Innovative Journal of Medical and Health Science
Received 20 June 2022 | Revised 18 July 2022 | Accepted 25 July 2022 | Published Online 09 August 2022

DOI: https://doi.org/10.52845/IJMHS/2022/12-8-1
Inno J of Med Health Sci 12 (08), 1946-1950 (2022)

RESEARCH ARTICLE

ISSN (P) 2589-9341 | (O) 2277-4939 IF:1.6

The unconventional life of Kary Mullis and the history of invention of Polymerase Chain Reaction (PCR): the begin of new era in Dentistry and Medicine scientific research

[1] Roberto Arrigoni | [2] Rosa Porro | [3] Roberto Pistilli | [4*] Michele Covelli

[1] CNR Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (IBIOM), 70125 Bari, Italy

[2] Department of Informatics, University of Bari "Aldo Moro", 70125 Bari, Italy

[3] Oral and Maxillofacial Surgery, San Camillo - Forlanini Hospital, 00152 Rome, Italy

[4] Interuniversity Research Center "Population, Environment and Health" - CIRPAS, University of Bari "Aldo Moro", 70122 Bari, Italy

Abstract

The impact that Polymerase Chain Reaction (PCR) has had and continues to have in the world of dental and medical biological scientific research is probably second to none. The inventor of this technique, Kary Mullis, is certainly a very particular figure in the scientific field, and without any doubt also deeply divisive, with his unconventional ideas - to say the least - regarding various topics. Here we want to briefly review the life of Kary Mullis and his contribution to scientific progress in dental and medical sciences.

Keywords: Dental molecular biology, PCR, DNA, Nobel prize.

1 | INTRODUCTION

The history of progress in the experimental sciences is normally characterized by small advances made by many researchers around the world; in this way, there is a slow, but constant and steady growth of knowledge. But every now and then there is the "miracle": a sudden, exponential, incredible progress due to the intuition of some genius, perhaps even an unlikely character: after all, the saying "genius and recklessness" arises with good reason.

And certainly, this is the case of Kary Mullis and the invention of the PCR technique, which has revolutionized the world of molecular biology and of all biological science in general. (Figure 1).

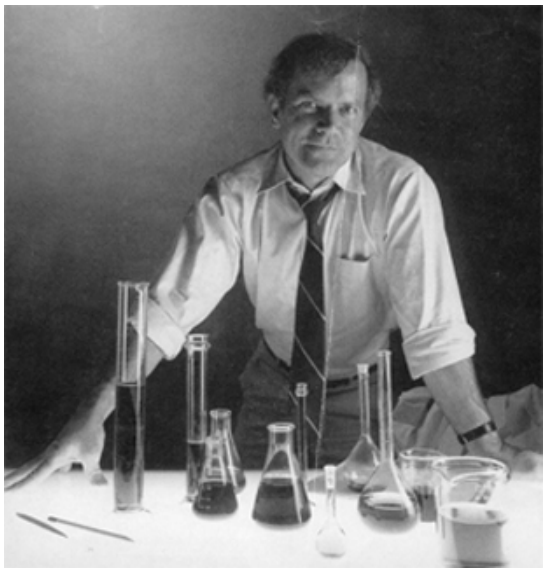

Figure 1. Kary Mullis in his "favorite place to play with DNA".

It is no overstatement to say that a modern laboratory of biochemical, genetic or cell biology research owes much of its existence to the Polymerase Chain Reaction (PCR), without which it would not even be possible to hypothesize the dizzying development that these experimental sciences have reached in the last 30 years. Grandiose, epochal projects such as the Human Genome (2001-2013), Microbiome (2008-2017), and the very recent completion of the human genome thanks to the international consortium called Telomere-to-Telomere (T2T) of (2019-2022), would not be in the least conceivable without the stroke of genius of Mullis [1, 2, 3].

