The discovery of DNA

A “silent” scientific revolution

More than half a century ago, on 25 April 1953, a short article just 900 words long, appeared on the scientific magazine Nature. It dealt with the discovery of the double helix structure of the DNA molecule, a molecule that contains genetic information and has a three-dimensional conformation. We will see why the latter is recognized today as a fundamental scientific discovery, but first let us get to know the history of ‘our scientists’ better and the scientific milieu they worked in.
The authors of the article were James Watson e Francis Crick: two young and ingenious English researchers who worked together in a laboratory of the prestigious university of Cambridge. J. Watson was 24 years old and was a vivacious and talented youngster, F. Crick was 36 years old and had worked as a physicist in the university. The discovery of the three-dimensional nature of the DNA molecule took place in February, two months before it was published publicly; at the moment, however, it did not draw great interest on the part of the scientific community of biochemists. Apparently it was not considered fundamental since not much importance was given to DNA yet. Many scientists of the Fifties believed that the DNA molecule was only an accessory respect to the mechanism of life but that proteins played a fundamental role. As a matter of fact, only later it was discovered that genetic information is contained and transmitted thanks to DNA that allows proteins to be built up and that proteins do not contain information to make other proteins. Hence at the beginning only a few researchers of J. Watson and F. Crick’s working environment understood and enriched the epoch-making discovery that the two scientists had revealed in just one page with a photograph. Indeed even the revolutionary article seemed to have had a very ‘domestic’ beginning. Odile, Crick’s wife, built the famous three-dimensional DNA model, made up of balls and sticks that appeared in the photograph along with the two researchers observing it; Watson’s younger sister, Elizabeth, instead, gave up a relaxing Saturday afternoon to type out the 900 words dictated by the two scientists to send to Nature.
J. Watson and F. Crick were the first to reveal the spatial structure of DNA thanks to an intuitive and fruitful reading and re-elaboration of many studies carried out earlier by various scientists, and indirectly thanks to another researcher who, in parallel, had managed to photograph the DNA structure with X-rays. This fundamental photograph, taken by Rosalind Franklin, was shown to them by a professor without her knowing. J. Watson e F. Crick analysed the picture and perceived what Franklin had failed to assume, i.e. that the DNA molecule has a three-dimensional structure. Years later, this discovery proved to be the most important brainwave in the field of Life Science of the last 50 years, since from then on it was possible to understand how genetic transmission from one generation to the next works.
Today it is possible to define the structure of DNA as a double helix made up of two intertwined filaments like the newels of a winding staircase whose rungs are made up of molecules of hydrogen that bind the nucleotides. What are nucleotides? They are the basic building blocks of DNA, each one is composed of a molecule of phosphoric acid, a sugar called deoxyribose and a nitrogen base. The nitrogen bases are of four different kinds: adenine (A), guanine (G), thymine (T) and cytosine (C). The pairing of the nitrogen bases that make up DNA follow a precise principle. In the Fifties all this had not been understood and explained yet, so let us see how what we know today was discovered, by getting to know the scientists and the various stages of their research.

The scientists and the stages

Why does the discovery of 1953 become a milestone in understanding the genetics of life and the evolution of Man and other living creatures? To understand the importance of the double helix of DNA it is interesting to retrace the main stages of the studies on DNA that were done before and after the publication of the first article by J. Watson and F. Crick on Nature. The first information regarding the existence of DNA goes back to 1869, **F. Miescher** mentions an acid substance that he calls nuclein that is contained in the nucleus of cells and is composed of protein and nucleic acid – this will subsequently be called DNA. From then on many studies are carried out on the subject. In 1938 the scientists **R. Signor**, **T. Caspersson** e **E. Hammarsten** understand that the DNA molecule is formed by long chains (sequences) of

