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Molecular biology in Veterinary Medicine: concepts and application

(Biologia Molecular na Medicina Veterinária: conceitos e avanços)

"Revisão/Review"

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Abstract
The discovery of the structure DNA by Watson & Crick (1953) started a new era in molecular biology. Tools of molecular biology have been used for research in many fields of biology and medicine. In veterinary medicine, molecular biology has been applied to the diagnosis of parasitic and infectious disease, development of vaccines and therapeutics, gene therapy, genetics of disease resistance, genotyping of pathogens etc. This article was written with emphasis on the concepts and application of molecular biology in veterinary medicine.

Key-words: *DNA, biotechnology, PCR*

Resumo
A descoberta da estrutura do DNA por Watson & Crick (1953) deu inicio a uma nova era na biologia molecular. As ferramentas em biologia molecular têm sido utilizadas para pesquisa em muitos campos da biologia e da medicina. Em medicina veterinária, a biologia molecular tem sido aplicada ao diagnostico das doenças parasitárias e infecciosas, desenvolvimento de vacinas e terapêutica, terapia gênica, genética da resistência a doenças, genotipagem de patógenos, etc. Este artigo foi escrito com ênfase aos conceitos e aplicação da biologia molecular na medicina veterinária.

Palavras-chave: *DNA, biotecnogia, PCR.*

Introduction

Molecular biology is a term designated for manipulation of DNA, RNA, protein and related molecules in the laboratory (NICHOLAS, 1996). Molecular research has been conducted in field of science, e.g. biology, medicine, chemistry, dentistry, ecology, zoology and paleontology. In veterinary medicine, molecular biology has been applied to the diagnosis of parasitic and infectious disease, vaccine and therapeutic protein production, gene therapy, genetic background on disease resistance and genotyping of pathogens.

At this time molecular epidemiological has become increasingly important because scientists have to know not only the diagnosis of some diseases but, also the molecular basis of the natural history of the disease (PAEZ et al., 2003). Some points for understanding molecular biology are included in this article.

1. Concepts

Heredity and Chromosomes

All living specimens have the ability to reproduce and transfer genetic information to the offspring specifying their structure and

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function. This information is contained in the chromosomes.

Chromosomes consist of mostly deoxyribonucleic acid (DNA) and a protein called histone. Histone has a binding and structural function, while the DNA constitutes the genetic information that is passed from parent cell to offspring cell. This occurs during the process of cell division by mitosis and also during the process of germ cell formation by meiosis.

DNA

Consist of two stands (WATSON and CRICK, 1953) each of which is a linear arrangement of nucleotides (Figure 1). There are four different nucleotides; each containing a nitrogen base: adenine: A; guanine: G; thymin: T; cytosine: C. The bases A and G have similar structure and are called purines: T and C have similar structure and are called pyrimidines. C always pairs with G and T always pairs with A. The sugar of each nucleotide is linked by phosphate group to the sugar of adjacent nucleotide forming the balance of double helix. The length of a short segment of DNA is usually measured in terms of the base pairs (bp). Longer segments are measured in terms of kilobases (1Kb = 1000 bases) or even megabases (1Mb = 1000 Kb).

The sequence of DNA bases of one strand acts as a template for the synthesis of a different nucleic acid (Ribonucleic Acid, RNA), which codes for protein. DNA is classified according to its repetitive structure. DNA that does not code for protein often has a repetitive structure. Two types of DNA have been characterized. In the highly repetitive (simple sequence) we have DNA with short sequences (5 -15 bp) repeated many times in tandem, and moderately repetitive DNA composed of tandem repeats of moderately long sequences (100-400 bp, sometimes up to 1000 bp). Again, variable numbers of these repeats at a given DNA locus are found from individual to individual; hence these are often called variable number of tandem repeats (VNTRs), according to Bliskovskii (1992) (Table 1). Simple Sequence Repeats (SSRs) or microsatellites are tandem-repeated sequence motifs of 1 to 6 base pairs. They play a significant role in genome evolution (BLISKIOVSKII, 1992).

