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Genetic test for identification of parents (parentage testing)

Laboratory examples

Modul no. 4: Precision livestock farming

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Analysis procedure

1. Genome DNA isolation of the test animal
2. Multiplex PCR - PCR for many products (microsatellites) at once in one tube, marking the microsatellite with the appropriate colour (one of the primers of each pair)
3. Fragmentation analysis (capillary electrophoresis) - precise separation of products by size (number of bp)
4. Peak sizes determination (by comparison with the standard calibration curve, automatically using the software)
5. Identifying alleles of individual microsatellites: distribution of the microsatellite according to the colour and known range of alleles (this corresponds to the number of repetitions in the allele)
6. Verification

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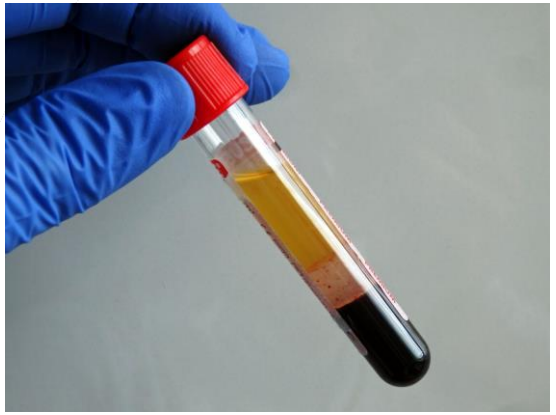
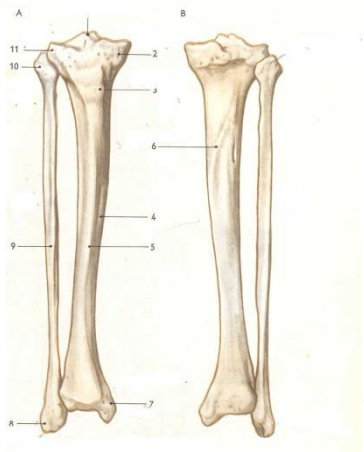
DNA isolation





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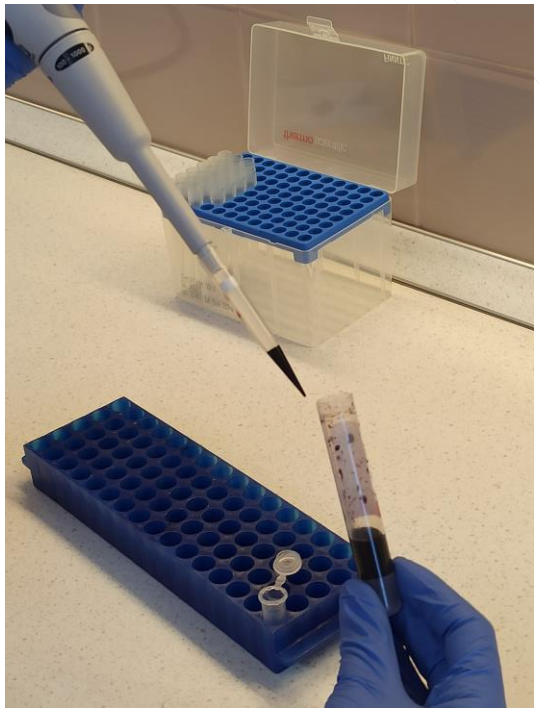
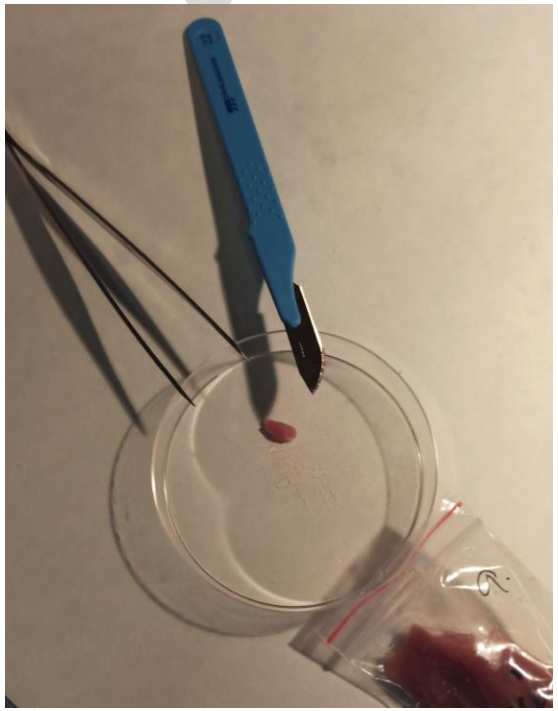
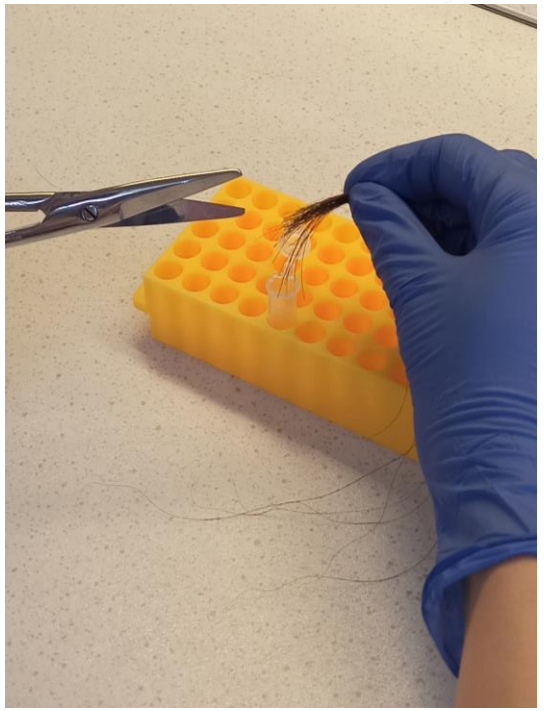
DNA isolation by spin-column method



