Which one scientific discovery or pathologist has had the most significant impact on medicine/pathology in the last 60 years?

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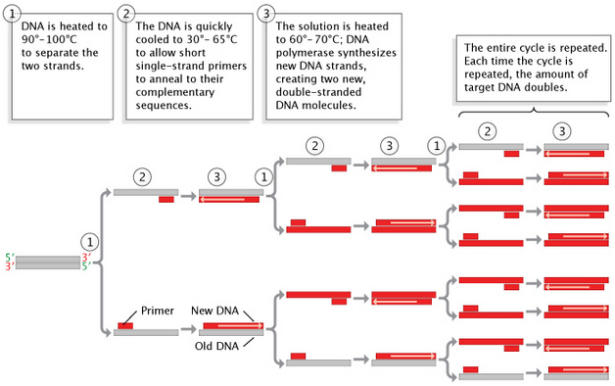
The story of pathology over the past sixty years has been shaped by nothing so much as the genomics revolution, touching all aspects of medical research and care. The birth of genomics can be traced back to the discovery of the polymerase chain reaction (PCR) technique in 1983 – and the discovery of heat-stable Taq polymerase – making this humble discovery in DNA replication technology by extension the most important of recent times.

Medical genetics and the discovery of PCR

What makes genetics such a rich field of science is the challenge of simultaneously trying to characterise how traits are expressed at the level of individuals, and how they are encoded at the microscopic level of molecular biology: the most notable example being the discovery of chromosomes by observing cells under the microscopes and relating the nuclear structures with the observation of traits being passed between generations of fruit flies[1]. By the 1970s, the field of genetics had discovered the complementary base pairing system, the structure of DNA[2] and even the triplet code[3]. Further developments were limited by the fact that creating enough DNA to analyse was challenging: the state-of-the-art technique was using bacteria as DNA cloning devices, but this was resource and time-consuming.



PCR changed the game by allowing DNA to be cloned without a living organism. Simply, PCR worked by using two primers – manufactured DNA sequences which bind to each end of the target sequence by complementary base pairing – and using a DNA polymerase protein to create the rest of the DNA sequence starting from the primers. The changing temperature in the technique allowed for the two strands of DNA to break, and for the cycle of primer binding and DNA polymerisation to begin again. Over time, the target DNA sequence would be produced[4].

There were three key breakthroughs. First was being able to select a specific DNA sequence to amplify by using a forward and backward primer. This meant that the DNA polymerase could only replicate sequences within the two primers, which reduced non-specific amplification of DNA. The second breakthrough was the discovery of a heat-stable DNA polymerase, Taq. This allowed the polymerase to survive the heat needed to break the DNA strands, meaning the process could be run continuously.

The third was that this process allowed for each new copy of DNA to act as a template, leading to an exponential increase in copied DNA and very fast cloning times[5].

PCR and the development of genomics

PCR was immediately understood to be a technological breakthrough and has formed the bedrock of modern genomics. In the field of histopathology, PCR techniques were being used to detect gene mutations until the last decade before the advent of next-generation sequencing (NGS) techniques such as Illumina sequencing[6]. The importance of PCR was underlined by its use in the Human Genome Project – an international effort to build a human reference genome – to clone known reference sites called sequence-tagged sites, for alignment of DNA fragments.[7] This project has allowed for the full usage of genomics in human disease, notably in projects such as The Cancer Genome Atlas[8] and Genotype-Tissue Expression Program[9] to understand cancer biology and the role of epigenomics respectively.

Even with the advent of NGS techniques, PCR continues to have the last laugh; to be used either as an adjunct to NGS to selectively amplify sequences of interest[10] or as a primary method in NGS. This is the case with emulsion PCR, where the PCR reaction is shrunk to the size of an emulsion droplet, allowing for a massive number of simultaneous PCR amplification reactions to occur with subsequent sequencing[11].

PCR in modern pathology

Despite nearly four decades since the development of PCR, it continues to be used in pathology.

Medical microbiology – a subspeciality dependent on amplifying small numbers of *in vivo* pathogens to be characterised – now uses PCR extensively for hard-to-culture pathogens such as *Neisseria* and *Chlamydia*[12]*.* Combining PCR with a reverse transcriptase – to detect RNA – and a primer attached to a fluorophore – to allow for detection of the number of DNA copies – has led to the development of RT-PCR, now famous amongst the lay public for its usage as a gold-standard diagnostic test for SARS-CoV-2[13].

The use of PCR is not restricted to pathogenic nucleic acids but is also used in cell-free fetal DNA detection in reproductive medicine. This technique is used to detect aneuploidy and the *RHD* genotype in the fetus for Rhesus factor compatibility[14].

PCR has also allowed for the development of DNA fingerprinting. Developed in 1984, modern DNA fingerprinting relies on the ability of PCR to amplify short tandem repeats – sections of DNA containing repeated base sequences which are highly variable between individuals – to then be used to identify relatedness between different individuals[5]. This has widespread applications in pathology such as criminal investigations in forensic pathology, and in paternity testing within reproductive medicine.

Why PCR?

It is initially strange to think that a discovery by an eccentric[15] molecular biologist in the 1980s to clone DNA would have such a profound effect on how pathology is practiced today. However, pathology has always benefitted from the development of new technologies which allow us to understand the biology of disease, starting with the light microscope and chemical stains in histopathology. It is clear looking back at so many of the highlights of the past sixty years in pathology – the characterisation of HIV/AIDS, the linking of *H. pylori* and peptic ulcers, the discovery of *BRCA1/2* in breast cancer, the development of imatinib and targeted cancer treatments and so many others – that modern pathology needed the new toolkit to detect pathogens and faulty cells that genomics provided. PCR is unique amongst discoveries: both being used every day by pathologists in the diagnosis of infections, cancers, and genetic identity; and being the ancestor for the genomic revolution we live with today.

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