Corresponding Author: Michele Covelli
Interuniversity Research Center "Population, Environment and Health" - CIRPAS, University of Bari "Aldo Moro", 70122 Bari, Italy EMail: dott.covelli.michele@gmail.com

Who was this scientist?:

Kary Banks Mullis was born on December 28, 1944, in Lenoir, South Carolina, near the Blue Ridge Mountains. His family was a typical American middle class and did not have a prominent scientific background: his father a furniture salesman, her mother was a housewife. However, since he was a boy, Mullis has been passionate about science, doing experiments of all kinds, particularly in chemistry, designing alternative method for the synthesis of various chemical substances. His academic career was brief: after graduating in Chemistry at the Georgia Institute of Technology of Atlanta in 1966, he received his Ph.D. in Biochemistry from the University of California Berkeley in 1972, about his research on protein structure and synthesis. Later Mullis moved to Lawrence, having won a research fellowship in pediatric cardiology from the University of Kansas Medical School. Subsequently he completed a two-year post-doc in Pharmaceutical Chemistry at the University of California at San Francisco (UCSF).

After those experience, Mullis became disillusioned about the academic world; he deemed himself unfitted for the role, because he did not find academic life sufficiently stimulating. Hence, Mullis's scientific path - as throughout his life -was also quite peculiar: after his doctorate he abandoned the university and choose to be a directing manager of a friend's bakery, and in his spare time dedicating himself to writing science fiction stories. Only later he returned to science, also thanks to the encouragement of his friend Thomas White, who helped him get a job at the Cetus Corporation, a biotechnology company based in Emeryville, California.

The history of PCR discovery:

At the Cetus his job was under White supervision, who was at the time the Cetus director of molecular research, and Mullis worked as a chemist. Eventually Mullis will work 7 years at Cetus, where in 1985 he will invent PCR (Figure 2) [4].

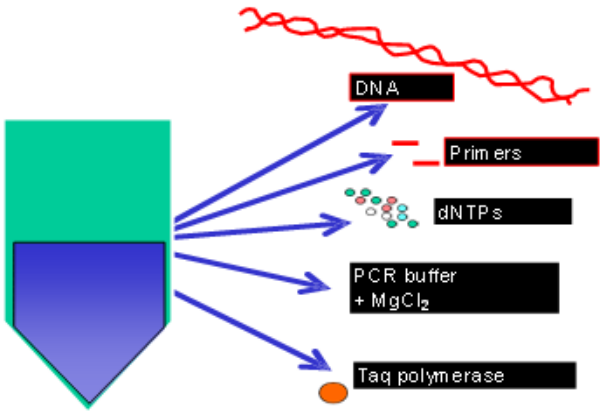

Figure 2. Schematic representation of PCR discovered from Kary Mullis.

Later he will become director of molecular biology of another company, Xytronyx, and in the subsequent years he will work mainly as a consultant on DNA and chemistry of nucleic acids in general for several companies, and also for forensic analysis: in his autobiography he recalls his role in the O.J Simpson trial, a hugely popular event in the USA.

In the late 90's and 2000's Mullis founded a company with the intent of selling jewelry pieces containing the amplified DNA of deceased famous people such as Elvis Presley and Marilyn Monroe.

It is not possible to write about Kary Mullis and not talk about his uniqueness: after all, it is quite unusual to find a Nobel prize winner that candidly admit his experience with LSD, even giving it credit for the birth of PCR.

We are somewhat used to think about a scientist like a rather serious, no frills- guy who is totally concentrated on her/his work, leaving very few moments for the rest of his life. To hear about the Kary Mullis life, filled with so many different interests, activities, is somewhat refreshing. A Nobel prize does not have to be a boring person: it can surf every day, it can be passionate about life, sport, and much more. In his writing, Mullis affirmed the importance of creativity, passion, and enthusiasm about science, encouraging especially the young scientist to have a multidisciplinary approach, who can allow to see relationships otherwise indistinguishable [5].

Yet the Kary Mullis life can teach us also something else about experimental science, or maybe better about the science publishing system. All of us are very familiar with the motto "publish or perish", i.e., the necessity for academic people to publish rapidly and frequently the results of their work; otherwise, they are destined to be at the margin of the scientific community, with no real possibility to access to financing and ultimately perform adequate experimental research.

The notorious Mullis experience with the Nature journal is quite amusing: they accepted a purely speculative article about matter of the universe and time regression [6] made by a young, unproven scientist at the beginning of his career; and yet 20 years later the same journal rejected a manuscript from Mullis that described for the first time the Polymerase Chain Reaction. If it hadn't really happened, no one could believe such an experience; definitely scientific publication procedure - in spite of his name –is not a perfect science, but rather a tumultuous process where even an article worth a Nobel Prize can be lost.