blood



tissue (meat)



hair bulbs (fur samples)

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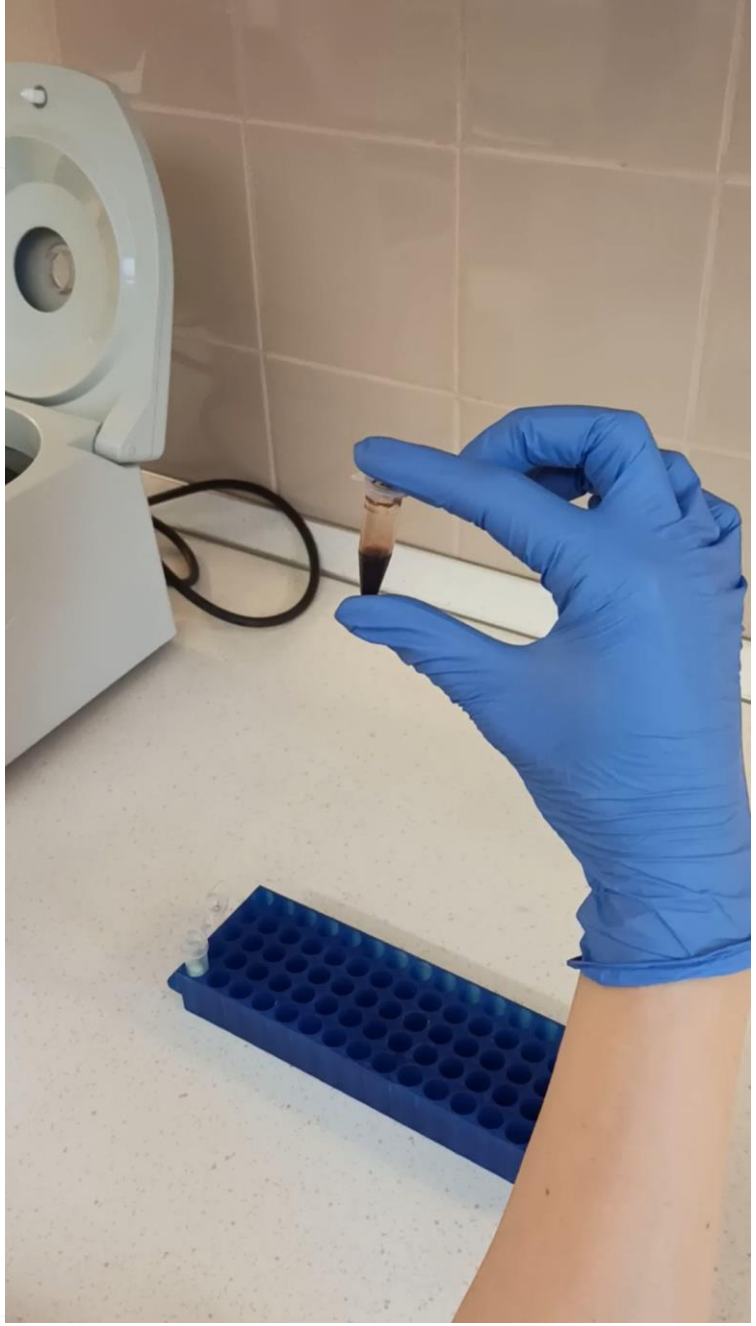
Cell lysis



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DNA binding and purification