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nucleotide bases. The nucleotide bases (or nucleotides) are, as we have already seen, 4 different building blocks that make up the long acid molecule of DNA and are called: adenine, thymine, guanine and cytosine. In 1944, other university researchers suggest for the first time that the DNA molecule contains genetic information. The genetic information (the set of instructions that cells need to build proteins) is assumed to be encoded in the different sequences of nucleotide bases that are repeated on the DNA chain.
Subsequently it is understood that it is in the DNA, that is transmitted from one generation to the next, from parents to children, that you find the genetic information that every cell of our body needs to carry out its specific function. Finally in 1952, **A. Hershey** e **M. Chase** establish that only the DNA molecules can be genetically transmitted to the next generation and not the proteins. In the very same year the scientist **R. Franklin** together with her colleague **R. Gosling** (a friend of J. Watson and F. Crick) produce a very well defined and clear map of **X-ray** diffraction obtained by bombardment of the DNA molecule. In fact, this is the famous photograph that inspired the intuition of 1953 on the three-dimensional nature of DNA.
In 1958 there is a breakthrough, **M. Meselson** and **F. Stahl** understand and illustrate the mechanism by which DNA reproduces: the double helix unwinds, it opens and two long parallel chains are obtained. Each filament is copied and in this way two identical copies of the original DNA molecule, that does not exist anymore, are obtained. It is only at this point really that the discovery of 1953, regarding the three dimensional structure of DNA, becomes very important because the exact knowledge of how the molecule is made in its three dimensions helps the understanding of its reproduction (with the mechanism explained earlier regarding the separation of the two chains) resulting in the formation of other double helixes that wind up and are identical to the original. At this point researches continue feverishly; in 1959 it is discovered that the filaments are copied and duplicated by an enzyme present in the cells of our body called DNA polymerase. Now the mechanism of genetic transmission is clearer and in 1961 the fact that specific molecules of DNA can conduct and instruct the construction, i.e. the synthesis, of specific proteins is understood.
Even though now it is clear that all these important discoveries are the outcome of the work of many researchers, the merit must certainly not be taken from those who had the first fundamental intuitions on the long path of learning that goes on even today. In fact, in 1962, James Watson, Francis Crick and Maurice Wilkins receive the Nobel Prize for their discoveries regarding DNA.

The discovery of the PCR method

Everyone knows the novel or has seen the film Jurassic Park in which, thanks to laboratory techniques, the blood of a dinosaur preserved in the buccal organs of a mosquito trapped in amber is extracted and so it becomes possible to reproduce the DNA of the long extinct animal. The scientists that work in Jurassic Park used the **PCR** method to bring to life dinosaurs that had been extinct for millenniums, from eggs produced in the present thanks to the DNA found in the mosquito! How was this possible?
Around the beginning of the Eighties in the world of biological sciences there is another powerful revolution. The setting is always a biochemistry, molecular biology or microbiology laboratory and the main character on which we are focusing our attention is still the DNA molecule. Few inventions have revolutionised the course of molecular biology as much and as fast as **PCR**, the acronym for **Polymerase Chain Reaction**. The polymerase chain reaction of DNA is a molecular biology technique that enables the multiplication, i.e. the copying (technically the ‘amplifying’), of a specific chain of DNA. When we talk about a specific chain of DNA we mean a fragment of nucleic acid, of which the initial and final nucleotide (the series of building blocks that make up the DNA filament) sequences are known, in other words the beginning and end of the fragment. This replication process takes place normally in nature, in the cells of our body, when the DNA contained in the nucleus unwinds from its double helix conformation and its filaments are copied by the DNA polymerase enzyme. This ordinary phase of cellular reproduction is imitated in a test tube by the PCR technique. In practise, a piece of ‘complete’ DNA with a double helix is reconstructed from a strand with a single helix. The revolutionary fact is that using this laboratory technique, man can choose to take a particular part of DNA that interests him and study and utilize it in compliance with the purpose of his research, outside the cellular mechanisms that take place in a living body.

