

Review Article

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DNA Cloning: The History of The Future

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Abstract

Here is reported the history, point by point, of the evolution of DNA cloning. The most important discoveries of the XX century that allowed the scientific community to begin to study cell functions, how they are regulated and how they can be "repaired". Apparently the future perspectives don't seem to have limits.

Keywords: Modern biology; DNA-cloning

Introduction

DNA cloning have been permitted the development of modern biology, in particular in the last 40 years we have passed from theorize DNA cloning to clone a gene, using fast techniques and widespread in all research laboratories, moreover there are company that can clone any DNA fragment, coding or regulative at relatively low cost; these clones can be used to transform bacterial cells and transfecting eukaryotic cells. To understand how these techniques of DNA manipulation have become so easy to use, a brief history can be made to understand what were the most important discoveries in this field.

Cloning Timeline

Modern biology started from the studies of the pioneers of science as Gregor Mendel, which in the XIX century postulated the laws on the genes segregation and the heredity of genetic factors; Friederich Miescher in 1869 discovered a weak acid in white blood cells, identifying the DNA; and Wilhelm Roux that in 1885 established the principle of tissue culture, in an experiment where was removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days.

In 1888, Roux tested the "germ plasm theory" for the first time. One cell of a 2-cell frog embryo was destroyed with a hot needle; the result was a half-embryo. This led him to propose his "Mosaic" theory of epigenesis: after a few cell divisions the embryo would be like a mosaic, each cell playing its own unique part in the entire design. At the beginning of XX century Thomas Hunt Morgan showed that genes were units of inheritance from his studies on *Drosophila melanogastes*. This concept was well described in the book "The theory of the gene" published in 1917 [1]. Successively, the embryologist Hans Spemann conduct many experiments on nuclear transfer of salamander embryo and in 1938 he published the results in the book "Embryonic Development and Induction" [2]. Spemann hypothesized that the next step for research should be the cloning organisms by extracting the nucleus of a differentiated cell and putting it into an enucleated egg.

In 1939 Andrey N. Belozersky began studies to demonstrate that DNA and RNA are always present in the cells, and successively that there is a correlation between their composition [3]. In 1941 Edward L. Tatum and George W. Beadle discovered the gene function, demonstrating that proteins are codified by genes. They formulate the central dogma of molecular biology: "one gene-one enzyme" [4]. Obviously, this proposal was an over simplification of the real situation [5], and in addition a class of eukaryotic proteins has been characterized, the DING family, for which seem to be not present in the genome its genes [6], and the same situation has been described in the archaea bacteria *Sulfolobus solfataricus* [7].

Starting from 1944 three fundamental evidences were found, for

first Oswald Avery with his co-workers Colin MacLeod and Maclyn McCarty found that the genetic information of cells was carried in DNA, in other words that DNA is the material of which genes and chromosomes are made [8]. Second, Robert Briggs and Thomas J. King (1952) made the first animal cloning by using nuclear transfer of embryonic cells of frogs (*Rana pipiens*) [9]. Third, in 1953 Francis Crick and James Watson, working at Cambridge's Cavendish Laboratory, solved the molecular structure of DNA [10]. This discovery has been one of the most important in biology because from the DNA structure was understood how it could replicate itself, as the genetic information could be maintained and other later discoveries such as the polymerase chain reaction, which is the basis of modern molecular biology.

In the 1962 John Gurdon had demonstrated the Spemann's hypothesis about the possibility to clone an organism, in fact he announced that he had cloned South African frogs using the nucleus of fully differentiated adult intestinal cells. This demonstrated that cells genetic potential do not diminish as the cell became specialized [11].

In the 1966 Marshall W. Nirenberg and successively Har G. Khorana described how is organized the genetic code, by using a series of elegant experiments they answered two questions: how DNA directed the expression of proteins, and what role RNA had in these processes [12-14]. The deciphering of the genetic code opened the door for the explosion of genetic engineering studies.

These years were crucial because there was an explosion of discoveries that started the modern molecular biology, in fact in 1967 was isolated the DNA ligase by Bernard Weiss and Charles D. Richardson [15]; in 1969 James A. Shapiro and Johnathan Beckwith announced to have isolated the first gene. The gene isolated from *E. coli* was *lacZ*, coding for the β -galactosidase [16]; in 1970 Howard Temin and David Baltimore, independently from each other, isolated the first restriction enzyme [17,18], and finally in 1972 the first recombinant DNA molecules was created combining the DNA of different organisms by Paul Berg and collaborators [19]. They constructed circular dimers of SV40 DNA virus containing the lambda phage genes and the galactose operon of *E. coli* [19].

