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Genetic test for identification of parents (parentage testing)

Lecture

Modul no. 1: Precision livestock farming

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Basic terms

parentage: parenthood

paternity: fatherhood

Individual identification: determining that the DNA sample belongs to a specific individual (molecular fingerprinting)

Genetic type: a set of genotypes (alleles) characteristic and unmistakable for everyone, also known as *genetic profile*

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Parentage testing and individual identification in animals

- importance - verification of origin and identification

- livestock in CZ (it varies by country)

    - pig: determining of the genetic type (identification) of boars in breeding farms

    - cattle: genetic type before selection for breeding (bull breeding), verification and certification of the origin of breeding bulls

    - horse: verification and certification of the origin of foals after insemination (always for English thoroughbred, even if there is no insemination)

    - other species: dog, exotic birds, protected animals CITES (origin and identification)

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Importance

Importance in animal genetics, forensic genetics and medicine

Animals:

- frequent errors in records
- incorrect parentage data to estimate breeding value
- you buy an animal that does not belong to the declared (quality) parents

Human:

- paternity disputes
- identification of the offender, or victims
- transplantation and other medical procedures where kinship is decisive

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Development and use of methods

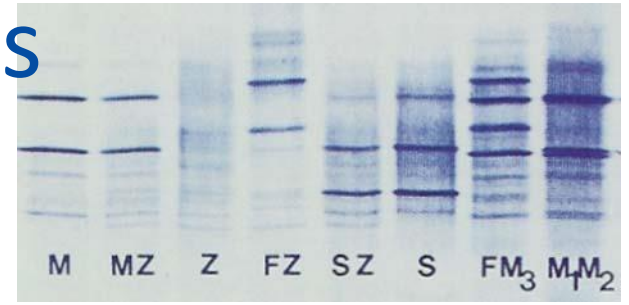
Previously: blood group polymorphism and protein

New: DNA variability (DNA fingerprinting)

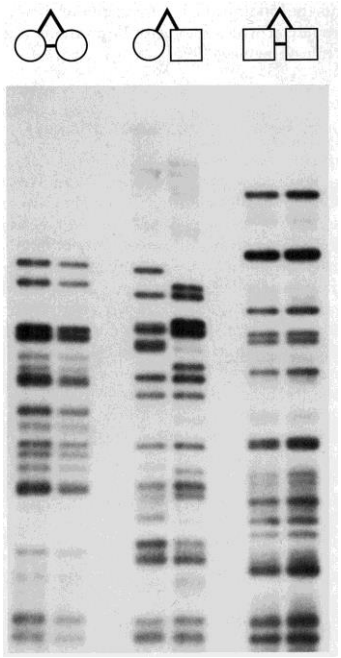
- VNTR (variable number of tandem repeats, minisatellites)

Currently:

- STR (short tandem repeats, microsatellites)
- SNP (latest, increasingly used)



protein IEF



VNTR

Requirements:

- high reliability of rejection and (or) confirmation of parentage
- relatively easy and quick laboratory testing
- high reproducibility
- the lowest price

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Microsatellites

- short tandem repetitions composed of mono, di, tri or tetra nucleotide motifs
- highly polymorphous ⇨ different alleles (5-20)

  e.g. $(GC)_n$ n = 5-25 repetitions

- lenght polymorphism - i.e. if the MS sequence is amplified by PCR, we detect by electrophoresis a different fragment size (allele) due to a different number of repetitions
- the allele designation is therefore the base pair size