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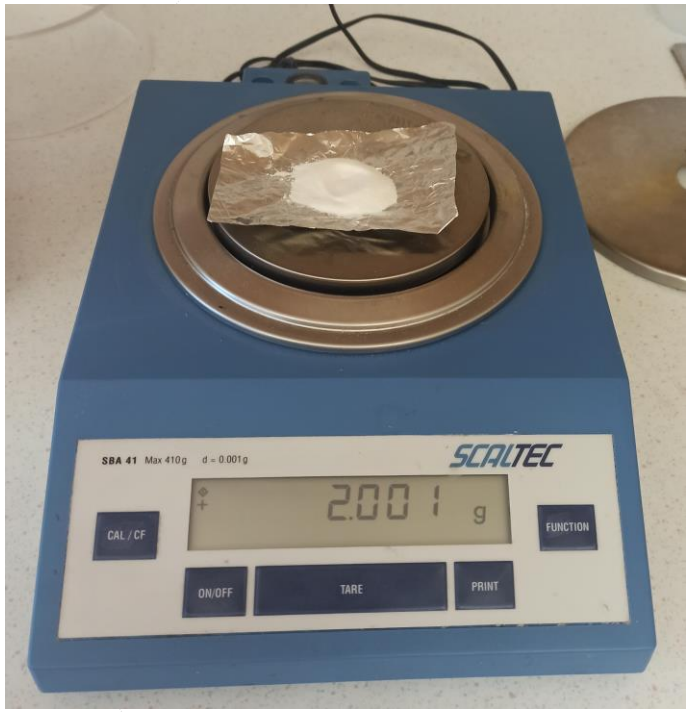
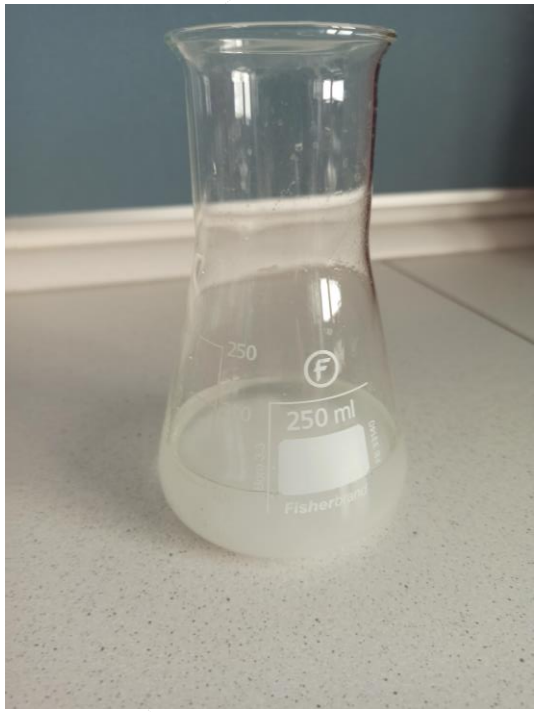
Agarose gel electrophoresis

verification of DNA quantity and specificity - preparation of 2% agarose gel





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Agarose gel preparation

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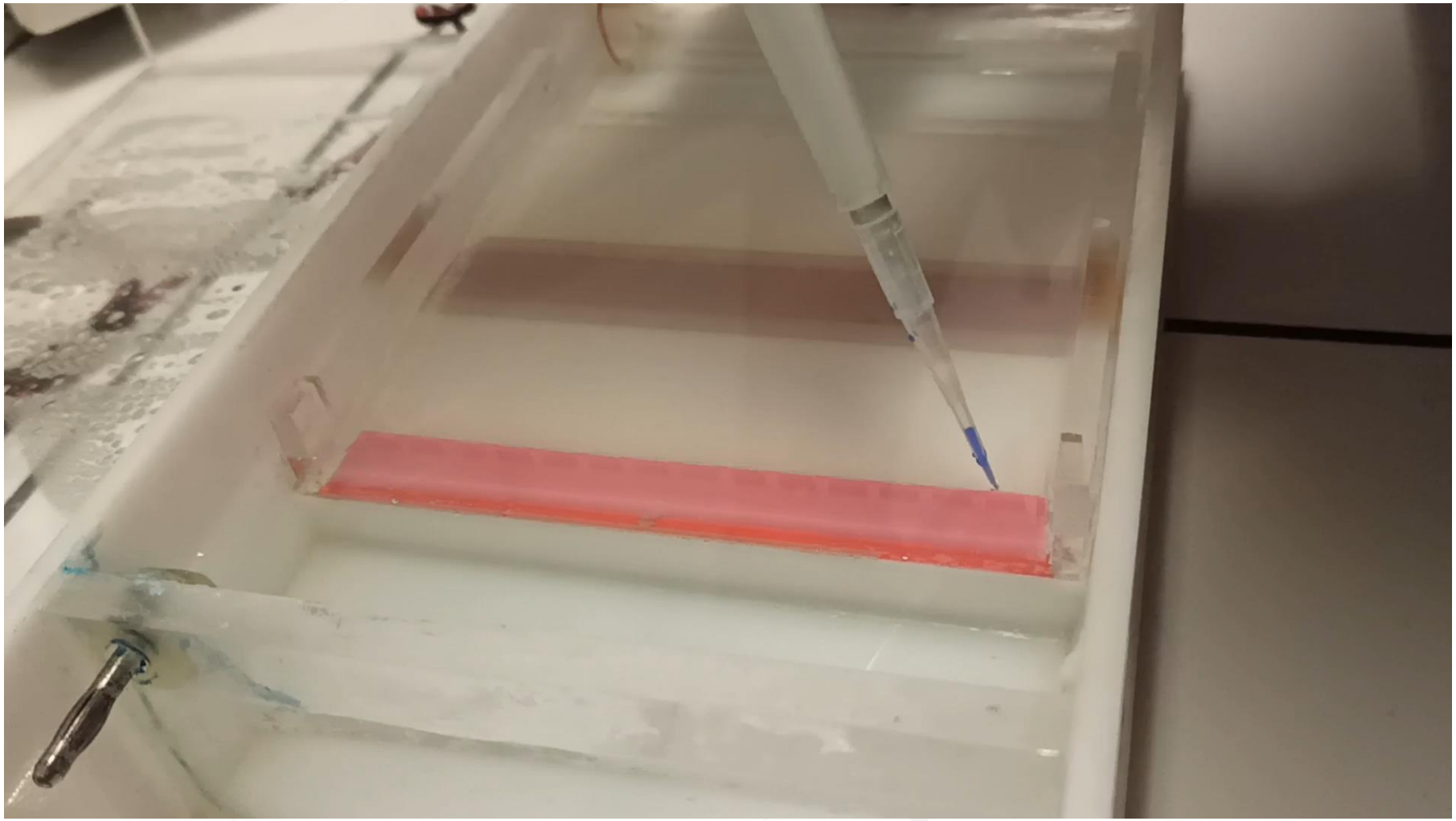
Agarose electrophoresis



