# A Comparison of Old and Modern Type DNA Marker Technologies and Their Impact on Animal Breeding Programs

Zühal GÜNDÜZ<sup>1</sup>, Onur YILMAZ<sup>2</sup>, İbrahim CEMAL<sup>2</sup>, Osman BİÇER<sup>1</sup>

**ABSTRACT:** In recent years, molecular genetic technologies allowed to identify genetic structure in farm animals have great advantages for animal breeding. Especially, in developed countries these methods began to be widely used to assist animal breeding studies. It can be said that there are various molecular genetic markers. These markers can be classified by taking into consideration a number of factors such as the principle of the detection technique, type of polymorphism. Although old type molecular genetic markers such as RFLP, AFLP, microsatellites are widely used today, the information obtained from them is more limited than modern molecular genetic markers. SNP chip technologies, which known as modern molecular markers and are one of the most important developments in the molecular genetics field, have provided genomic breeding value estimation and genomic selection in farm animals. In this review, old and new types of molecular markers were compared and their usage in animal breeding were discussed.

Keywords: Animal breeding, molecular markers, SNPs



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## Eski ve Modern Tip DNA Marker Teknolojilerinin Karşılaştırılması ve Bunların Hayvan Islahı Programlarına Etkisi

ÖZET: Son yıllarda, moleküler genetik teknolojiler hayvan ıslahı anlamında çiftlik hayvanlarının genetik yapısının tanımlanması için oldukça önemli avantajlar sağlamıştır. Özellikle gelişmiş ülkelerde bu yöntemler hayvan ıslahı çalışmalarında yaygın bir şekilde kullanılmaktadır. Çok fazla sayıda moleküler genetic işaretleyiciden bahsetmek mümkündür. Bu işaretleyiciler polimorfizm türü ve tarama tekniği gibi bir çok faktör dikkate alınarak sınıflandırılabilir. Eski tip moleküler genetik işaretleyiciler günümüzde yaygın olarak kullanılmasına rağmen bunlardan elde edilen moleküler bilgiler modern olanlara göre oldukça kısıtlıdır. Moleküler genetik alanda en önemli gelişmelerden olan ve modern genetik işaretleyici olarak bilinen SNP çip teknolojisi çiftlik hayvanlarında genomik damızlık değer tahminlerinin yapılmasına ve genomik seleksiyona olanak sağlamaktadır. Bu derlemede eski ve yeni tip moleküler işaretleyiciler karşılaştırılmış ve hayvan ıslahında kullanımları tartışılmıştır.

Anahtar Kelimeler: Hayvan ıslahı, moleküler işaretleyiciler, SNPs,

<sup>2</sup> Adnan Menderes Üniversitesi, Biometri ve Genetik A.B.D., Zootekni, Aydın, Türkiye Sorumlu yazar/Corresponding Author: Onur YILMAZ, oyilmaz@adu.edu.tr

Mustafa Kemal Üniversitesi, Hayvan Yetiştirme ve Islahı A.B.D., Zootekni, Hatay, Türkiye

#### INTRODUCTION

Until recently, phenotypic selection or estimated breeding value (EBV) based on phenotype has been used for animal breeding to improve genetic progress for quantitative traits, without which genes affect the property or the effect of each loci (Walsh, 2000; Naqvi, 2007). Recent developments in molecular biology and statistics have prepared the opportunity of identifying and using genomic variation and QTL that affected the genetic improvement of livestock (Montaldo and Meza-Herrera, 1998). Molecular markers have a significant role in animal breeding in terms of animal identification and to determine the genetic diversity by levels of DNA polymorphism. The increasing availability of molecular markers in farm animals such as cattle, sheep, goat, poultry and swine allows the detailed analyzes and evaluation of genetic diversity, and furthermore the detection of genes influencing economically important traits.

Although the majority of molecular markers used nowadays with high-throughput systems are microsatellite markers (simple tandem repeat, STR) and Single nucleotide polymorphisms (SNPs). Many molecular genetic markers such as random amplified polymorphic DNA (RAPD) markers, single-strand conformation polymorphisms (SSCPs), restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs) markers are widely used in farm animals for the determination of genetic diversity, paternity analysis, detection of major genes and mapping of quantitative trait loci (QTL) (Kinghorn et al., 1993; Roher et al., 1994; Kinghorn 1997; Vignal et al., 2002).