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Mikrosatelites

$(GC)_n$
PCR size

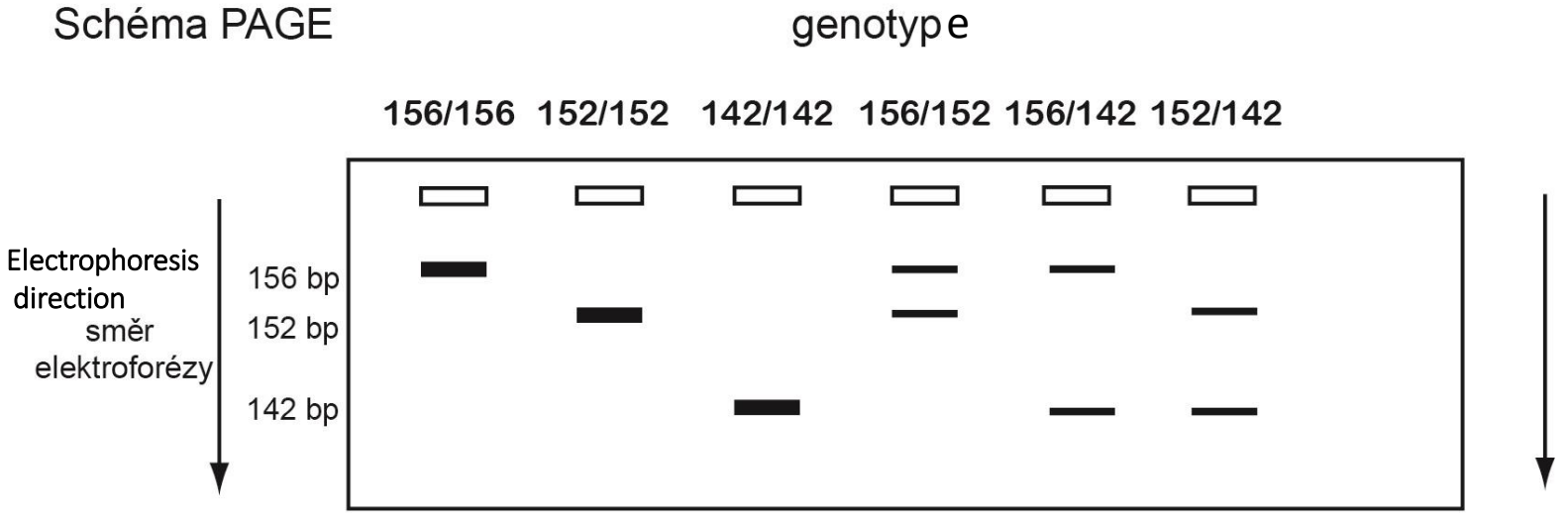
↓primer forward mikrosatelite primer reverse ↓

5´CATTGAATCGGTATCAT$(N)_{60}$AGCGCGCGCGCGCGCGCT$(N)_{40}$ACGTTAATTCATTCGTG 3´ n=8 152 bp

5´CATTGAATCGGTATCAT$(N)_{60}$AGCGCGCT$(N)_{40}$ACGTTAATTCATTCGTG 3´ n=3 142 bp

5´CATTGAATCGGTATCAT$(N)_{60}$AGCGCGCGCGCGCGCGCGCGCT$(N)_{40}$ACGTTAATTCATTCGTG 3´ n=10 156 bp

PAGE scheme
Schéma PAGE



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Used microsatellites

Examples:

Pig (10x) – S0068, S0107, SW24, SW840, SW353, SW936, SW353, S0070, SW72, TNFB

Cattle (11x) – TGLA53, TGLA122, TGLA126, TGLA227, ETH3, ETH10, ETH225, BM1824, BM2113, INRA23, SPS115

Horse (17x) – VHL20, AHT4, HTG4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS2, HMS3, ASB17, LEX3, HMS1, CA425

Dog (18x) – AHTk211, CXX279, INU055, REN169018, REN54P11, AHT137, AHTh260, AHTk253, INRA21, REN169D01, AHT121, AHTh171, FH2054, REN162C04, REN247M23, FH2848, INU005, INU30

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Additional panels

- raptor 5 MS
- goat 14 MS
- sheep 13 MS
- deer, cats, beavers, nutria, hens, geese, llamas

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Analysis procedure

1. Isolation of the genomic DNA of the tested individual
2. Multiplex PCR - amplification of a panel of microsatellites
3. Fragmentation analysis using fluorescence capillary electrophoresis
4. Determination of peak sizes
5. Determination of alleles of individual microsatellites: discrimination by color and known range of alleles
6. Evaluation - verification of parentage

