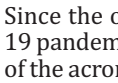

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What is PCR?

Since the outbreak of the Covid-19 pandemic, everyone has heard of the acronym PCR or the phrase PCR testing. But what is PCR technology? What is it used for and how has it revolutionised molecular biology?

An American biochemist, Kary Mullis, who worked at Cetus Corporation in Emeryville was responsible for synthesising oligonucleotides. Oligonucleotides are short single strands of synthetic DNA or RNA used in molecular biology serving multiple applications from forensic research to genetic testing. In this case, they aided in identifying point mutations in human genes like those in the HBB gene in sickle cell anaemia. To study a specific gene within the whole human genome, he needed a full copy of that gene sequence which was proving impossible at the time since upon trying, the primer would bind to too many places in the DNA making it impossible to make a copy of the desired gene.

Luckily, one day while driving his Honda Civic a thought occurred to him that if he used two primers and performed several cycles of denaturation, annealing and polymerisation he might be able to copy the desired sequence of DNA. This intellectual leap set him on the path to eventually be known as the father of PCR which was developed in May 1983. In 1993, Mullis was awarded a Noble Prize for his pioneering work in this field.

In simply words, PCR is a sensational technique that enables one to amplify a specific sequence of DNA and thus produce multiple copies from a single DNA sample. In fact, whereas the idea of gene cloning, DNA profiling and DNA-based diagnostics were considered impossible or ineffective, thanks to Mullis, today they have become day-to-day tests in laboratory biology. Thus, this breakthrough in the scientific community opened endless doors of opportunity and led to the boom in the field of biological research and biotechnology.

PCR is an inexpensive and fast test to molecular photocopy specific sequences of DNA. For instance, if a strand of hair at a crime scene is found and it is believed to be that of the suspect, that strand of hair is taken to a forensic science laboratory and a DNA sample is extracted from it. However, the amount of DNA extracted is not sufficient to study it closely and cross-analyse it with the DNA samples taken from the three possible suspects. Therefore, the scientist performs PCR on that sample to make several identical copies. The sample is first heated to denature the DNA or separate it into two different pieces of single-stranded DNA. Then, an enzyme, Taq polymerase, derived from the bacterium Thermophilus aquaticus, will synthesise two new strands of DNA by using the original DNA strands provided from the sample as a template. This will result in the duplication of the original DNA once the process is done and the DNA strands have annealed. Therefore, one PCR cycle results in two DNA copies where each of the copies has one old and one new strand of DNA. Then, each of these strands can be used to create two new copies, and so on. The cycle of denaturing and synthesizing new DNA is repeated around 30 to 40 times producing more than 1 billion identical copies of the original DNA sample. This whole process occurs in a few hours in a machine called a thermocycler which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis. Therefore, the scientist is now able to cross-analyse the DNA sample that was at the crime scene with those of the potential suspects and identify the person that was responsible for the crime. This is one example of how PCR is applied in forensics among others.

DNA paternity testing is done to determine if an individual is the biological parent of another individual and one of the processes involved in this test is PCR. First, a swab is rubbed against the insides of the individuals' cheeks (ex. Man A, Man B and Child X) for about 30 seconds. The swab's tip grabs loose cells for DNA testing. This swab is then taken to a laboratory for the DNA to be extracted from these cells. PCR then follows to amplify the DNA extracted into several copies of 16 to 18 genetic systems which are also called markers or loci to make one DNA profile consisting of 15 to 17 markers that are useful for the test. This is done for each individual. Each individual has different sizes/lengths of DNA fragments in the genetic system and a special software will then measure and compare the different sizes of the DNA sections of the individuals. Now, a child's DNA profile is a 50-50 combination of the mother's and father's DNA. Thus, if Man A is the father of Child X, Man A's DNA profile should match that of the child unlike that of Man B.

In Covid-19 testing, quantitative PCR is used. This is a process in which fluorescent dyes are added to the PCR process to determine the amount of genetic material from the SARS-Cov-2 virus in a sample. The PCR process that occurs is similar to those previously described however in this case the primers will only connect to a genetic sequence that is specific to the viral DNA, ensuring that only the viral DNA is being replicated. Thus, in a positive result, the individual is infected since many copies of the viral DNA were replicated. In a negative result, since the primers won't match with the genetic material, no DNA is copied since no viral genetic material is present, indicating no infection. However, in a false negative result, the person is infected but not enough viral genetic material was present in the sample for the PCR to detect it. This can also be applied in detecting other diseases and genetic conditions as well as monitoring the progression of diseases by looking for certain changes in a gene or chromosome, such as in cancer.

In agriculture and food-testing, PCR is used for the detection of pathogens, recognising allergens, product developments, detecting genetically modified organisms, the identification of fishery products, grain processing and rice cultivar identification. Product development considers seed quality control, gene analysis and discovery and gene cloning. For instance, gene cloning via PCR allows farmers to breed animals or cultivate plants for several generations having a specific beneficial characteristic without it being lost over the years. While this can be ethically debated as one is playing with nature and preventing the natural course of evolution, it may be more profitable for farmers in the long run unless a disease or pest wipes out that generation of unadaptable crops/animals.

Albert Einstein once said that "most of the ideas of science are essentially simple" and PCR is a true testament to that. By unlocking the basic concepts of DNA replication, over the years scientists have learned how to improve PCR technology to make the utmost of it. We have come a far way from when it was first developed and have witnessed how it has reshaped the biotechnological industry enabling accurate disease diagnosis, personalised and faster treatments as well as enhancing forensic research, food quality control and much more. One can only imagine what the future holds and how PCR technology may continue to elucidate problems we currently have no solution for.

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