different annealing temperatures for each pair used. This increases the sensitivity of the reaction, since the substrate for the second reaction is the product generated in the first. If you intend to detect two or more pathogens simultaneously, the suggestion is to use a multiplex PCR, where the annealing temperature is the same for all primers used (19)

And what is the difference with real-time PCR? Easy! Conventional PCR necessarily involves an electrophoresis system that allows visualization of the amplicon generated after repeated cycles, using a photographic record. Real-time PCR, on the other hand, records the presence or absence of the expected product at all times (20).

Finally, Kary Mullis's ingenious idea can be complemented with online biotools that allow sequence alignment, optimal primer generation, or pathogen identification based on a sequence obtained in conjunction with the GenBank database.

Conclusion

There's no doubt that the technique known as PCR has been used for many other purposes not mentioned above, including forensic medicine and paternity studies. It's no coincidence that it's said that medicine is divided before and after PCR (21), the brilliant idea of Kary Mullis (1945-2019).

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Conflict of Interest

None.

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