Impact of alternative RNA splicing on β-casein transcripts in mare's mammary gland

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Photo: K. Potočnik

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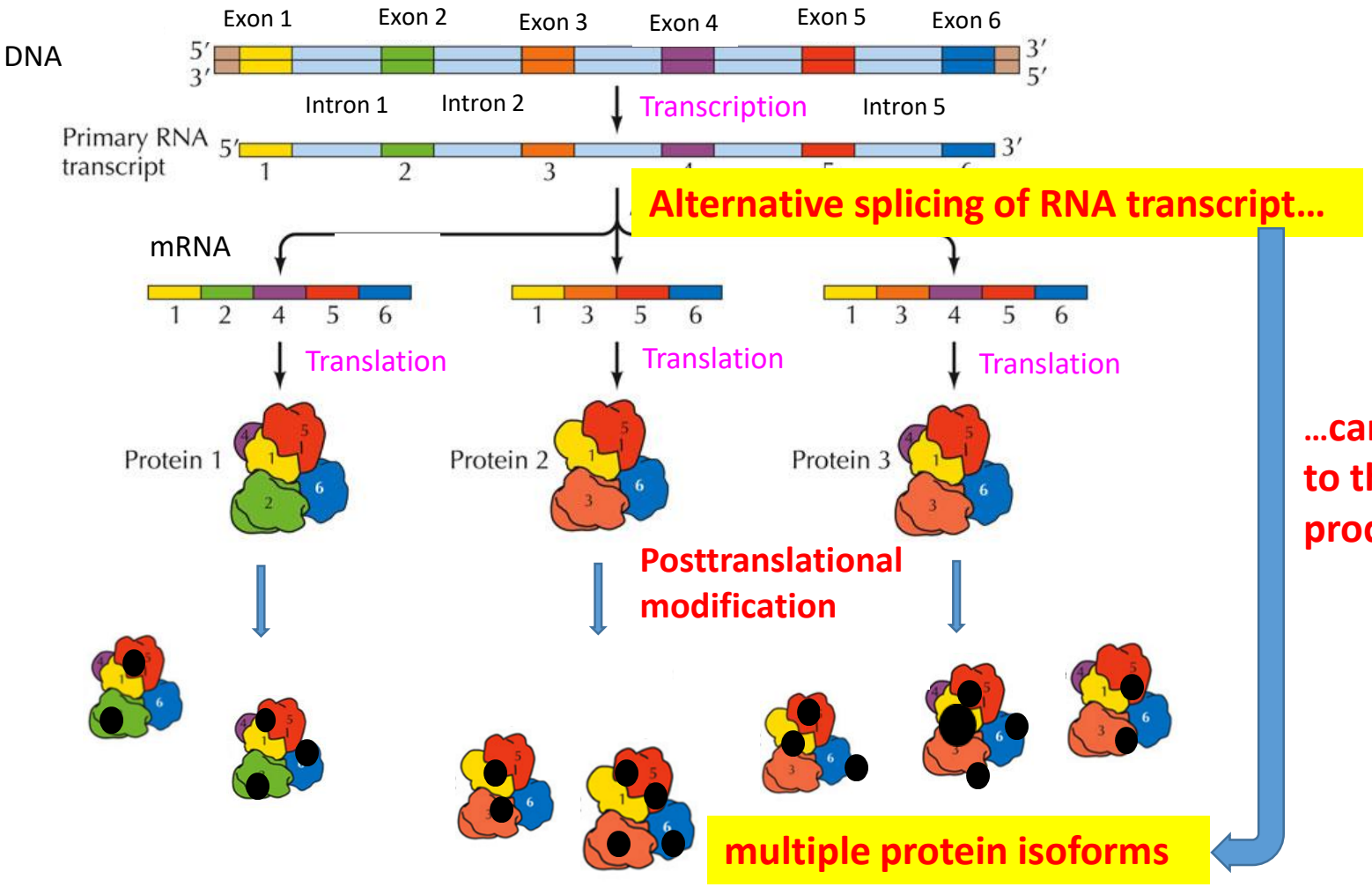
One gene = one protein?

