SEVEN
publicações acadêmicas

Molecular biology - Some techniques and applications: Literature review

Crossref doi
https://doi.org/10.56238/uniknowindevolp-075

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ABSTRACT

Molecular Biology is an area of Biology that is dedicated to the molecular structure of intracellular chemical and biological reactions and was established due to the study of the structure of DNA, RNA, protein synthesis and various molecular techniques. The evolution of these techniques has enabled the detection of diseases and genetic errors more quickly and effectively for diagnosis and treatment. Given this, this work uses a literature review on the use of Molecular Biology, in order to highlight its positive and negative points. Molecular study is strongly linked to the function of genes and gene products, which makes the approach to molecular biology expansive. By sequencing the genome of several organisms, it is concluded that nucleotide sequences alone are not sufficient to fully understand the functions of genes. Already, in relation to the techniques, it is known that the PCR have its techniques delineated around the Central Dogma of Molecular Biology executing in vitro processes of Transcription by Polymerase Chain Reaction. After data analysis, it is concluded that there was an evolution of Molecular Biology techniques as well as their application, so the positive points stand out in relation to the negatives.

Keywords: Deoxyribonucleic acid, Ribonucleic acid, Polymerase Chain Reaction.

1 INTRODUCTION

Molecular Biology is an area of Biology that is dedicated to the molecular structure of intracellular chemical and biological reactions, defined by Astbury and Florence (1938) as not only the refinement of morphology, but in-depth study that is specifically concerned with the forms of biological molecules, their conception and function.

Molecular Biology was established due to the discovery of structures and techniques such as, for example, the structure of DNA, techniques with ribonucleic acid (RNA) and new equipment that automated the process of sequencing the genome of living organisms (OSADA; COSTA, 2006).

A milestone in the history of Molecular Biology is the discovery of Watson and Crick, who in 1953 proposed in their article published in the journal Nature, *"Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid*" (WATSON et al., 1953) the structure of Deoxyribonucleic Acid (DNA) with antiparallel strands in double helix, which became conventional in textbooks and scientific (CHAKRABORTY, 2018; GUASTELLI et al., 2018; FREITA et al., 2020). However, it is currently known that there are other DNA structures.

Recent discoveries have revealed the existence of new DNA structures that challenge this traditional view. These include G-quadruplexes, four-stranded DNA structures formed by stacked guanines. These structures have been extensively studied due to their role in biological processes, such as the regulation of gene expression and the maintenance of genomic integrity (YANG et al., 2010). In addition, i-motifs, double-stranded DNA structures that form an i-shaped conformation, have also been the subject of investigation due to their occurrence in regulatory regions of genes. These findings have significant implications for understanding DNA function and scientific research (SERIKAWA et al., 2018).

Molecular techniques have been applied in various sectors such as the food industry, plant genetic improvement, the application of biotechnology by the use of microorganisms in the safe production of food, among others (SOARES *et al.*, 2017; VARGAS *et al.*, 2018).

However, despite assisting in other sectors, Molecular Biology focuses largely on the health area with its greater focus on diagnosis and screening of diseases such as cancer and Human Visceral Leishmaniasis (HVL), for example (NONNENMACHER *et al.*, 2002; VARGAS *et al.*, 2018; SOUZA, 2019).

The evolution of these techniques makes it possible to detect diseases and genetic errors more quickly and effectively for future treatments. In addition, these molecular techniques are also very widespread in the scope of scientific development that, by using little genetic material, but with high specificity become viable to identify, genotype and catalog microorganisms and viruses of medical importance (VIEIRA *et al.*, 2011; MENESES *et al.*, 2019).

Given this, this work uses a literature review on the use of Molecular Biology, in order to highlight its positive and negative points, as well as studies and applications in various media.

2 METHODOLOGY

The research uses the method of bibliographic review, of the dissertation type, with data and scientific foundation. Thus, it was possible to analyze previous articles that contribute to the understanding of the subject.

The data are the results of a bibliographic review, carried out in the databases of articles *SciELO* (*Scientific Electronic Library Online), PubMed*, using the descriptors Applied Molecular Biology; Innovations in Molecular Biology; Molecular Biology Techniques and Molecular Biology Studies, from June to August 2021.

After reading the titles and abstracts of the articles found, articles in Portuguese and English were included that were fully available and that discuss Molecular Biology and its applications in the period, published between 2016 and 2021. All articles were separated according to year of publication and language.

3 RESULTS AND DISCUSSIONS

A total of 815 articles were found on the *PubMed* platform, of which only three were written in Portuguese and 812 in English (Table 1).

Table 1 - Articles according to descriptors in the *PubMed platform*

Descriptors and Language	**Portuguese**	**English**	**Total**
Molecular biology Applied	3	792	795
Innovations in Molecular Biology	0	0	0
Biology Techniques molecular	0	20	20
Biology studies molecular	0	0	0
Total	3	812	815

Source: own author.

In *the SciELO indexer, 75 complete articles were found, of which 17 are in Portuguese and 21 in English; 37 articles from* the SciELO platform *were excluded because they were in another language, as shown in Table 2.*

Table 2 - Number of articles found in search on the *SciELO* platform according to the search descriptors, language of publication in the years 2016 to 2021.