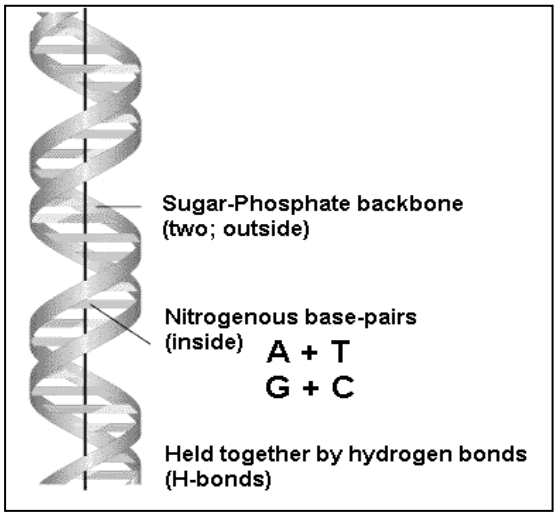

Figure 1 - The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis. Figure diagrammatic by WATSON & CRICK, 1953).

Restriction Enzymes

Since the description of double helical structure of the DNA molecule by Watson and Crick (1953), some reports about it have been published (GIUDICE and LAVERY, 2003).

Knowledge about the structure of DNA has made possible and the discovery of enzymes which enable not only the modification and linking of DNA molecules but also the synthesis of new molecules. Restriction enzymes are DNA-cutting enzymes found in some microorganisms, that recognize specific 4-8 bp sequences and cut DNA where that sequence exists (Table 2). Change in DNA sequence will change some of these "restriction sites". Therefore DNA will be cut into different size. These differences can be detected as polymorphism via method called RFLP (Table 1).

Table 1 - Terms used in molecular biology.

Abbreviators	Used Names	What is it?
RFLP	Restriction Fragment Length Polymorphism	Difference in DNA sequence between some individuals, detected as changes in fragment size when DNA is digested with restriction enzyme
AFLP	Amplified Fragment Length Polymorphism	Similar to RFLP, but uses PCR which increases power of the method
RAPD	Randomly Amplified Polymorphic DNA	Technique that allows to find the difference between similar individuals using PCR with non-specific primers
SNP	Single Nucleotide Polymorphism	Common variations of a single nucleotide among the DNA sequence of individuals
SSR	Simple Sequence Repeat (Microsatellite)	Short repetitive DNA sequences in the genome
STS	Sequence Tagged Site	Short DNA sequence from a known chromosomal location identifying the position of a gene that can be amplified by PCR

The Genetic Code

The information necessary for producing a specific sequence of amino acids is contained in code-form within the sequence of bases in a segment of DNA. This sequence is called the genetic code (NICHOLAS, 1996). Each specific sequence is called a codon and is made up of three nucleotides.

Choice of enzyme for any particular situation is dependent on whether the recognition sequence is present, the template being used (GRAHAM and HILL, 2001) and cost.

Recombinant DNA and DNA Cloning

DNA replication is the principal tool in molecular biology, which enables production of an unlimited number of copies of a particular segment of DNA. Two techniques have been used for this process. The first is called DNA cloning, and the second is called the Polymerase Chain Reaction (PCR), according Mullis (1990).

In order to determine the precise sequence of nucleotides in a template some steps are important to know. First of all, the role of the vector is to propagate and maintain the DNA fragments generated by restriction digestion. Additional requisites of vectors are the efficiency and simplicity of inserting and retrieving the DNA fragments (SCHMIDT et al., 2001; COURT et al., 2002; KAHN et al., 2002; ROSANDER et al., 2002; McDERMOTT et al., 2003; NAKANISH et al., 2003).

Depending on the size of the insert DNA and the purpose of research, different types of cloning vectors (Table 3) can be used, e.g. bacteriophage vectors are frequently used for the propagation of either genomic and complementary DNA. Plasmid vectors allow easier manipulation of cloned DNA sequence than phage vectors (COOPER, 1997).

On the other hand libraries are classified as a repository of DNA fragments (CAMPBELL and CHOY, 2002; JORDAN, 2002), cloned in their vectors. Libraries can be classified based on the cloning vector – e.g. cosmid, BAC or YAC. Two types of libraries can be found, genomic and cDNA libraries. Genomic libraries are produced when the total genomic DNA is digested and the fragments are cloned into an appropriate vector. cDNA means complementary DNA which is generated from mRNA transcripts, using the enzyme reverse transcriptase. cDNA is then cloned into an appropriate vector to make a library.

Table 2 - Sample of some restriction enzymes.