The aim of this study was to discuss the comparison of molecular markers and its potential use in the animal breeding.

# DNA Marker Technologies and Their Use in Animal Breeding

Litter size and production traits (milk, meat, wool etc.) in farm animals, considered as quantitative characters, are generally polygenic. These traits are influenced by many factors such as genes and environment. It has known that quantitative genetics approaches are important to increase the possibility of choosing the right animal to be parents (Nicholas, 1996). Molecular genetic techniques to identify the genetic structure and diversity in farm animals have shown rapid development in recent years and began to be widely used. Various molecular genetics marker technologies have been developed to reveal selection decision, genetic structure, and diversity.

#### **Older Types of Molecular Markers**

RAPDs (Random Amplification of Polymorphic Length DNA). **AFLPs** (Amplified Fragment Polymorphisms), **SSCPs** (Single Stranded Conformation Polymorphisms), RFLP (Restriction Fragment Length Polymorphisms) and microsatellites defined as the older type markers can be described in three main categories. They can be sorted as bi-allelic dominant (RAPDs, AFLPs), the bi-allelic co-dominant (RFLPs, SSCPs) and the multi-allelic codominant (microsatellites) (Vignal et al., 2002).

#### **Bi-allelic dominant markers (RAPDs, AFLPs)**

Although, use of RAPDs and AFLPs, described as dominant markers, do not seem that interesting to use at a first glance, they have great advantageous in terms of ease of use. RAPDs PCR technique, also known as AP-PCR (Arbitrarily Primed PCR), described by Williams et al., (1990) and Welsh et al., (1990), does not require any specific information of the DNA sequence for the targeted genome and is implemented using the randomized primers. The low reproducibility is one of the main disadvantages of RAPDs technique

RAPD technique is quite tightly dependent on the laboratory procedure therefore it must be very careful preparation of design of laboratory protocol.

Despite having a low-reliability method, RAPDs widely uses to identify genetic similarity and diversity, to measure inbreeding in population and the construction of genome map in farm animal (Rao et al., 1996; Bhattacharya et al., 2003; Ali, 2003; Ahmed, 2005; Binbaş, 2006; Elmaci et al., 2007; Kumar et al., 2008).

The amplified fragment length polymorphisms (AFLPs) technique, which is a cost-effective fingerprint technique and presents more information, is based on selective PCR amplification of a group of

DNA fragments resulting from cutting with restriction enzyme. Hundreds of highly replicable markers from DNA of any organism are generated by this technique.

Amplified fragment length polymorphism (AFLP) technique allows for the identification of variations caused by SNP and indels which is very important for the identification of genetic diversity studies. For these reasons, this technique is widely used in genetic relationship studies, QTL analysis, linkage mapping, and profiling of gene expression using cDNA genetic diversity studies (Barendse et al., 1994; Otsen et al., 1996; Nijiman et al., 1999; Moreno et al., 2002; Foulley et al., 2006; Negrini et al., 2007).

Although, RAPDs and AFLPs markers are dominant and generated at random. Both of them are good choice for QTL mapping or diversity studies in species (Negrini et al., 2006).

### Bi-allelic co-dominant markers (RFLPs, SSCPs)

Restriction Fragment Lenght Polymorphisms (RFLP) technique was developed following the discovery of restriction endonucleases in the 1960s. A simple and useful way of testing for a mutation is RFLP analysis, uses an enzyme with a recognition sequence created by the mutation (Simm, 1998). There are approximately more than 300 restriction enzymes that are isolated from bacteria and cut DNA wherever specific short sequences (Montaldo and Meza-Herrera 1998; Babalola, 2003). PCR-RFLP technique described just a polymorphism with each probe, is cheap and widely used more than another marker system such as RAPD, SSCP. This method is commonly used in nucleic acid hybridization definition, identification and diagnosis, description of polymorphisms on the gene construction of a genetic linkage map and recombinant DNA technology in farm animals (Solak et al., 2000; Vignal et al, 2002; Schlötterer, 2004; Turner et al., 2004; Cemal et al., 2009; Sevim et al., 2012; Yilmaz et al., 2013; Yilmaz et al., 2014).

