Topic 4: Genetic test for identification of parents (parentage testing)
Lecture

At the beginning it is necessary to explain the basic terms that will be used during this presentation. The first one is parenthood - the correctness of both parents, father and mother, is verified. In the case of paternity, only the father's authenticity is verified for an individual, which is the most important thing for humans. If we are going to identify an individual, we will use molecular genetic methods (called molecular dactyloscopy) to determine whether the DNA sample belongs to the individual being examined. The term genetic type or genetic profile represents the set of all alleles (genotypes) that is characteristic, unmistakable and unique to each individual.
The purpose of genetic parentage testing is to verify the origin and identification of an individual. In the case of livestock bred in the Czech Republic, the testing is governed by Act No.154/2000, called as Breeders Act. For pigs, the genetic type of boars in breeding farms is determined, for cattle, the origin of breeding bulls is verified, and their genetic type is determined before selection for breeding, for horses, the origin of foals after insemination is verified.
Parentage determination is of great importance not only in animal genetics, but also in forensic genetics and medicine. There are mistakes in breeding pedigrees that could cause inaccuracy in the estimation of breeding value. The price of animals is often based on the genetic quality of their parents, and it is therefore necessary to be able to verify parentage. Customers may also be cheated. In the field of forensic genetics, paternity disputes are commonly dealt with, and many offenders are now convicted based on DNA traces. Even victims of violent crimes or natural disasters can be identified from their DNA. Relationship verification is a necessity, especially in transplantation.
In the past, before the development of molecular genetic methods, blood groups and then protein variability were used to verify parentage. However, these methods were not very reliable. Therefore, methods based on the detection of DNA variability (DNA fingerprinting), initially based on the variable number of tandem repeats (VNTR) in minisatellites, began to be used. Currently, methods associated with DNA amplification by PCR, called STRs, are used for microsatellites. The advantage is that a very small amount of biological material is sufficient for analysis. This most widely used method will be the subject of the following explanation. The detection of single nucleotide polymorphisms (SNPs) is also increasingly used in conjunction with modern large-scale animal testing methods. The basic requirements for modern methods are high reliability of rejection and or confirmation of parentage, speed and simplicity of testing, high reproducibility and, finally, the lowest possible cost.
What are microsatellites? They are short repetitive sequences, tandem repeats, that consist of moni, di, tri or tetra nucleotide motifs. They are highly polymorphic, i.e. that the number of their allelic forms is very high. We classify them as length polymorphisms. We can amplify the sequence with microsatellites by PCR and then detect their length by electrophoresis, which is determined by the different number of repeats. The designation of alleles of individual microsatellites is determined by the size of this sequence in numbers of bases.
In the figure you can see an example of only 3 alleles of one microsatellite, which differ in the number of repeats of the GC repeat and the size of their PCR product varies, and their combination can give a total of 6 different genotypes. The size of the allele is determined by gel electrophoresis, here is a diagram of the gel electrophoresis for illustration.
Different sets of microsatellites, or panels, are used in different animal species, with a minimum number of 10, which is sufficient for highly reliable parentage verification. In humans, 15 to 22 of these markers are used for paternity.

Other microsatellite panels are being developed for other animal species and allow studying the genetic diversity of populations in addition to parentage tests.
DNA isolation is possible from any biological material that contains even a minimal amount of DNA. This is followed by a multiplex PCR reaction that specifically multiplies the individual microsatellites together in one tube. Their size is then determined by precise capillary electrophoresis. The alleles and genotypes for each microsatellite are then determined. By comparing the genetic type of parent and offspring, parentage can be verified.
The idea of multiplex PCR is that multiple DNA fragments are amplified in a single reaction, depending on the number of primers added to the reaction. One of the primers of a given pair must always be labelled with a specific fluorescent colour. When designing primers, care must be taken to ensure that the allele size ranges of different microsatellites in a single colour do not overlap, otherwise they would not be able to be differentiated from each other. The reaction takes place in a thermal cycler. The result is a microtube containing amplicons of different lengths, so that the entire analysis can be performed in one tube.
The mixture of PCR fragments of many different microsatellites needs to be distinguished by size and colour. This is done by fluorescence capillary electrophoresis. This allows a very precise determination of the size of the individual fragments, i.e. alleles, and is performed using a genetic analyser.
The specific size of individual alleles is determined by comparison with the calibration curve of a size standard. It is a mixture of DNA fragments of known length. The size standard is always separated with each sample together and is distinguished by the orange colour.
The result of electrophoresis can be monitored using raw data. On the electrophoretogram we can see the peaks corresponding to the individual microsatellites (red, blue, green and black) and the size standard (orange).
The genetic analyzer software enables automatic evaluation – determining the sizes of individual peaks that correspond to alleles. We thus obtain the genotypes of the individual microsatellites of the individual, the so-called genetic type. By comparing with parents we can verify parentage or by comparing with another sample we can identify the individual.