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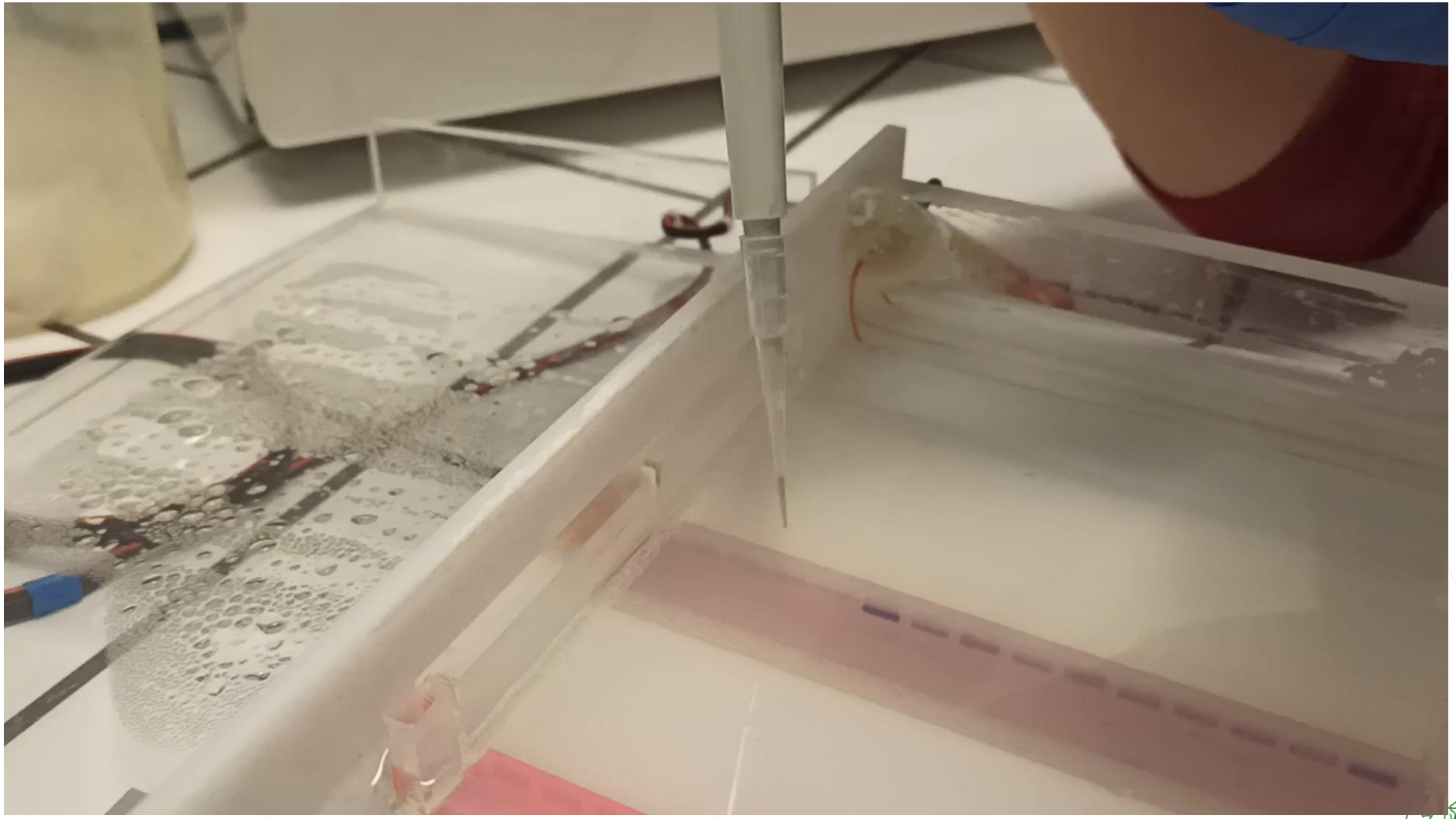
Agarose electrophoresis