The utilization of PCR therefore enables the 'amplification' of a specific tract of DNA, multiplying i.e. reproducing it, billions of times in a very short time, a little over an hour, and obtaining a great number of copies.
This laboratory technique, worked out in the first half of the Eighties by the brilliant mind of Dr. **Kary B. Mullis**, has enabled us to better understand the genetic information contained in the DNA of each individual, with crucial consequences in wide-ranging fields, from laboratories where pure research is carried out to hospital analysis laboratories and to courtrooms. A confirmation of the importance of this achievement is the Nobel Prize for Chemistry that Kary B. Mullis received in 1993.
Before trying to understand how a PCR 'reaction' (this is how a procedure that takes place in a test tube is called) applied to a specific tract of DNA works, let us learn about the life and the personality of its inventor, a charming genius of our times.

The witty genius

Since when he was at the *Dreher High School of Columbia* (USA), Dr. Mullis proved to be an impertinent youngster gifted with a strong sense of humour and soon became a leader among the students. In the years that followed he accumulated academic titles and important assignments and devoted himself to paediatrics, biochemistry and cardiology. In 1979 he worked for the *Cetus Corporation of Emeryville*, California and as a chemist specialized in DNA he worked at a research on the synthesis of oligonucleotides, i.e. the building blocks that compose DNA and he invented the polymerase chain reaction (PCR).
He had the real intuition about how to multiply DNA billions of times in 1983. The legend narrates that Dr. Mullis was going home from the laboratory on an evening like many others and his mind was lost in hazy thoughts but suddenly he noticed that the path he was walking on was made up of many sections arranged one after the other like the rungs of a ladder. He went on walking and the path divided at a junction. This reminded him of the two strands of DNA that divide before replicating, and then, suddenly, the street lights that lined the path came on one after the other because it was getting dark. The brainwave hit him in a second: a way to make infinite copies of DNA strands from a single portion came to his mind. He turned around and ran back along the illuminated path to the laboratory to try and put his ingenious idea into practise.
Dr. Mullis' career continued brilliantly, he received the **Nobel Prize** and another very prestigious prize, the **Japan Prize**. He worked on both technological researches and on those regarding the photochemistry of DNA. In one of his most successful books, 'Dancing Naked in the Mind Field', written in the year 2000, his sense of humour and brilliance can be appreciated in disparate sections: he writes about poisonous spiders, about science and parapsychology, about astrology and the Hiv virus.

Inside the test tube

Making use of his vast knowledge on DNA and of all the laboratory equipment available in the Eighties, Dr. Mullis started carrying out experiments to carry through the brainwave he had on the path that evening. His aim was to define a method that allowed him to copy a specific piece of DNA many times 'in vitro', i.e. in a test tube. The PCR method works on a theoretical principle that we will now describe.
With a bit of imagination we can compare the implementation of a PCR laboratory experiment with the preparation of a cake. To start preparing the cake we arrange the required ingredients on the table (eggs, milk, butter, baking powder, flour, etc.) and then we mix them in a bowl. In the case of the preparation of a PCR reaction we arrange the various biological and chemical components (the cake ingredients) on the laboratory counter in their proper containers such as test tubes, pipettes and jars. Some preliminary steps must be carried out before starting the preparation of the cake such as dividing the egg yolk from the albumen, or working at the butter when it is too cold. In the case of a PCR too some preliminary steps must be carried out before mixing everything in just one last test tube (the mixing bowl).

DNA extraction

First of all we have to extract the DNA from the nucleus of the cell in which it is contained. For example the DNA must be

extracted from bacterial or blood cells or from the remains of a human or animal mummy. The procedure entails various stages with some chemical compounds and subjecting the cell sample to suitable treatments. In other words we have to demolish the cell structure, i.e. break all the cells of the sample so as to make the DNA come out. Then it is necessary to digest the proteins that are associated to the DNA molecule, this means that at the end of the procedure a solution that contains only the DNA that has been separated from the digested proteins is obtained. These proteins have been transformed into amino acids (the bricks that make up proteins). At the end of this initial phase we will have a test tube with DNA dissolved in a liquid such as ethanol. In this environment DNA is not soluble and its filaments are visible in suspension in the test tube.