To For the complete development of modern molecular biology

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other two techniques were fundamental: the DNA sequencing, method developed by Frederic Sanger in 1975 [20], and the Polymerase Chain Reaction (PCR) in 1983 by Kary B. Mullis [21].

Easiness of Cloning

As said, two techniques have increased exponentially the rate and reduced the difficulty of DNA manipulation: DNA sequencing with the Sanger method and the PCR. In 1981 was obtained the first complete sequence of a genome, that of human mitochondria, a circular genome of about 16,500 base pairs [22]. In 1995 has been sequenced

the first complete genome of an organism capable of independent life, the genome of the eubacteria *Haemophilus influenzae*, of about 1,83 Mb [23]. Up to date have been sequenced the genomes of about 2400 viruses, 3000 bacteria and 700 eukaryotic organisms, including humans, and these numbers are constantly growing.

In 1983 Kary B. Mullis developed the Polymerase Chain Reaction (PCR), a simply and powerful method to obtain a rapid amplification of a designed fragment of DNA (Figure 1a), at beginning they used the *E. coli* DNA-polymerase, but successively the amplification reaction was developed on the use of the thermophilic DNA-polymerase from

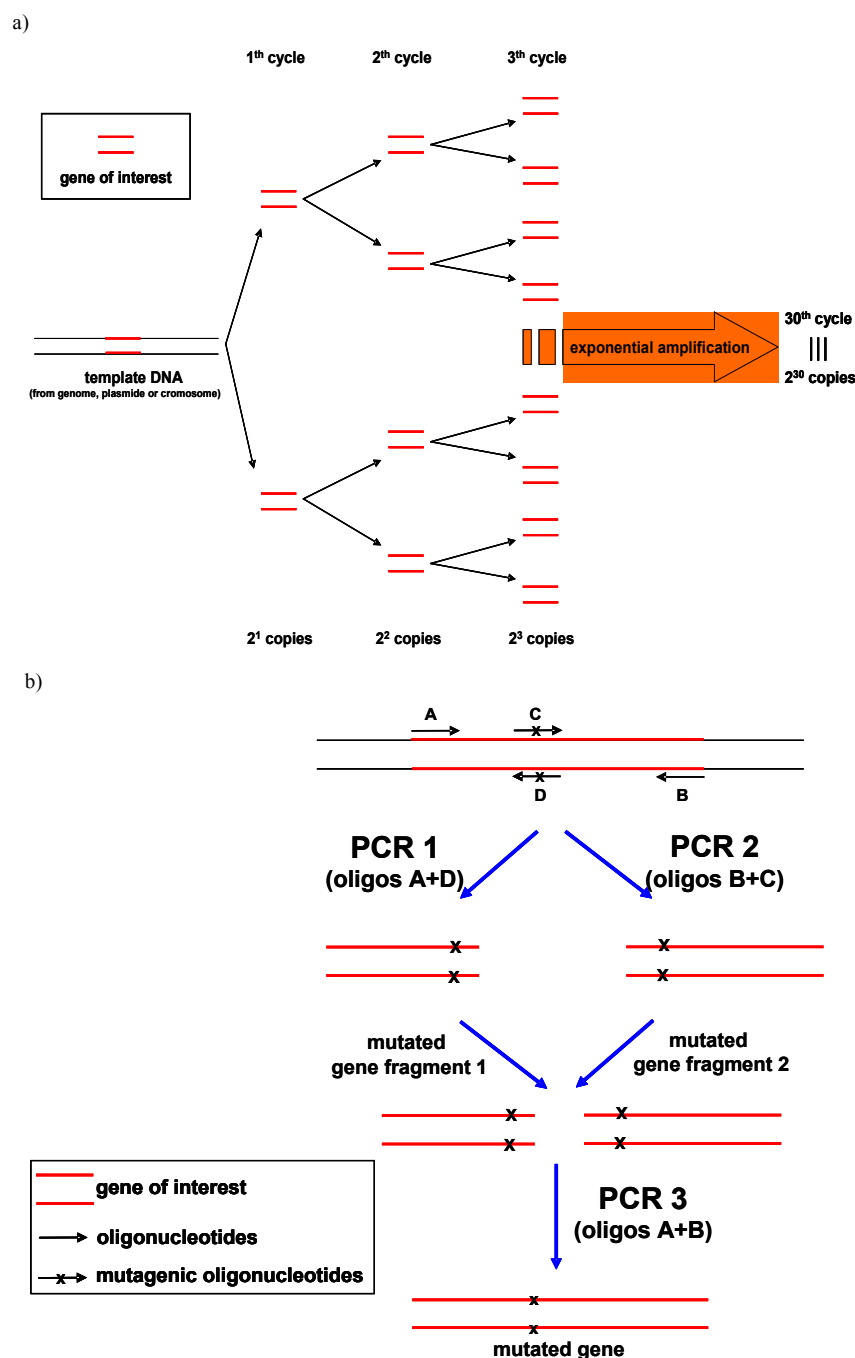
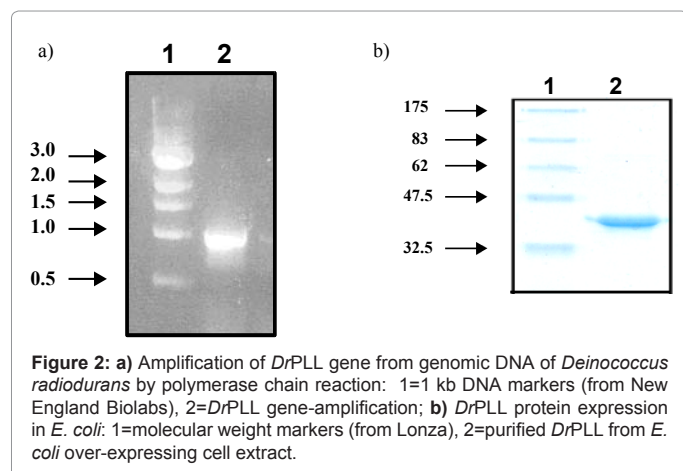