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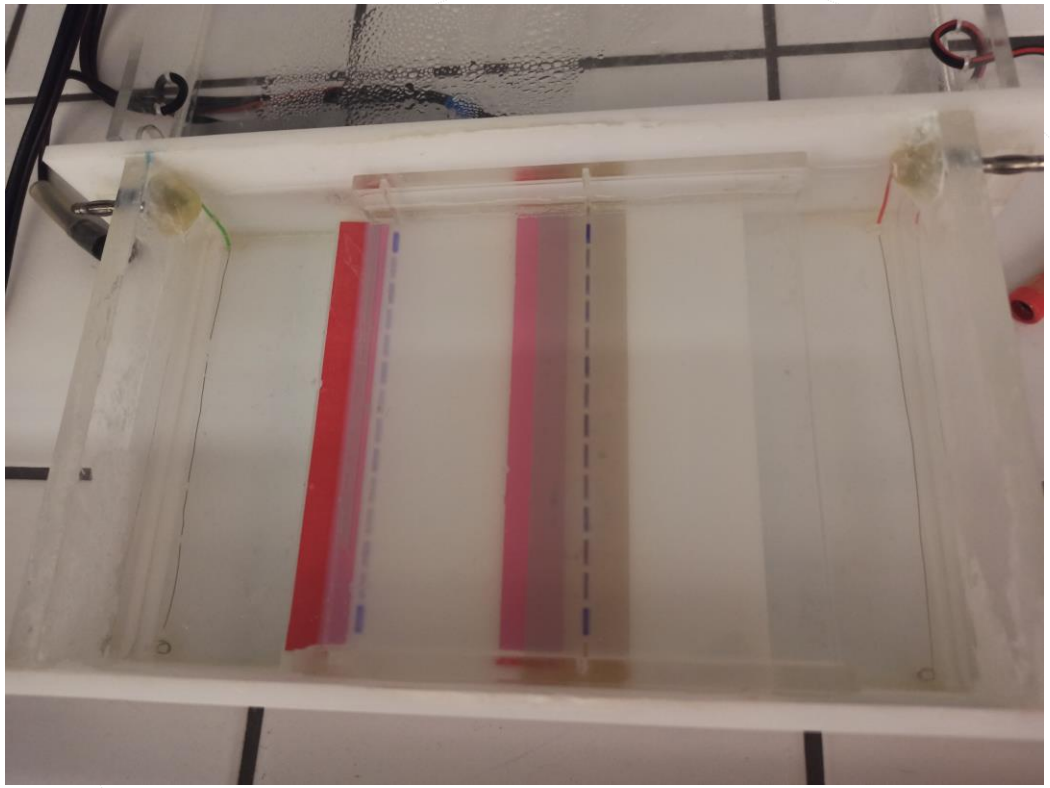