Descriptors and Language	**Portuguese**	**English**	**Total**
Molecular biology Applied	0	0	**2**
Innovations in Biology Molecular	0	0	**0**
Biology Techniques molecular	8	13	**56**
Biology studies molecular	9	8	**17**
Total	17	21	**75**

Source: own author.

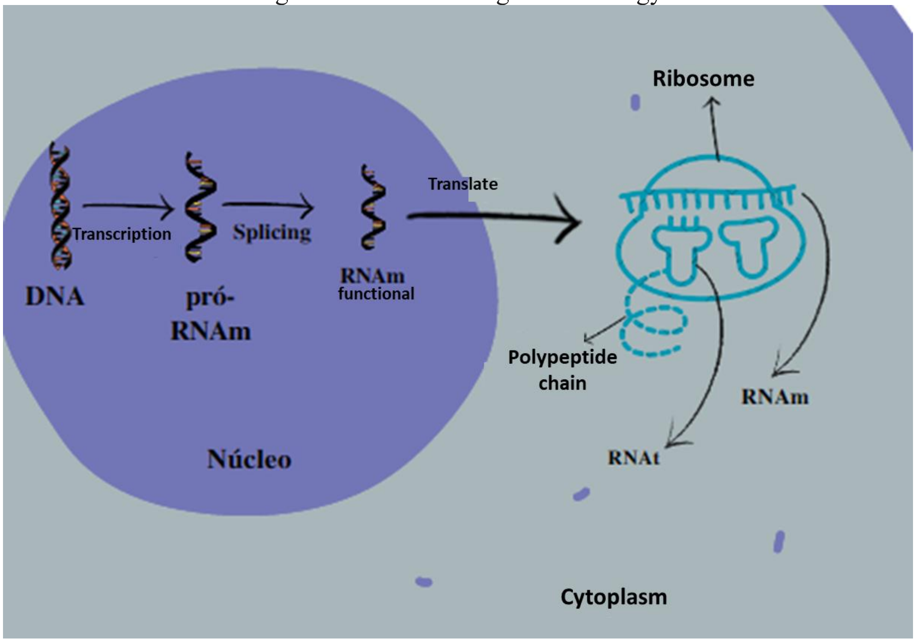
It is observed that when the results of the two platforms are added together, the number of articles published in Portuguese was reduced in relation to publications in the language

Englishwoman. It is also observed that in the two indexers for the descriptor "Innovations in Molecular Biology" no publication was found.

For a better understanding of the concepts of molecular biology it is necessary to deepen in genetics. Classical genetics is a specialty that studies the characteristics of organisms passed between generations and genetic variations between species and organisms; the patriarch, Gregor Mendel, in studies with pea species observed factors that identified what is now called heredity (ASTRAUSKAS et al., 2009; STOLL, BIERHALZ, 2020).

Among the subareas of Genetics is Molecular Biology, whose Central Dogma is the flow of information from the genetic code (Figure 1). This theme has evidence disseminated in the scientific scenario in recent years and still grows with constant publications of new discoveries and updating of techniques already used. The molecular subfield of genetics is intended for the investigation of the composition and action of a particular gene within the genome, through tools such as biochemistry and cell biology to better understand it (PAWLOWSKI, MILOS, MENDEL, YAVOR, KAISERMANN, JOHN. 2020).

Figure 01 - Central Dogma of Biology



Source: The authors.

The molecular study is strongly linked to the function of genes, to explain the complex functioning of organisms, which makes the approach of Molecular Biology expansive, in addition to focusing on the research of several genes and gene products together. Therefore, it promotes knowledge related to the polypeptide production generated from RNA translation, through the relationship between the gene product and its corresponding gene (STRACHAN; READ, 2016).

By sequencing the genome of several organisms, it is concluded that nucleotide sequences alone are not sufficient to fully understand the functions of genes and that, to achieve this knowledge, complete studies of the proteins expressed from the genes are necessary, since the genome is directly linked to proteins. Within the process of protein production, modifications occur after transcription of DNA into messenger RNA (mRNA) and after translation of this into protein. This process holds the physiology played by the product of gene action, so it is of paramount importance more studies on proteomics and transcriptomics to understand the actions of certain genes (DA SILVA NETO *et al.*, 2019).

Proteomics is the area of Molecular Biology that is intended for the exponential analysis of proteins expressed by a given organism or cell under special conditions to monitor and identify proteins expressed by cells and tissues in an ideal manipulated environment or in natural expression (WESTERMEIER; Naven, 2002; PORTIS *et al.*, 2019). The monitoring of the expression of these proteins is able to show that they can undergo changes in their structure and demand according to the stimulus suffered even in different tissues within the same organism (PORTIS *et al.*, 2019).

Proteomics has developed in the scientific and health field with research on therapies, diagnosis and development of new drugs, it is also applied in microbiology and biochemistry to enable the identification and catalog of specific molecules, called biological markers, which contribute immensely to the diagnosis, future treatment and better prognosis of certain diseases (EMÍDIO *et al.*, 2015).