Enzyme abbreviators	Scientific name	Organism from which derived	Recognition sequence (cut at *) 5′ → 3′
AluI	*Arthrobacter luteus*	Bacteria	AG*CT TC*GA
Ava I	*Anabaena variabilis*	Cianobacteria	C* C/T C G A/G G
Bam HI	*Bacillus amyloliquefaciens*	Bacteria	G* G A T C C
Bgl II	*Bacillus globigii*	Bacteria	A* G A T C T
Eco RI	*Escherichia coli* RY 13	Bacteria	G* A A T T C
Eco RII	*Escherichia coli* R245	Bacteria	*C C A/T G G
Hae III	*Haemophilus aegyptius*	Bacteria	G G * C C
Hha I	*Haemophilus haemolyticus*	Bacteria	G C G * C
Hind III	*Haemophilus inflenzae* Rd	Bacteria	A * A G C T T
Hpa I	*Haemophilus parainflenzae*	Bacteria	G T T * A A C
Kpn I	*Klebsiella pneumoniae*	Bacteria	G G T A C * C
Mbo I	*Moraxella bovis*	Bacteria	*G A T C
NotI	*Nocardia otidis caviarum*	Bacteria	GC*GGCCGC
Pst I	*Providencia stuartii*	Bacteria	C T G C A * G
Sma I	*Serratia marcescens*	Bacteria	C C C * GGG
SstI	*Streptomyces stanford*	Bacteria	G A G C T * C
Sal I	*Streptomyces albus* G	Bacteria	G * T C G A C
Taq I	*Thermophilus aquaticus*	Bacteria	T * C G A
Xma I	*Xanthamonas malvacearum*	Bacteria	C * C C G G G

There are two ways to find a specific or target DNA sequence in a library, a sample of DNA, or whole genomes. These are hybridization and amplification. Hybridization is based on the pairing affinity of complementary single strand DNA molecules and amplification is bases on the PCR.

Specific sequence of DNA, RNA or protein can be identified by hybridization protocols (Table 4). In these protocols, template is separated by size using electrophoresis and then a probe is used which hybridizes to the sequence of interest.

PCR is based on using a special DNA polymerase to make copies of a specific DNA fragment. The choice of which DNA fragment will be amplified by the polymerase is determined by the primers (short pieces of synthesized DNA called oligonucleotides) that prime the polymerase reaction (COHEN, 1994).

2. Application of Molecular Biology in Veterinary Medicine

The manipulations of DNA can be used for a variety of purposes, including mapping, cloning, expression studies, creation of novel products, and achieving a better understanding of heredity and the genetic component of phenotypic variation.

RFLP techniques have been used to study the patterns of virus in different populations of chickens, genetic defects in bovine embryos and commercial broiler lines (BOULLIUO et al., 1991).

In the field of food safety, hybridization methods were used to detect *Campylobacter* sp. (WEGMULLER et al., 1993), *Toxoplasma* (MacPHERSON and GAJADHAR, 1993), *Listeria* (ROSSEN et al., 1991), and *Coliform bacteria* (BEJ et al., 1990).

For PCR, samples can be collected

from blood, cells fluids, or even paraffin embedded tissue. PCR has been used to diagnosis of viruses, bacteria and viruses such reoviruses, bluetongue and epizootic hemorrhagic disease have been studied by PAGE profiles (DANGLER, 1996).

Table 3 – Types of cloning vectors available.

Vector	Insert size
Plasmids	0.5 – 3 kb
Lambda phage	10 – 20 kb
Cosmids/phagemids	40 – 50 kb
Bacterial Artificial Chromosomes (BACs)	100s of kb
Yeast Artificial Chromosomes (BACs)	100s of Mb

Hybridization studies have been conducted in avian leucosis virus, bluetongue (CHINSANGARAM et al., 1992), canine distemper (ZURBRIGGEN et al., 1993) rabies (JACKSON and WUNNER, 1991), anaplasmosis (AMBROSIO et al., 1988), leptospirosis (VANEYS et al., 1988), mycoplasmosis (McCULLY and BROCK, 1992), babesiosis (JASMER et al., 1990), eimeriosis (PROFAUS-JUCHELKA et al., 1988) and toxoplasmosis (SAVVA, 1989).

Table 4 – Tools of Recombinant DNA can be used by hybridization protocols

Hybridization Technique	**What do template needs to do it**
Southern	DNA
Northern	RNA
Western	Protein

Molecular biology also has been used in small and large animal clinics as an excellent tool in ophthalmology, such as gene therapy (STOPA, 2002) and for diagnosis and prevention of equine infectious diseases (DESMETTRE, 1999).

In the field of preventive veterinary medicine, the molecular biology can be applied to discover the identification of microorganisms and diagnosis of virus, bacteria, protozoa and helminthes, molecular studies, focusing stuff of epidemiology, evolutionary biology of microorganisms, host resistance (PRICHARD, 1997), vaccine development (KNOX et al., 2001) and chemotherapy.

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