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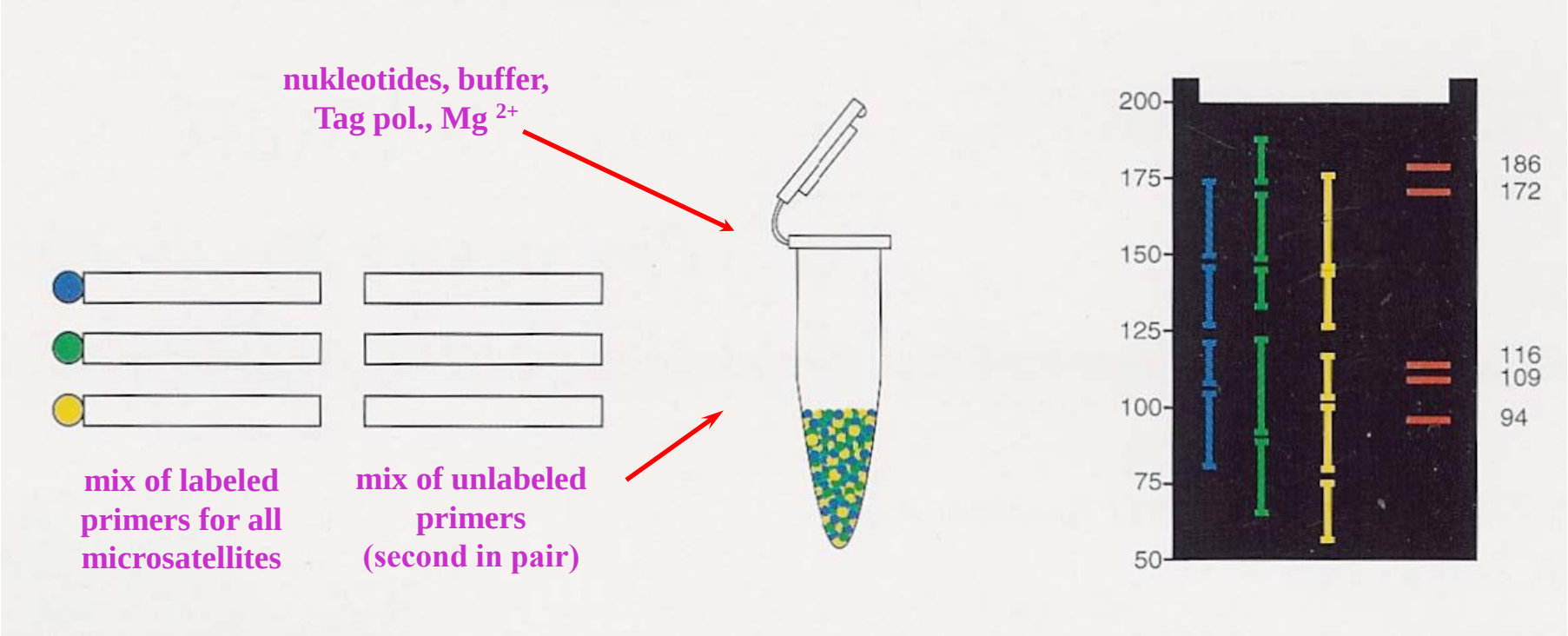
Multiplex PCR



the allele size ranges of different microsatellites in the same color must not overlap

Fluorescent colours(G5):

FAM - blue

VIC - green

NED – yellow

PET - red

LIZ – orange (size standard)

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Fragment analysis

Fluorescence capillary electrophoresis on a genetic analyzer - separation of microsatellites according to size