The components of the PCR reaction

These are the ingredients of the cake when they have not been mixed yet. On the laboratory table we have the extracted DNA with the exact sequence that has to be copied which is called the target and the aim of the experiment is to multiply this specific piece. From now on we will use the 'convention' that the sequence that has to be copied is called '**target**' so that it will not be confused with other DNA fragments that take part in the PCR.

In addition two more fragments of DNA called **primers** are used; their function is to provide a starting place for copying and replication of the target sample, i.e. they help to trigger off the beginning of the replication. During the PCR reaction, the target filament is heated and its double helix unwinds: two single strands of the DNA target are obtained in this way. Here the primers are added and they bind to the single stranded DNA, in practise they arrange themselves close to each single strand. This is the starting place for replication.

But for replication of the target to begin, we need yet another ingredient, DNA polymerase that, as we have already seen, is a cell enzyme. DNA polymerase also has the function of replicating and repairing DNA. This enzyme can elongate a primer by adding nucleotides (that are the bricks that make up a DNA strand) one at a time. In the recipe, a mixture of small, loose bricks (the basic structures of the DNA filament) must still be added; these nucleotides will be used to build up the new DNA filaments. We have already described what nucleotides are in point 1.

A mixture of other supporting elements (such as salt and grated lemon peel in a cake) must also be added, such as magnesium that helps to create the right chemical conditions for the reaction to take place.

The thermocycler

This is the oven to cook the cake in. We have all the necessary ingredients and so we mix them together in one test tube just like we pour the cake mix into a cake tin. To bake the cake we put the tin into the oven and cook it for a fixed time and at a specific temperature, for the PCR we put the test tube containing all the compounds into the thermocycler. This laboratory equipment has a thermostat: thus it is possible to regulate and programme the temperature variations and length of time of each of the three phases of the amplification, i.e. the three steps that are necessary to copy the target sample many times (the baking of the cake). The functioning of the thermocycler is based on different systems of heating and cooling by means of air, liquids, electrical resistances etc. The thermocycler has a small drawer in which the test tubes where the PCR reaction is going to take place are inserted; there are different types on sale, of varying sizes depending on the requirements of the laboratory.

The three phases of a PCR

Finally it is time to bake the cake; we take the test tube and insert it in the thermocycler. What happens next? The baking consists of subjecting the test tube to a certain number of thermal variation cycles. Each thermal cycle is made up of three phases. Periods of heating are alternated with periods of cooling.

1st phase: denaturation

In this first phase of a PCR reaction the total separation of the two DNA filaments of the target sample that has to be replicated takes place. Usually the temperature applied to the test tube must reach 94°C for 30-60 seconds. By warming the DNA the unwinding of the double helix and the separation of the two filaments takes place; it is said that the DNA is denatured.

2nd phase: hybridization of primers

This is the most delicate moment in which the primers have to 'stably hybridize' to the DNA template. What does this mean? It means that the primers that have been added to the mixture have to bind to the single separated strands of the original DNA target sequence. Usually the procedure involves various experimental attempts in order to find the ideal temperature that promotes the binding of the primer strands to the target strands according to the type of DNA sample that has to be replicated. Usually the temperature is lowered to about 30 – 55 °C.

3rd phase: the extension of the new DNA strand

The ideal temperature for polymerization, i.e. the construction of DNA filaments identical to the DNA target depends on the DNA polymerase enzyme utilized. At present an enzyme called Taq polymerase is used and a temperature of 65 - 72°C is applied for 5 minutes. In this phase the polymerase enzyme physically attaches the building blocks of the filament (the nucleotides) to the primers that are coupled and complementary to the target filament. In practise, the copying of each single target filament takes place. DNA polymerase takes care of correcting any mistakes in the event that a 'wrong' nucleotide is incorporated, an eventuality that can take place since the mechanism is never perfect. Thus the DNA polymerase is capable of eliminating an error and creating a correct pairing with the corresponding nucleotide present on the filament that is being copied and replicated.