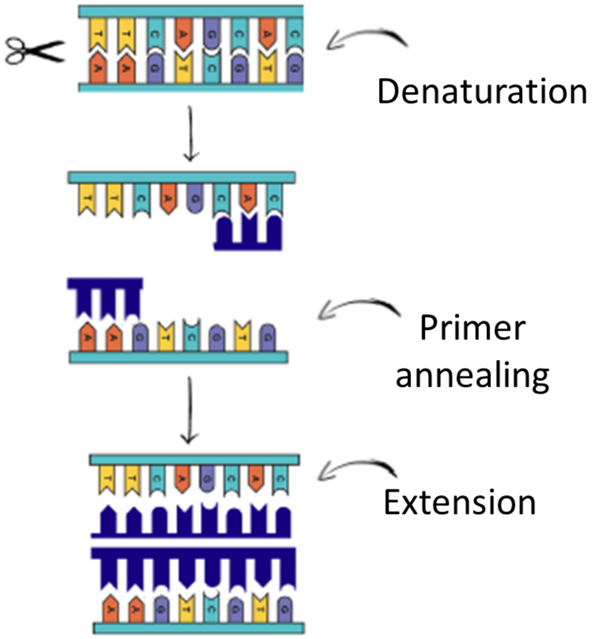
On the other hand, the study of the transcriptome goes through the set of DNA transcriptions in RNA directly linked to gene expression. One of the tools used for the study of the transcriptome is the technology of DNA microarrays that allows quantifying, identifying and evaluating the mRNA coming from the transcription of certain genetic material as described by Falchetti *et al*. (2019), in a research that used DNA *chips* for diagnosis of idiopathic Parkinson's through peripheral blood.

On the other hand, sequential RNA (RNAseq) is a method of mRNA sequencing, which analyzes the transcriptome, and compares the difference in gene expression in transcriptomes of various species. Through bioinformatics and the tools inserted in its processes, space is opened for the study of gene networks of co-expression and with this, it makes it possible to describe the correlation patterns of gene sets, which are grouped into niches within a gene translation network (MERCÊS, 2020).

Additionally, the Polymerase Chain Reaction (PCR) tests have their techniques outlined around the Central Dogma of Molecular Biology by executing *in vitro* processes of Transcription by polymerase chain reaction (FREITA *et al.*, 2020).

PCR technology is based on nucleic acid molecules and their enzymatic activity, to promote *in vitro* replication of DNA strands. The gene amplification of the studied material is possible from the division into stages: denaturation, annealing and extension, through different temperatures on the genetic material, as shown in Figure 2 (PEREIRA, 2018; CANTANHEDE, 2021).

Figure 02 - PCR phases.



Source: The authors.

There are some variations within the PCR technique described in the book, "Introduction to conventional, real-time and digital PCR techniques", which show the existing differences and the factors that influence their execution (PEREIRA, 2018).

Amplification techniques are used for multiple purposes, with a broad spectrum of applicability. Advances in technology strengthen the improvement of traditional PCR techniques, which has resulted in the emergence for example of real-time PCR (qPCR), which can quantify, track and monitor the reaction in real time. Thus, compared to conventional PCR, the results are analyzed more quickly and in addition to the high specificity and sensitivity, the risk of contamination is low (BREZINA et al., 2016; CANTANHEDE, 2021).

NestedPCR is a technique with higher sensitivity and specificity than other technologies, as it is about 38% more specific than conventional PCR. The technology aims, in principle, at the amplification of the sequence present in the previously amplified fragments, by means of two different *primer groups*. After the first amplification, the product obtained will be submitted to the new PCR, to increase the specificity of the technique (SELIGOVÁ *et al.*, 2020).

At the end, it is concluded with the technique of electrophoresis in polyacrylamide gel and reading of the observed test. As a negative point, the time spent for its application is highlighted, however, as a positive point it promotes the increase of the detection capacity as a consequence of the double passage by PCR (CARVALHO *et al.*, 2010).

Another technique also used is the Reverse Transcriptase Polymerase Chain Reaction (RT PCR) which aims to convert the single strand of mRNA into complementary DNA (cDNA), by the action of the reverse transcriptase enzyme. Through reverse transcription, a copy of cDNA is synthesized from the viral RNA (genome or intermediate product of the replication process). This new synthesized molecule will be used as a template for the conventional PCR reaction (HENRIQUE *et al.*, 2005; CUNHA, 2018).

Given this, it is noted that there are several techniques of Molecular Biology and the choice for their application will depend on the objective for which the technique will be applied.

4 CONCLUSIONS

In agreement with the objectives it is concluded that there was an evolution of the techniques of Molecular Biology as well as their applications. In addition, the positive points described by the evaluated studies stand out in comparison to the negative ones.

The evaluation of new advances in molecular biology and biotechnology techniques is of paramount importance for the development of new scientific research, more accurate and faster diagnoses, for the production of medicines and vaccines, among others. Therefore, these advances have a positive impact on improving public health.

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