The principle of Single-strand conformation polymorphism (SSCP) analysis based on PCR is a method used to separate DNA fragments of the same size. DNA polymorphisms and mutations at multiple regions in the single loci can be detected by SSCP as a mutation scanning technique (Orita et al., 1989; Bastas et al., 2001). Denaturing high-performance

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liquid chromatography (DHPLC) known as improved model of the SSCP technique is used for the separation of the heteroduplex and homoduplex strands (Liu et al., 1998). Reported results from SSCP studies are always particular to specific fragments and sequence changes; generalizations can be problematic. Mutations that show no mobility shift under one set of conditions may be revealed under different conditions (Hayashi, 1991; Fan et al., 1993; Sheffield et al., 1993). SSCPs uses to detect sequence variations, and screening of mutation in farm animal.

## Multi-allelic codominant markers (Microsatellites)

Microsatellites markers, which are among the most widely used molecular genetic methods, are short tandemly repeated DNA sequences that are present in variable copy numbers at each locus and throughout the genome (Ashley and Dow, 1994; Forbes et al., 1995; Bruford et al.,1996; Ellegren et al.,1997; Montaldo and Meza-Herrera, 1998; Schlötterer, 1998; Schmid et al., 1999; Toth et al., 2000; Beuzen et al., 2000; Hancock, 2001;).

Microsatellites, which had spread a whole genome, are DNA sequences consisting of short repeats of highly variable number. Microsatellites have several advantageous such as highly polymorphic, co-dominant inheritance, easy genotyping and scored. For this reason microsatellite markers are widely used in genetic diversity and paternity analysis studies. (Bruford et al, 1996; Montaldo and Meza-Herrera, 1998; Beuzen et al.; 2000; Sancristobal et al, 2003; Schlötterer, 2004; Togan et al., 2005; Acar, 2010; Jyotsana et al., 2010; Arora et al., 2011; Kusza et al., 2011; Lasagna et al., 2011; Agaviezor et al., 2012; Alvarez et al., 2012; Yilmaz and Karaca, 2012; Cemal et al., 2013; Yilmaz et al., 2013, Öner et al. 2014; Yilmaz et al. 2014).

### **Modern Types of Molecular Marker**

New genetic technologies developed rapidly have found applications in animal production. Identification of gene regions with an effect on complex quantitative traits of economic importance will increase genetic gain and its proportion per year. The SNPs genotyping technologies provide powerful resources for animal breeding programs. Genomic selection using SNPs is a new tool for choosing the best breeding animals. In addition, the high density maps using SNPs can provide useful genetic tools to study quantitative traits genetic variations (Koopaee and Koshkoiyeh, 2014; Yılmaz et al., 2015).

#### Single nucleotide polymorphisms (SNPs)

Old type molecular markers were widely used to determine genetic diversity, paternity analysis and other molecular genetic studies in the last two decade. Nowadays, scientific studies have focused on singlenucleotide polymorphisms (SNPs) to identify genetic variations. SNP is defined single nucleotide changes in a specific base position that occurs in around 1% of a large population.

SNPs have emerged as a powerful tool in marker technology, was first proposed by Lander (1996), it refers to a sequence polymorphism caused by a single nucleotide mutation at a specific locus in the DNA sequence (Akey et al., 2001, Yang et al., 2013). SNPs, forming the 90% all of genetic variation, are the most modern method of genotyping with a greater sensitivity and ease of automation (Landegren et al., 1998). SNPs have low mutation rates and can be amplified easily for testing (Lipshutz et al., 1999; Beuzen et al., 2000; Stoneking, 2001; Vignal et. al., 2002).

SNPs provide convenience in genetic disease studies, paternity testing, traceability, estimation of genomic breeding values (GEBVs), genetic mapping for various livestock species. Today, genomic selection has become possible with determining millions SNP by a single analysis in various animal species (Hayes et al., 2007; Goddard and Hayes, 2007; Hayes et al., 2009; Bolormaa et al., 2010; Slack-Smith et al., 2010; Bolormaa et al., 2011; Daetwyler et al., 2012; Eggen, 2012)

#### CONCLUSIONS

Molecular markers have been developed and potential tools for animal breeding. Nowadays molecular genetic techniques, provided an important contribution to the quantitative theory, have become a vital tool for animal breeding program. Molecular markers are very important for the determination of genetic variation within and between populations, re-construction of pedigree data, measurement of the effective population size, identify admixture populations, providing of evolution history in population genetics.

In summary, for molecular techniques make a great benefaction to livestock production system we need a joined-up strategy addressing genetic progress as well as conservation, rather than gradually proceeding classical breeding methods.

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