During the first cycle of the PCR reaction new filaments are synthesized which, after denaturation i.e. the division of the double helix and separation of the parallel strands, can bind themselves to the primers. These products accumulate arithmetically but it is only from the second cycle on that two single helix products are formed that will make up a fragment identical to the double helix target DNA.

Thus with repeated denaturation cycles, hybridization of primers and their extension an exponential increase of the copies of the target DNA segment is obtained.

During each 'cooking' cycle the number of fragments of DNA that have to be copied (i.e. those present between two primers) doubles. After just 32 repeated cycles, millions of copies of double helix DNA fragments identical to the target are already formed.

At this point the cake is ready and we can take it out of the oven. It will have risen and cooked and will be ready to be tasted. In the same way the test tube removed from the thermocycler at the end of all the thermal cycles of 'cooking' and cooling to which it has been subjected, is ready, the PCR is over. The numerous copies of DNA, identical to those of the original sequence we wanted to replicate, are available to be subsequently analysed and utilized in different ways according to the requirements of the experiment.

Thermostable DNA polymerases

Dr Mullis understood that a decisive limit to the success of the amplification reaction was the destruction by heating of the DNA polymerase enzyme that had to continually be added during each cycle of the process since it did not endure the high temperatures of the PCR cycles. In fact, when he started experimenting in the Eighties he used a DNA polymerase extracted from the micro-organism E. coli, that was thermolabile and got destroyed at 94°C.

Hence another revolution took place when a few years later a thermophilic micro-organism, Thermus aquaticus was discovered. The latter was capable of living even in boiling water springs. The thermoresistant polymerase of these bacteria, called Taq polymerase, remained active even at 94° C: therefore it could be added just once, at the beginning of the reaction.

Nowadays, in all laboratories in which PCR analyses are carried out on DNA samples, thermostable DNA polymerase enzymes are used.

Nowadays the DNA polymerase, isolated from the thermophile bacterium Thermus aquaticus, discovered in the thermal springs of Yellowstone Park, is produced with a technique of recombining DNA in E. coli. In practise, bacteria of E. coli are used as producers of the enzyme Taq polymerase thanks to genetic manipulation that induces E. coli to produce an enzyme identical to the one that Thermus aquaticus produces in the extreme conditions in which it lives.

What in PCR for?

In just 20 years from its invention, the PCR method, thanks to the great progress of technology and research, can now be used to find a solution to problems that first could not be solved.
Due to its great sensitivity, PCR is presently applied whenever very small quantities of DNA are involved. In the field of medical diagnosis, the amount of DNA contained in a drop of blood is more than sufficient to amplify and verify the possible presence of a genetic mutation associated to a hereditary sickness. With this method tumour cells can be identified, for example in the case of liquid tumours that are very difficult to locate. Moreover, since DNA is a very stable substance, even after thousands of years, as in the case of Egyptian mummies, it is possible to study it with the PCR method. Thanks to the discovery of this method, a new scientific discipline was born: paleobiology that involves the study of DNA in plant and animal fossils.
In conclusion, it would be impossible to even just outline all the possible variations of the PCR method and their respective fields of application, so in this context we will just consider some examples.

A help in the Police and Carabinieri Corps investigations

Even in courtrooms now it is normal to talk about DNA analyses using the PCR method. In forensic medicine, the finding of organic material (such as hair, skin fragments or blood) on the scene of the crime or of an accident allows the identification of the victim or of the culprit.
In the Biological Investigations Laboratory of the Forensic Department of Police in Rome (Laboratorio d'Indagini Biologiche del Servizio Polizia Scientifica di Roma), researchers have created a PCR method suitable for solving those crime cases which apparently seem to have no clues on the crime scene. The possibility, on the part of the forensic team, of discovering some clues on how the mysterious facts took place and the identity of the culprits has greatly increased from when it has become easier to analyse the DNA. Moreover, work is being carried out to create an Italian DNA data bank in which complete DNA profiles of dangerous individuals or those who are being investigated, are kept.
From around 1996, the application of the PCR technique allows the analysis of DNA even in those cases in which the samples are very small or in bad conditions such as hair fragments or very old bone material.
American television serials and lately even Italian ones often show teams of biologists, biochemists and other scientists, side by side with detectives, carrying out investigations that are far from reality. In fact, on the scene of the crime, it is normal to see expert biologists in white suits and half masks scraping at walls, collecting biological samples from tooth brushes or discovering traces of blood or sperm that were hidden or invisible.