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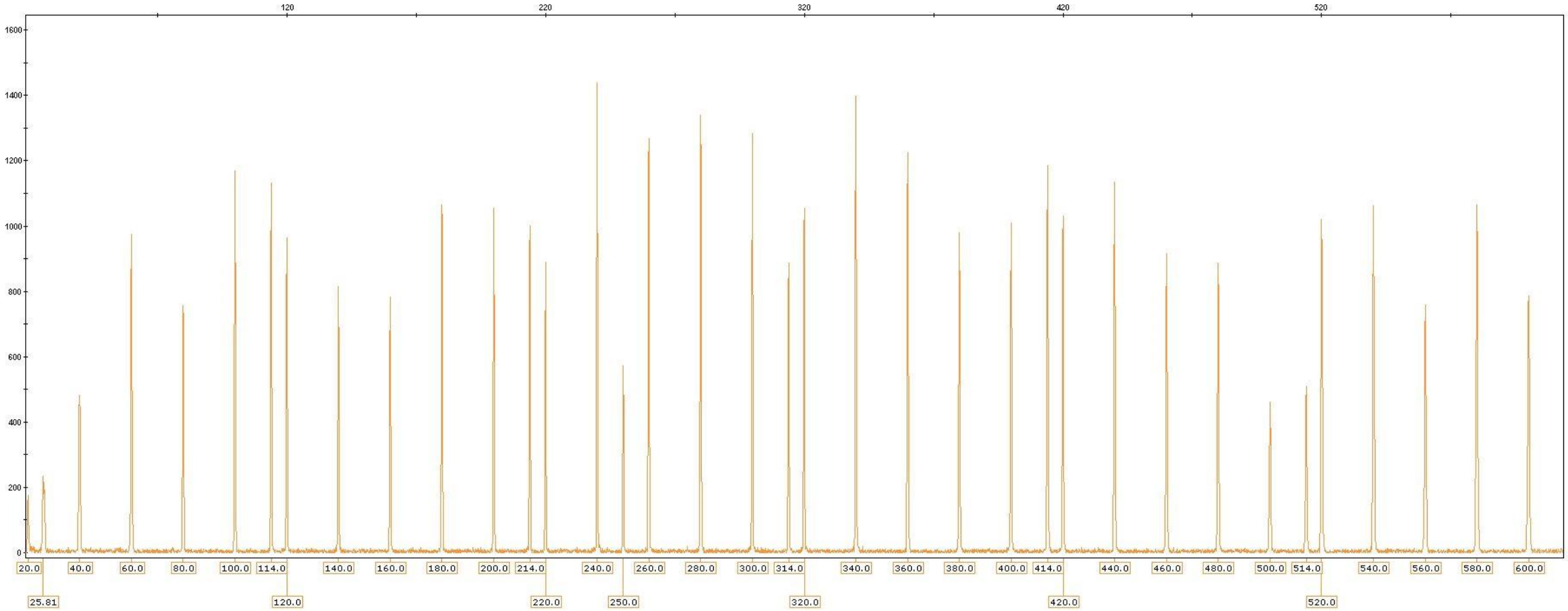
Size standard



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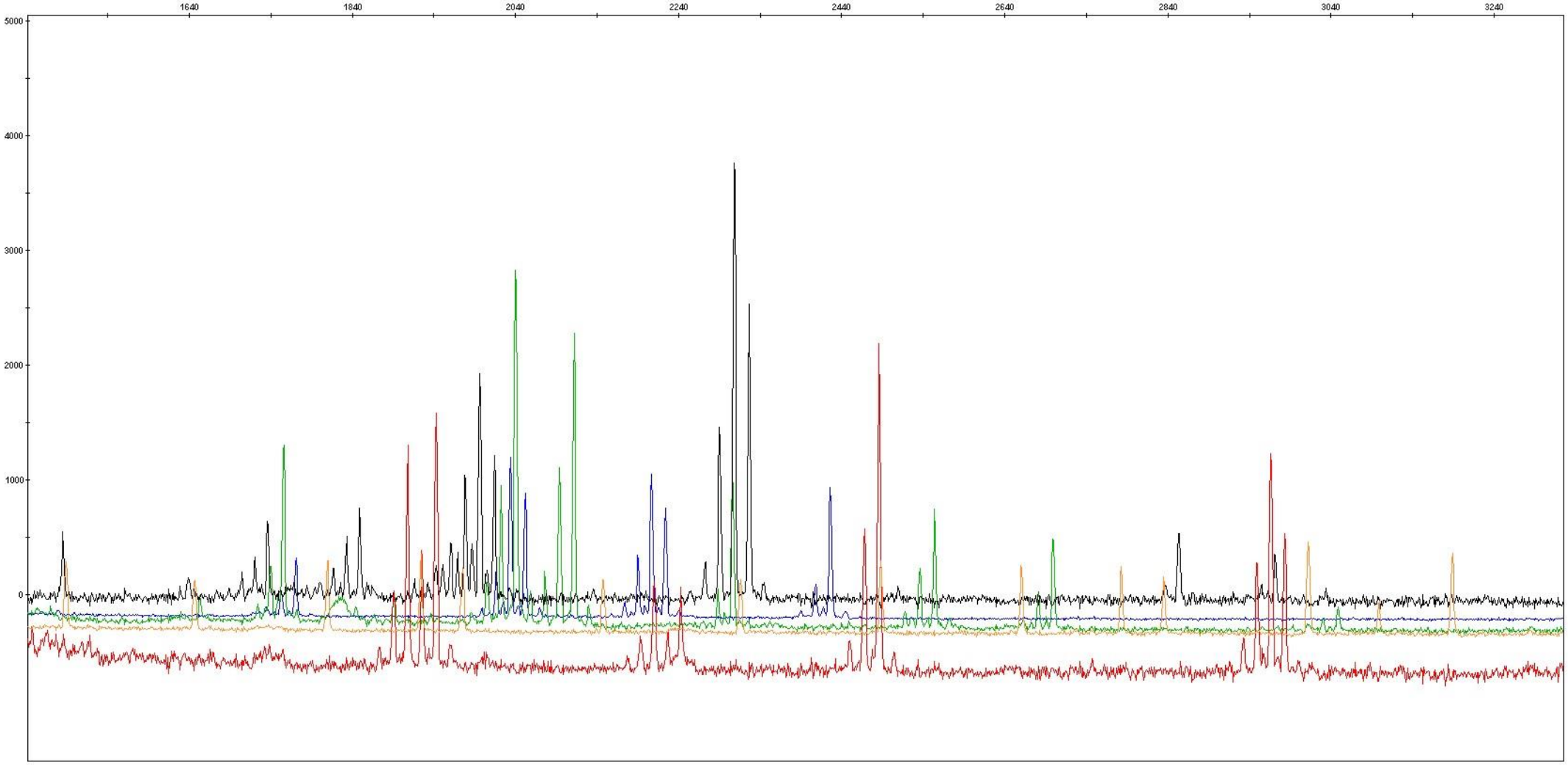
Raw data - capillary electrophoresis