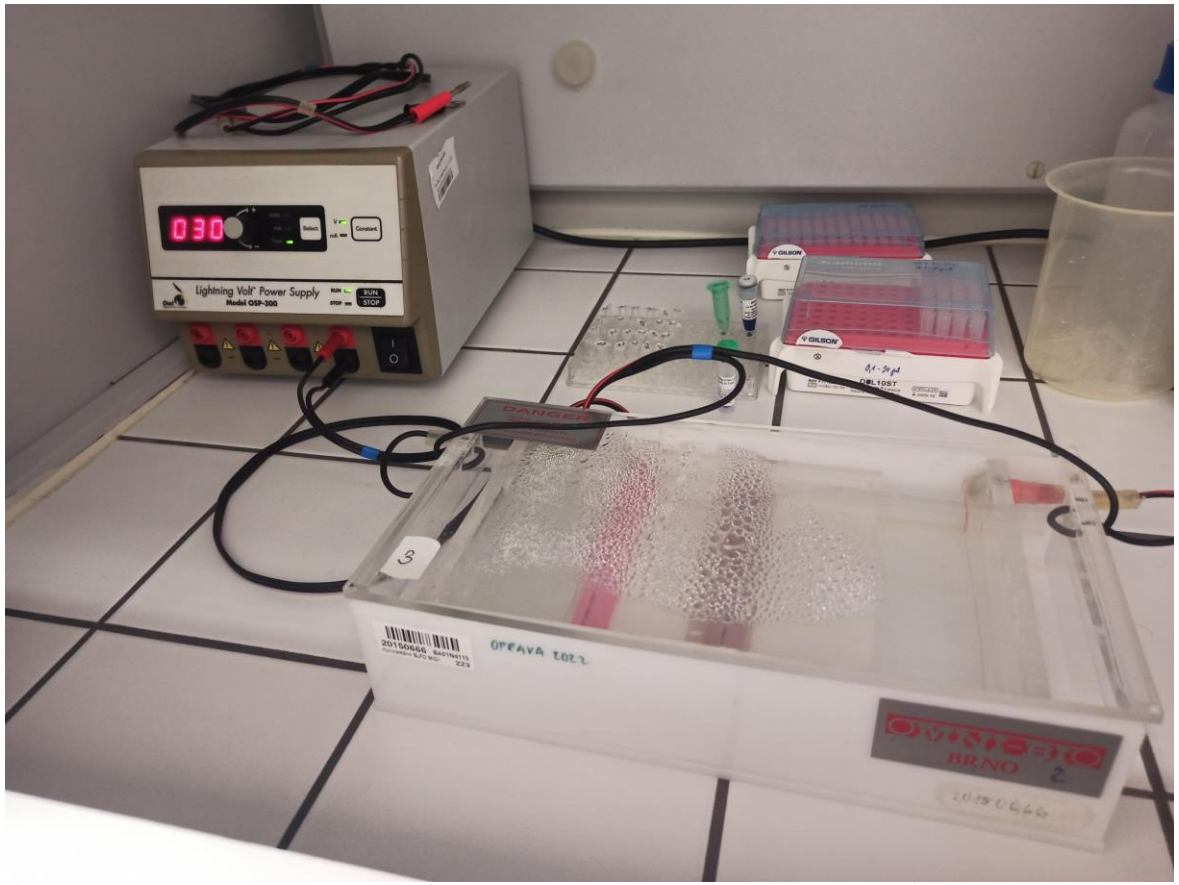
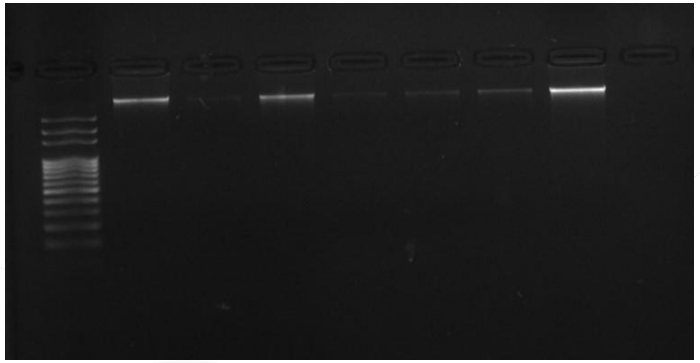
Agarose electrophoresis