Figure 1: a) Scheme of DNA amplification by polymerase chain reaction. b) Scheme of mutagenesis by using polymerase chain reaction.



Thermophilus aquaticus [24]. By using this techniques is possible to clone any DNA fragment, mutagenize a gene in a site-direct (Figure 1b) or random mode [25], to study the genes expression and their regulation by quantitative real time PCR [26]. Take together these two techniques represent the history and the future of modern molecular biology, in fact in all the laboratories it's possible to reproduce this techniques to identify genes by in silico analysis or by sequencing of DNA isolated from the environment, than amplify it by PCR and cloning in the opportune plasmids. In figure 2a is reported the amplification of a *Deinococcus radiodurans* gene, the ORF DR0930, its cloning in the expression vector pT.7-7 was made in just 1 week, and its over expression in *E. Coli* and purification in 2 weeks (Figure 2b) [27].

Currently thousands genes have been cloned starting from phages, viruses, bacteria, plants, fungi, to human. Hundreds of transgenic mice have been obtained to study the effect of particular genes during embryonic development and in the adult animal.

What Future

Gene cloning is a carefully regulated technique that is largely used in many labs worldwide. Over the last 50 years, scientists have conducted cloning experiments in a wide range of animals using a variety of techniques. However, cloning raises important ethical issues, especially as related to the humans. In fact, the cloning of the first mammal from mature (somatic) cells of a 6-years-old sheep made in the 1996 by Ian Wilmut and Keith Campbell, researchers at the Roslin Institute in Scotland [28], generated many questions in the scientific community and in public opinion on what should be the limits of science. This is a fundamental question that is not easy to answer. For the advancement of science there should not be limits in the research, but in the use of some results probably yes.

The best use of scientific discoveries is the application for the treatment of diseases. Researchers hope to use embryonic stem cells, which have the unique ability to generate virtually all types of cells in an organism, to grow tissues in the laboratory that can be used to grow healthy tissue to replace injured or diseased tissues. There are many preclinical studies demonstrating the efficacy of gene and cellular therapy [29], but switching to the clinical trials showed a mix of positive and negative results, indicating that the way even if it is right is still long.

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