Parentage verification in equines through genomic markers

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Introduction

Since ages the knowledge of biological pedigree information holds immense importance for all races including animals specially domestic animals with proven economic gains. Pedigree information includes information of mother (maternity testing) and father (paternity testing) of the offspring.

This information holds special attention for genetic gain over the year's alongwith selective breeding of animals. A paternity testing is to determine the biological father. Similarly, maternity testing is performed to identify the biological mother of the offspring. The maternity testing is less common, because at least during childbirth, except in the case of a pregnancy involving embryo transfer or egg donation, it is obvious who the mother is. So, the information regarding the scientific analysis of paternity testing is more in demand. But, the maternity testing can be useful for the condition that the mother is long time separated from her children and the proofs of biological relationship are required. Nevertheless, the technique of paternity and maternity verification is same. Parentage testing can be done by older methods including ABO blood group typing, analysis of various other proteins and enzymes, or using human leukocyte antigen (HLA). However, a DNA testing has become more formal and most appropriate method for the parentage testing now days.

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The DNA of an individual is almost exactly same in each somatic cell. Sexual reproduction brings the DNA of both parents together randomly to create a unique combination of genetic material in a new cell, so the genetic material of an individual is derived from both their parents. A DNA based parentage test determines and examines the allele found in the mother, the child and the alleged father. The accuracy of the DNA test results depends on the extent and accuracy of statistical analysis of the DNA testing process. With sufficient testing, DNA technology provides an extremely powerful method of discrimination between fathers and non-fathers. Since the DNA molecular structure and genetic characteristics of a child are inherited from or determined by the DNA structure of the biological mother and father, DNA identification provides a conclusive and definitive way to establish biological relationships. Therefore, genetic markers became the most acknowledged method to determine parentage. The genetic testing methods involve polymerase chain reaction (PCR), short tandem repeat (STR) and restriction fragment length polymorphism (RFLP), with which exclusion or inclusion of a parent's DNA to that of a child results in a probability factor of 99.999999% or 1 in 10 billion of the population.

Microsatellites and Other Genetic Markers

A genetic marker is an amplified locus that is informative in terms of showing polymorphism between individuals underlying the genetic variability. In the field of equine genetics, low-density single nucleotide polymorphisms (SNPs), microsatellites, and amplified fragment length polymorphisms (AFLP) have long been the preferred types of genomic data for

parentage assignment due to their low cost and established technological correctness. Generally, the basis of parentage assignment rests on exclusion- and likelihood-based methods. Exclusion-based methods rely on their ability to exclude false parent–offspring combinations when the offspring's candidate parents' genotypes violate Mendel's laws. These methods are often used due to their ease of interpretation, but the number of expected exclusions depends on allele frequencies in the population and on genotype call rates and error rates.

Microsatellites are short segments of DNA that consist of repeating nucleotides and are therefore also referred to as Short Tandem Repeats (STRs). The repeat units can range from two to six base pair motifs and the entire microsatellite can range in size from fewer than ten to hundreds of bases; depending on the number of repeat units. The nature of the repeat element can be categorized as simple $(AC)_n$; compound (two or more microsatellites found in close proximity) or complex (containing repeat units of several nucleotides), either of which may be interrupted or not. Microsatellites are inherited in a Mendelian fashion and CA-repeats are the most common motif in most mammalian genomes. Microsatellites markers are abundant and evenly spread throughout the genome although they are often associated with non-coding DNA. Genetic markers can be classified into two types: genes with known functions (Type I) and anonymous DNA fragments (Type II). Type II markers include marker systems such as AFLP, Random Amplified Polymorphic DNA (RAPD) and microsatellites. Whole genome markers such as Restriction Fragment Length Polymorphism (RFLP) and AFLP rely on the digestion of genomic DNA by restriction enzymes to produce variable patterns between individuals. These approaches are gel based and therefore not suited to high throughput genotyping. Being dominant markers, it is also impossible to distinguish between homozygotes and heterozygotes. This makes the discriminatory power of these methods less than ideal, although RFLP has been used successfully for parentage analysis in Thoroughbred horses. RAPD is another dominant marker system which is not whole genome-based but relies on the detection of polymorphisms with a few nucleotide mismatches. Another repeat-based marker is the Inter Simple Sequence Repeat (ISSR) which produces similar profiles to RAPD but uses primers anchored to microsatellite sequences. Microsatellites are co-dominant which means that heterozygotes can be distinguished from homozygotes. In addition, microsatellites can have many different alleles at a single locus. These traits make them highly polymorphic and ideal in identification. Microsatellite polymorphism is generated through interplay of a high mutation rate and polymerase slippage during DNA replication.

Importance of parentage testing in horses

Many of the traits we value in horses are highly heritable. Breeders have long known that offsprings tend to resemble their parents. Consequently, pedigrees and parentage are important. These heritable valuable traits such as endurance, coat color, athletic stamina etc in horses provide high economic gain to the breeders. Most horse breed registries have instituted rules that require some form of genetic testing to verify the parentage of registered horses.

ISAG comparison tests

The International Society of Animal Genetics (ISAG) provides list of approved markers and technologies for genotyping and parentage analysis. When two or more laboratories identified the same genetic variants in two subsequent comparison tests, the ISAG committee would assign official nomenclature for the marker. In the 1990s, the genetic markers (DNA) replaced blood groups and biochemical markers for parentage testing. The ISAG comparison

tests are conducted on a biannual basis with the goal of standardizing the recognition of genetic markers. Because horses are shipped internationally, coordination among laboratories is important at the international level. At the 2010 ISAG horse parentage testing workshop, the laboratories agreed to test the following microsatellite markers for the parentage testing of horses: AHT4, AHT5, ASB17, ASB2, ASB23, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, and VHL20. These markers provide sufficient power to identify horses and to resolve most questions of parentage. However, most laboratories also test for additional markers to resolve disputed parentage, or to address special needs for the horse populations under study.

Parentage testing at NRCE

Since, microsatellites have been used for parentage testing and individual identification because of their virtue of being highly polymorphic and show abundant sequences dispersed throughout most eukaryotic nuclear genomes, work has been initiated at ICAR- National Research Centre on Equines, Hisar, Haryana. For developing the facility of parentage verification of equine foals the blood samples were collected from the foal and its known reported parents. Genomic DNA was extracted from the blood and hair follicles and analysed with a panel of 21 Microsatellite markers which were found highly polymorphic and heterozygous in equines. Results from over 300 animals were found correctly matched (100%) to their parents upon genotyping. The parentage testing facility of NRCE will be soon available to equine breeders.

Applications of microsatellite based genetic testing for parentage and identity

The power of genetic testing to address questions of parentage and identity depends on the accuracy of methods used and statistical analysis. Currently, hundreds of alleles are evaluated using the microsatellite tests. The genome projects have invented more powerful tools as well, but these are not necessarily better for application to parentage and identity. There are two issues that drive the choice of parentage test for horses: (i) the cost of running the test; and (ii) the ability to use the genetic information that has been derived from previous tests. To do a parentage analysis, the genetic results from the parents and the offspring must be available. Currently, microsatellite results are stored in an extensive computerized database, and it is these records that are used in parentage analysis. So once a horse has been tested, it need not be tested again. If the exercise of parentage verification becomes widespread among horse breeders, then it might become economically more viable. This genetic marker evaluation also holds promise for other performance traits along with parentage assignment.