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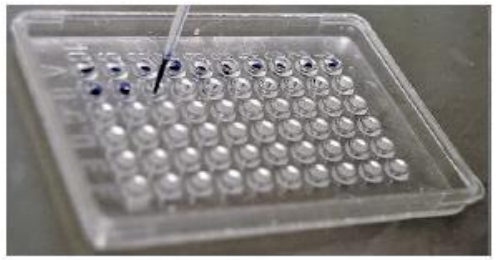

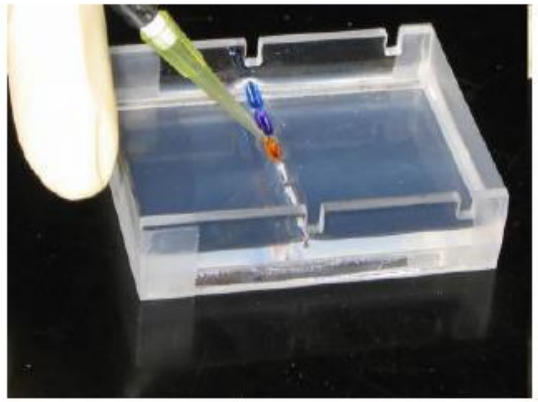

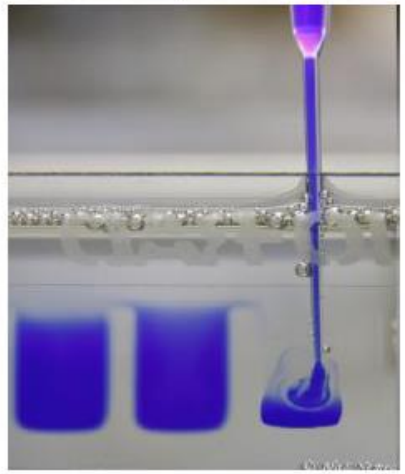

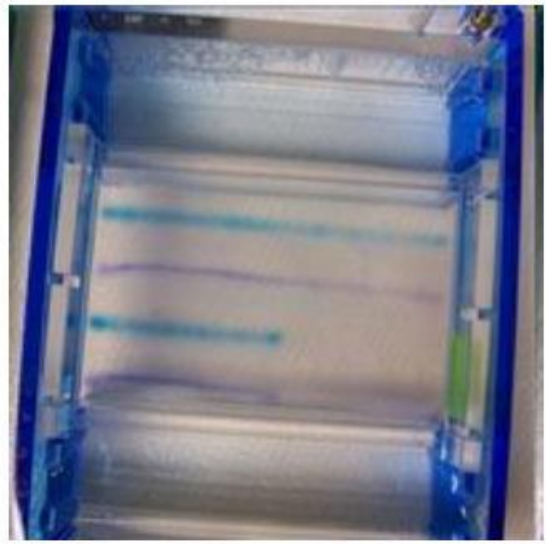

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Multiplex PCR

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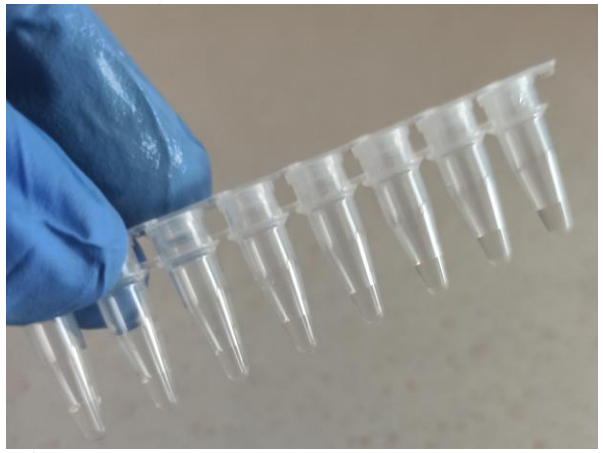
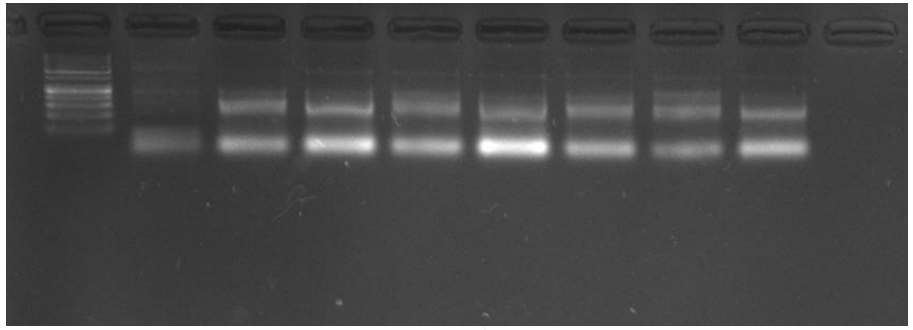
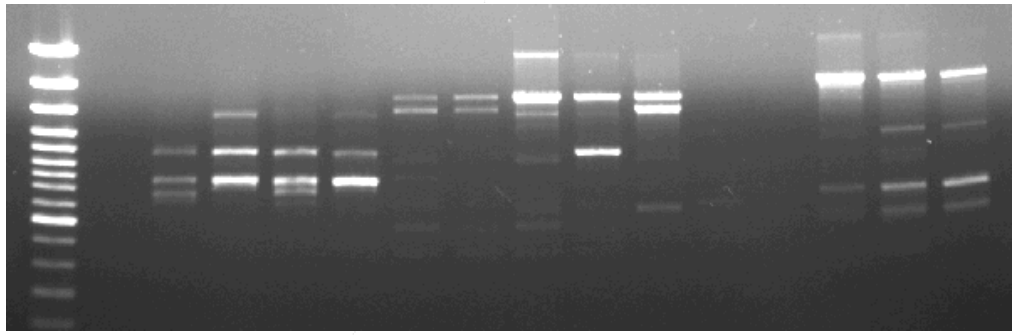
Multiplex PCR