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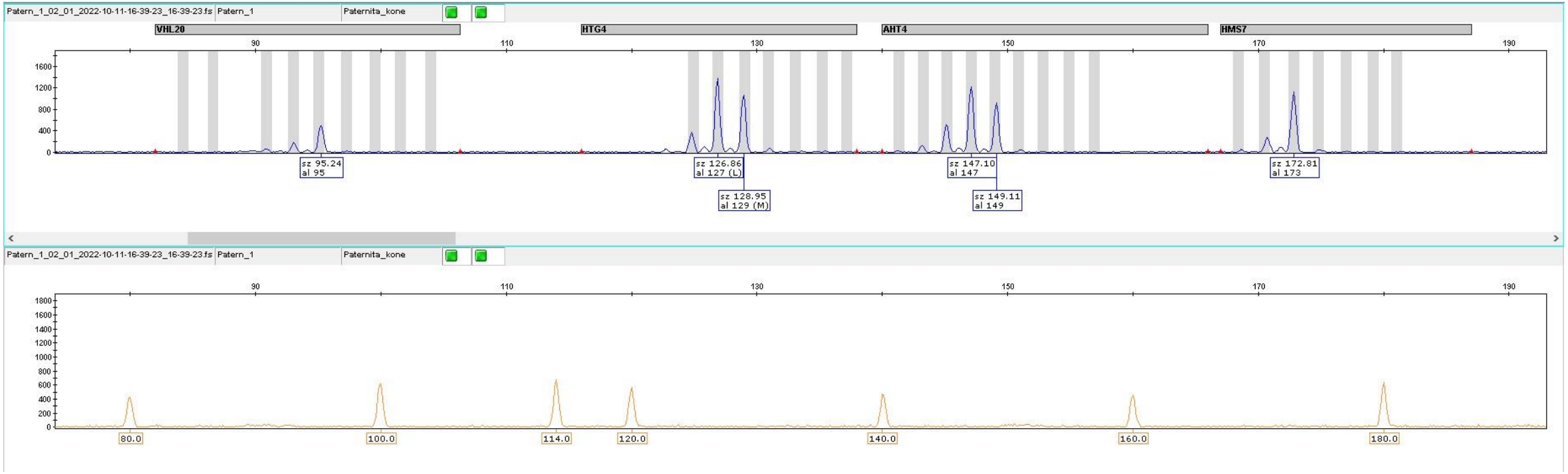
Evaluation - genotypes

Example of one of the colours (6-FAM) and size standard (LIZ)



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Partners:

Thank you for your attention!

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