Topic 4: Genomics of farm animals/Sequencing demonstration

Practical example

In this presentation we will be looking at genomics in livestock and in particular sequencing. We will use the example of the laboratory process of Sanger sequencing on an automated sequencer. This presentation is part of module 1, animal genetics. The creation of this presentation was supported by an ERASMUS+ KA2 grant within the ISAGREED project, Innovating the content and structure of study programmes in the field of animal genetic and food resources management using digitization.

The actual laboratory procedure of Sanger sequencing involves several steps. The first is the preparation of a suitable sample, i.e. DNA. The sample can be DNA cloned in a vector and obtained by isolation from bacterial cells. Universal primers are used for sequencing and therefore the result is usually of high quality. However, cloned DNA is not always available. More often we use the PCR product as template. Here, the purity of the PCR product is critical (the PCR product must be purified), as well as its quantity. If we have a good quality sample, we can proceed to the second step - the actual sequencing reaction. This is an enzymatic reaction similar to PCR, but only one primer and a commercially available reaction mixture containing specific fluorescently labelled ddNTPs (each base a different colour), called terminators, must be used. The result is a mixture of fragments, each terminated by a terminator which indicates by its colour the base at the corresponding site in the sequence. For a detailed description of the method, see the lecture on this topic. This is followed by removal of the free nucleotides and precise capillary electrophoresis. The final step is the software evaluation of the data and assembly of the final sequence.

One option for removing free nucleotides, especially free color-labeled terminators, is to use an emulsion of a porous reagent that absorbs these nucleotides and thus purifies the solution. The reagent must be thoroughly mixed by vortexing before adding it to the sequencing mixture and then removed with a sheared tip. To do this, the sequencing mixture and emulsion should be allowed to shake for 30 minutes. After centrifugation, the supernatant is removed into a sequencing plate and is ready for analysis in the sequencer.

The basis of the automatic sequencer is fluorescence capillary electrophoresis. This picture shows the basic components of the instrument - the anode section with the polymer pump on the left, the detection chamber in the middle, the capillary array on the top right and the sample plate and cathode section on the bottom. Prior to each separation, the pump pushes fresh polymer into the capillaries, then the sample is electroinjected into the capillary and separation proceeds from the cathode to the anode. The fluorescence signal is picked up in the detection section, with shorter DNA fragments arriving before longer ones.

This picture shows a close-up of the pump, polymer bag, anode and anode buffer container.

Glass capillaries filled with polymer are the basis of precise DNA separation. Polymer is a special gel with separation ability. The DNA travels through the capillary and separates according to size. Smaller fragments reach the window earlier than longer ones and the instrument reads the corresponding signal (fluorescent colour).

Insertion of fresh anode electrophoresis buffer must be done very carefully. You can see the block with the pump, which always fills the capillary with fresh polymer before the run. The bag on the right contains a separation polymer.

Autosampler extends and is ready to load the sample plate.

Snap the plate retainer (cover) onto the plate, septa, and plate base.

First, we insert the cartridge with cathode buffer (left side) and washing solution (right), and subsequently plate assembly. After closing, the sequencer will automatically move to the correct position.

We can insert a maximum of 2 plates with samples, i.e. a total of 192 samples.

After the washing step, the electrodes are immersed in the samples and electroinjection into the capillaries takes place, then the electrodes are placed in the cathode buffer and electrophoresis begins.

The reading of the fluorescent signal takes place gradually as the relevant fragments travel to the sensor

After the electrophoresis is finished, we will check the raw data.

The evaluation is carried out using Sequencing analysis software. The color of the peak corresponds to the corresponding base in the sequence shown above. Here we also see an example of a heterozygote, it can be recognized by the fact that there are peaks of two different colors at the same position.

Specific software can be used to compare the sequences of a large number of samples and thereby find differences - polymorphisms. In the mentioned bee samples, there are 2 types of polymorphisms – deletion on the left and single nucleotide on the right. Evaluation of this result allowed us to distinguish different mitochondrial DNA haplotypes in our bee population that are related to their origin.

We can export the finished sequence in a suitable format for further use.

For comparing finished sequences, e.g. the Clustal program is suitable. We can thus find disease-causing mutations, alleles influencing performance trait in farm animals, determine genetic diversity in animal populations, kinship, and many other applications.

And that's all for this short presentation explaining the basics of DNA sequence determination. I believe that the illustrative examples in this video will help you understand one of the basic laboratory methods without which modern genetics and genomics cannot do. Thank you for your attention.