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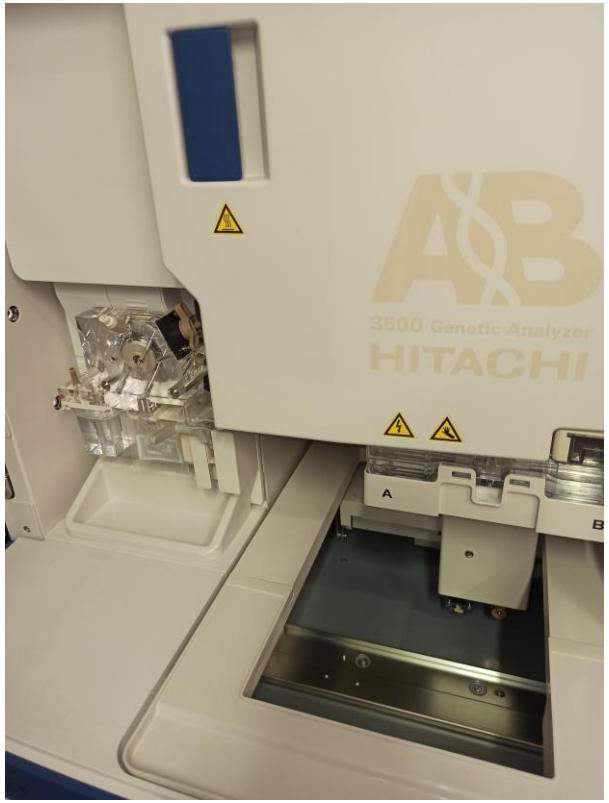
Fragment analysis

- capillary electrophoresis + fluorescence detection on a genetic analyzer



Demonstration of genetic analyzer control videos (sequencers) - see lecture module 1 - sequencing

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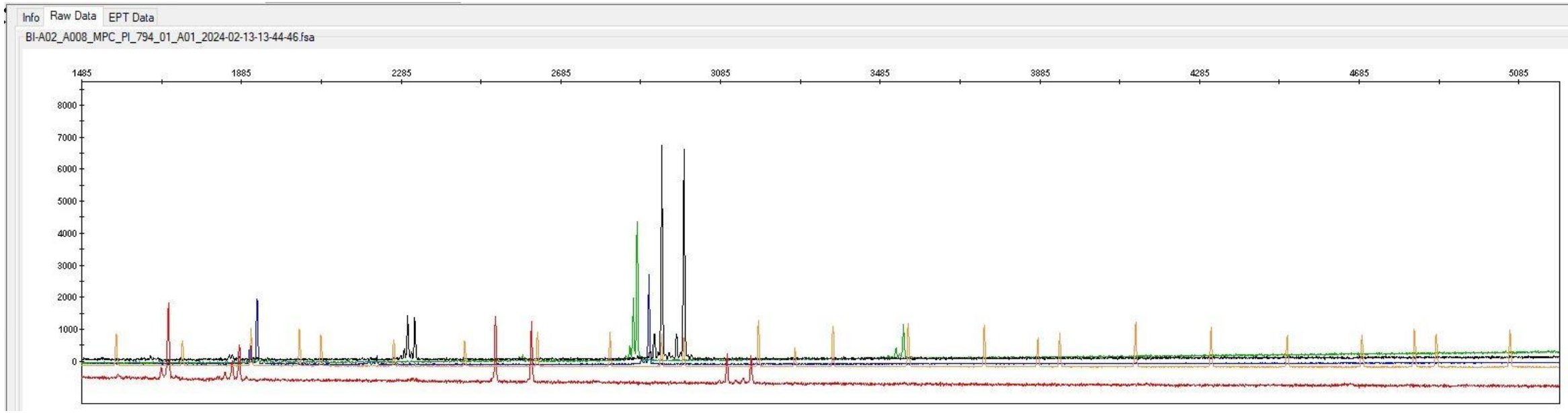
Raw data - evaluation



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Output from the automatic analyzer

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Example of parentage verification in horses

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Sire

Locus	Genotype
VHL20	LL
HTG4	KL
AHT4	HO
HMS7	MN
HTG6	JO
AHT5	MM
HMS6	MN
ASB23	IJ
ASB2	KM
HTG10	OO
HTG7	OO
HMS3	IM
HMS2	HM
ASB17	NO
LEX3	IH
HMS1	MM
CA425	JM

Descendant

Locus	Genotype
VHL20	LM
HTG4	KK
AHT4	HJ
HMS7	KM
HTG6	OO
AHT5	MN
HMS6	MP
ASB23	IK
ASB2	MQ
HTG10	IO
HTG7	OO
HMS3	MP
HMS2	KM
ASB17	NN
LEX3	HI
HMS1	JM
CA425	JN

Dam

Locus	Genotype
VHL20	LM
HTG4	KL
AHT4	HJ
HMS7	KK
HTG6	GO
AHT5	NN
HMS6	PP
ASB23	KS
ASB2	OQ
HTG10	IM
HTG7	OO
HMS3	PP
HMS2	KL
ASB17	NN
LEX3	II
HMS1	JJ
CA425	NN

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Thank you for your attention!

*This presentation has been supported by the Erasmus+ KA2 Cooperation Partnerships grant no. **2021-1-SK01-KA220-HED-000032068 "Innovation of the structure and content of study programs in the field of animal genetic and food resources management with the use of digitalisation - Inovácia obsahu a štruktúry študijných programov v oblasti manažmentu živočíšnych genetických a potravinových zdrojov s využitím digitalizácie"**. The European Commission support for the production of this presentation does not constitute an endorsement of the contents which reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.*

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