Mullis therefore decided to publish his work in a less heralded journal, the Cold Spring Harbor Symposia on Quantitative Biology in 1986 [7]. The scientific community did not wait too long to recognize the potential of PCR: within a few years, it became a technique used daily in everyone. biological research laboratories, and in the following years Mullis received numerous prestigious awards, including the Robert Koch Prize in 1992 and the Japan Prize in 1993. Also, in 1993 Kary Mullis received the Nobel Prize in Chemistry together with Michael Smith, for their eminent contribute to DNA analysis: Mullis for developing the Polymerase Chain reaction, and Smith for setting up an efficient method for site-directed mutagenesis [8].

Today it is inconceivable to imagine a biological laboratory without a PCR thermocycler: there is no biological field that does not use PCR - classical or real time - in its experiments. Just think about an actual or future scientific breakthrough: preventive screening for a conspicuous amount of genetic disease, gene therapy, vaccine production, personalized medicine, genome, and microbiota analysis, CRISPR, SARS COV2 virus detection, to name a few….: without this simple but elegant technique, none of them would be possible [9-10].

PCR allows selective amplification of any DNA region of our choice, starting even from a single DNA molecule [11]. The PCR technique consists in cycles, usually repeated 20-30 times. Every cycle of the PCR reaction consists in 3 steps:

1. Denaturation: the double-strand helix must be separated;
2. Annealing: the primers – their sequence designed to be specific for the chosen DNA sequence - bind the complementary DNA region;
3. Extension: by using a DNA polymerase the elongation of the newly formed DNA strands begins.

By cycle repetition, the number of copies of the desired DNA sequence grows exponentially, reaching billions of copies in just over an hour (Figure 2).

To pinpoint the breakthrough that the PCR was for the biological research, we can divide the research in before and after the PCR development. Without this technique, all the work on genetic material was rather amateurish, with no clear possibility to study DNA molecule in detail, to know the genetic sequence, the gene expression, to clone a DNA sequence in a vector, and so on [12, 13, 14]. New research fields like molecular biology, genetics practically were born with the PCR.

By no means Kary Mullis was a role model: the habitual use of several drugs – LSD included – [15, 16], eccentric ideas (to put it politely) about aliens kidnapping – [17], self-admitted livestock taunting with electric fences [18], are really questionable. Even at the beginning of his work at the Cetus company, his behavior was so creep that he was not fired because of his friendship with the director White [19]. Also some of his position about scientific topics – he was casting doubts about the HIV as the cause of AIDS -, are somewhat controversial[20].
Was the invention of the PCR a stroke of luck for Mullis, one night while driving in the hills of Mendicino in California [21], probably - by his own admission - under the influence of drugs? We don't know, we can't say for sure, and perhaps the answer to that question isn't even that important.

2 | CONCLUSIONS

The eccentricity of Mullis' life and thoughts does not in any way diminish the value of his contribution to scientific progress: we will be eternally grateful to him for allowing us to effectively manipulate DNA, opening the doors to new knowledge and research, therapeutic and clinical possibilities hardly imaginable without him.