Discovering unknown micro-organisms

From the environmental point of view, PCR is applied to trace undesired microbes in drinking water, rivers and sea water.
To monitor and identify a microbial community, samples of micro-organisms whose genus and species are unknown must be collected from their natural environment and possibly cultivated in the laboratory. To get to know the bacteria collected, the utilization of the different PCR methods can be really decisive because it permits the sure identification of their DNA.
The advantage of utilizing methods that analyse and recognise the DNA of bacteria is that the experiment times are much shorter than when classic microbiology techniques are used; moreover, the results are more precise and repeatable. In fact, many groups of micro-organisms that live in very different surroundings have been discovered and studied in acceptable times and with reliable results: sea waters containing oil or other hydrocarbons that are metabolized (eaten) by some species of bacteria, soil polluted with zootechnical sewage (animal sludge), milk, cheese and natural environments in which food products are left to season.

DNA is sought for in dishes too!

In the food industry, to prepare various meat-based products it is common to mix different animal species. This practise is allowed so long as the different species are indicated in the ingredients. This implies that on the label of a salami for example, the animal species that have been used, must be indicated clearly, in a decreasing order depending on the

quantity of each species that is present.
The determination of the animal species present in a food product is carried out during food controls carried out by the Veterinary Police that has the duty of performing biological and chemical analyses with the objective of defending consumers rights.
A precise definition of the species is of great importance even considering the preferences of consumers who may express the need to know the animal and plant species present in a food product for health reasons, in the case of specific allergies to some kinds of meat, or the wish to comply with food limitations for religious reasons. Some religions prohibit the consumption of pork or other food products in certain periods of the year; thus, whoever wants to comply with these rules has the right to be protected and to trust what is written on the label.
Food frauds take into account practically all productive activities, from the sale of fish products to the preparation of canned meat. More often than one would think some valued and costly fish are substituted with other very similar species of lower cost, for example, small sharks are sold as dogfish or crab and lobster meat are substituted by similar but lower quality products.
The macroscopic identification, i.e. visual, of meats that are similar in colour and shape when they are frozen is extremely difficult. The same unreliability on identification can occur with fresh meat of some species. When controls are carried out by Veterinaries or by the Carabinieri in a slaughterhouse, in a fish market or even in a seed storeroom it is not easy to tell ‘with the naked eye’ if a product is alright and normal.
DNA analyses of animal and plant species using PCR have greatly helped and speeded up the control procedures of the Authorities on suspected food products. For example, food controls regarding the search of GMOs (in rice, pasta or vegetables) are now carried out with PCR techniques in all analysis laboratories.
Summing up, in these last years, methods that involve the use of PCR are developing fast. The main advantage of using PCR is represented by the possibility of copying fragments of target DNA with just a few bases, i.e. very short. In the field of the food processing, this capacity of identifying very small strands of DNA proves to be useful since in cooked and treated foodstuffs very small quantities of degraded, damaged and non-purified DNA are found.
Moreover, as far as other applications of PCR methods in food processing are concerned, it is greatly utilized to search for pathogenic micro-organisms (dangerous for Man and animals) in food and raw materials (milk, eggs): for example, *E. coli*, *Salmonella*, *L. monocytogenes* and other bacteria that can provoke sickness when ingested. In these cases too, the search for DNA so as to identify the bacterium in a rapid and safe way, can be very useful in hospital structures to provide the most efficient cure to victims of food poisoning.