3 | BIBLIOGRAPHY

1. Green, E. D., Watson, J. D., & Collins, F. S. (2015). Human Genome Project: Twenty-five years of big biology. Nature, 526(7571), 29–31. https://doi.org/10.1038/526029a
2. Integrative HMP (iHMP) Research Network Consortium (2019). The Integrative Human Microbiome Project. Nature, 569(7758), 641–648. https://doi.org/10.1038/s41586-019-1238-8
3. Nurk, S., Koren, S., Rhie, A., Rautiainen, M., Bzikadze, A. V., Mikheenko, A., Vollger, M. R., Altemose, N., Uralsky, L., Gershman, A., Aganezov, S., Hoyt, S. J., Diekhans, M., Logsdon, G. A., Alonge, M., Antonarakis, S. E., Borchers, M., Bouffard, G. G., Brooks, S. Y., Caldas, G. V., … Phillippy, A. M. (2022). The complete sequence of a human genome. Science (New York, N.Y.), 376(6588), 44–53. https://doi.org/10.1126/science.abj6987
4. Wade, Nicholas (September 15, 1998). "Scientist at Work/Kary Mullis; After the 'Eureka', a Nobelist Drops Out". The New York Times. p. F1. ISSN 0362-4331.
5. https://www.karymullis.com/science.shtml
6. Mullis, Kary (May 1968). "Cosmological Significance of Time Reversal". Nature. 218 (5142): 663–664. Bibcode:1968Natur.218..663M. doi:10.1038/218663b0. ISSN 1476-4687. S2CID 4151884.
7. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51 Pt 1:263-73. doi: 10.1101/sqb.1986.051.01.032. PMID: 3472723.
8. Shampo MA, Kyle RA. Kary B. Mullis--Nobel Laureate for procedure to replicate DNA. Mayo Clin Proc. 2002 Jul;77(7):606. doi: 10.4065/77.7.606. PMID: 12108595.
9. Fueller, J., Herbst, K., Meurer, M., Gubicza, K., Kurtulmus, B., Knopf, J. D., Kirrmaier, D., Buchmuller, B. C., Pereira, G., Lemberg, M. K., & Knop, M. (2020). CRISPR-Cas12a-assisted PCR tagging of mammalian genes. The Journal of cell biology, 219(6), e201910210. https://doi.org/10.1083/jcb.201910210
10. Ide, S., Hayakawa, K., Yamamoto, K., Tsuzuki, S., Tanuma, J., Ohara, K., Yamada, G., Okuhama, A., Kanda, K., Suzuki, T., Akiyama, Y., Miyazato, Y., Nakamura, K., Nomoto, H., Nakamoto, T., Ujiie, M., Saito, S., Morioka, S., Ishikane, M., Kinoshita, N., … Ohmagari, N. (2021). Positive Ratio of Polymerase Chain Reaction (PCR) and Validity of Pre-Screening Criteria at an Outpatient Screening Center during the Early Phase of the COVID-19 Epidemic in Japan. Japanese journal of infectious diseases, 74(5), 481–486.
11. Analytical Methods Committee Amctb No. PCR - the polymerase chain reaction. Anal Methods. 2013 Dec 19;6(2):333-336. doi: 10.1039/c3ay90101g. PMID: 33985286.
12. Matsuda K. PCR-Based Detection Methods for Single-Nucleotide Polymorphism or Mutation: Real-Time PCR and Its Substantial Contribution Toward Technological Refinement. Adv Clin Chem. 2017;80:45-72. doi: 10.1016/bs.acc.2016.11.002. Epub 2017 Jan 4. PMID: 28431642.
13. Mehta P, Kaushik R, Singh KP, Sharma A, Singh MK, Chauhan MS, Palta P, Singla SK, Manik RS. Establishment, Growth, Proliferation, and Gene Expression of Buffalo (Bubalus bubalis) Transgenic Fetal Fibroblasts Containing Human Insulin Gene, and Production of Embryos by Handmade Cloning Using These Cells. Cell Reprogram. 2018 Apr;20(2):135-143. doi: 10.1089/cell.2017.0013. Epub 2018 Feb 15. PMID: 29446977.

14. Liu W, Tang X, Zhu X, Qi X, Zhang N, Si H. Genome-wide identification and expression analysis of the E2 gene family in potato. Mol Biol Rep. 2019 Feb;46(1):777-791. doi: 10.1007/s11033-018-4533-9. Epub 2018 Dec 7. PMID: 30535894.
15. McDonald, Coby (Winter 2019). "Intolerable Genius: Berkeley's Most Controversial Nobel Laureate". California Magazine. Cal Alumni Association.
16. Gross, Michael (2001). Life on the Edge: Amazing Creatures Thriving in Extreme Environments. New York: Basic Books. p. 103. ISBN 978-0-7382-0445-1.
17. Arnaud, Celia Henry (August 21, 2019). "Kary Mullis dies at age 74". Chemical & Engineering News.
18. Mullis, Kary Dancing Naked in the Mind Field (1998) 0679774009 (ISBN13: 9780679774006)
19. McDonald, Coby (Winter 2019). "Intolerable Genius: Berkeley's Most Controversial Nobel Laureate". California Magazine. Cal Alumni Association. OCLC 939087276. Retrieved August 24, 2021.
20. https://www.nobelprize.org/prizes/chemistry/1993/mullis/biographical/
21. Mullis KB. The unusual origin of the polymerase chain reaction. Sci Am. 1990 Apr;262(4):56-61, 64-5. doi: 10.1038/scientificamerican0490-56. PMID: 2315679.