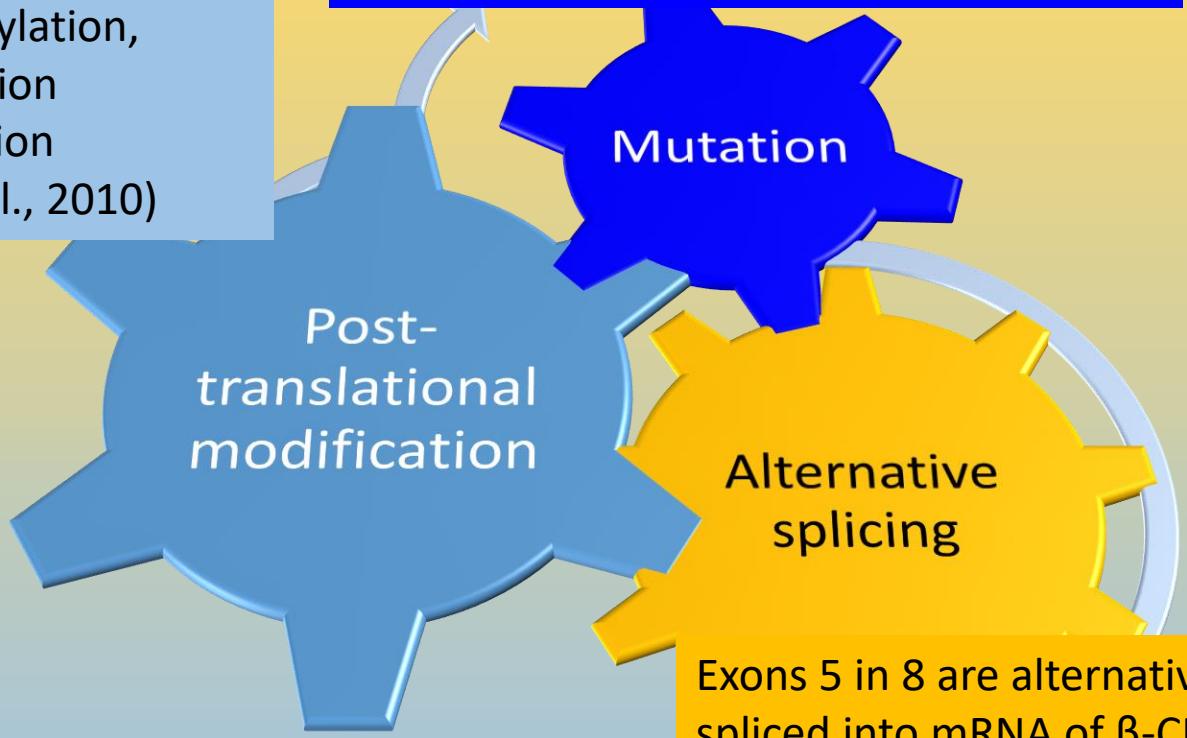


The mechanism of equine casein gene expression

Post-translational modifications:
- phosphorylation,
- glycosylation
- deamidation

(Matéos et al., 2010)

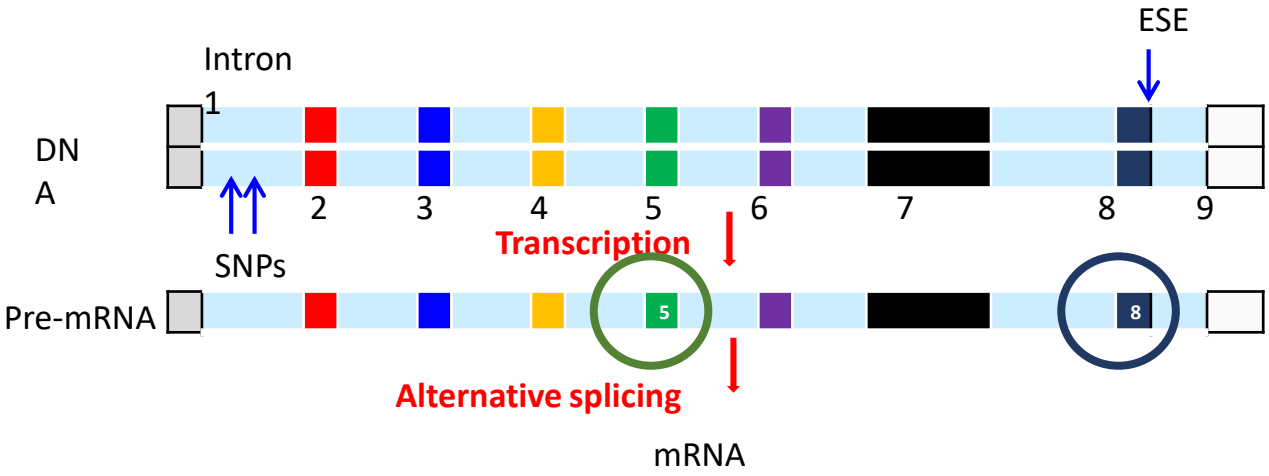
Polymorphism effects equine casein gene expression (Lenasi et al., 2006)



Exons 5 in 8 are alternative spliced into mRNA of β-CN and determined 3 different splicing patterns (Lenasi et al., 2006)

The result of alternative exon splicing of equine β-casein (β-CN) gene transcripts are different variants of mRNA for β-CN

(Lenasi et al., 2006)



Splicing pattern (1)

Full-length variant β-CN:

Splicing pattern (2)

Alternative variants β-CN:

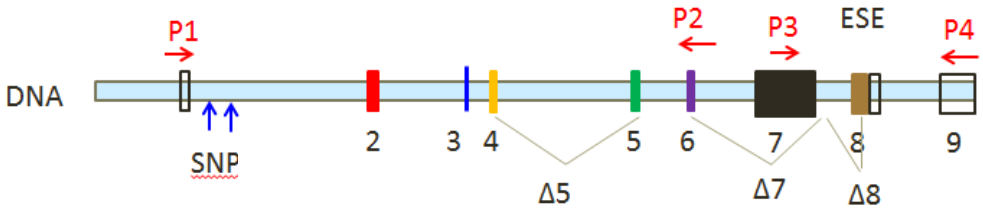
Splicing pattern (3)

Short variant β-CN:

Translation

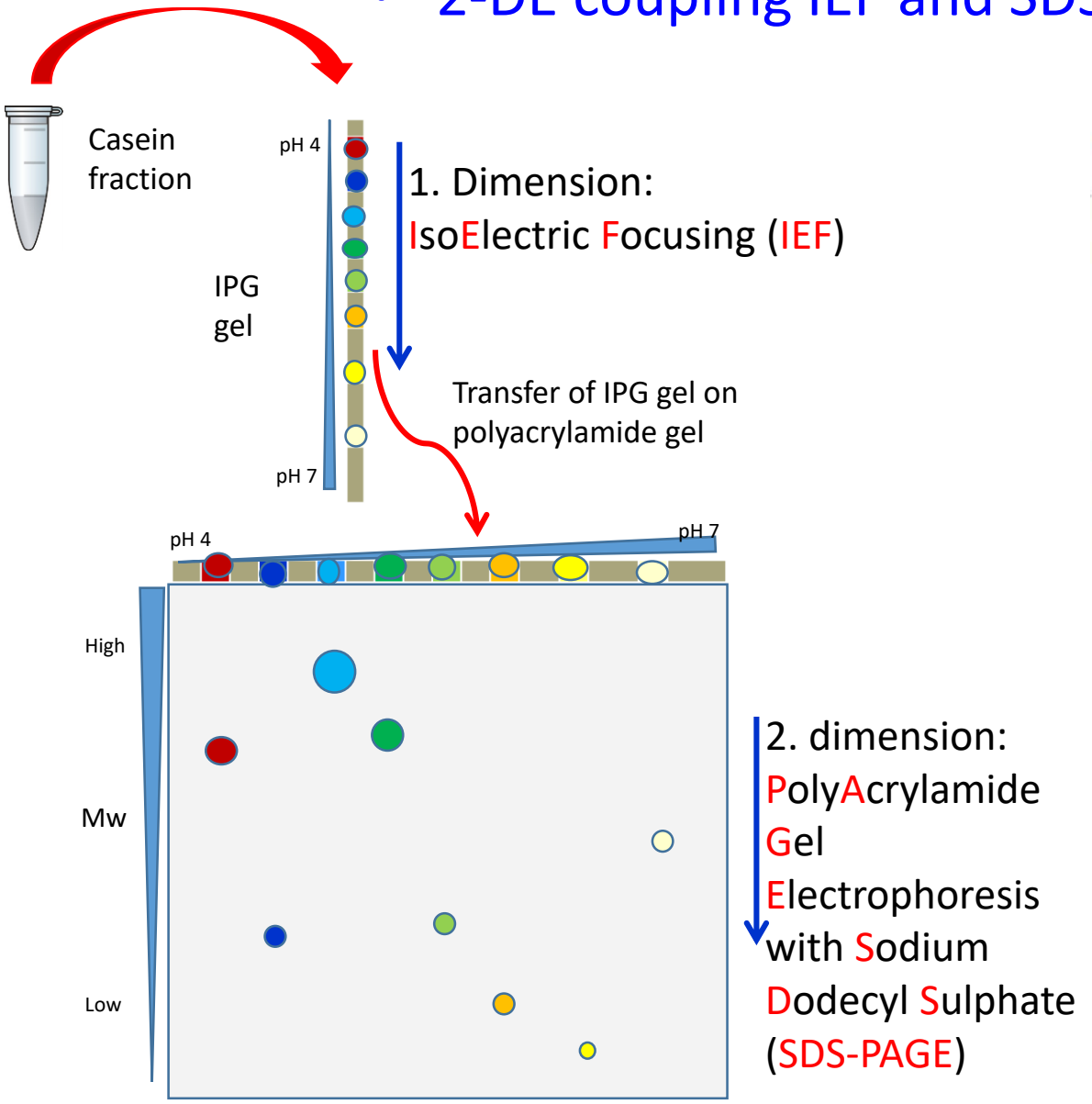
The aim of the study: Whether a correlation between alternative RNA splicing pattern and equine β-CN variants on protein profile can be established?

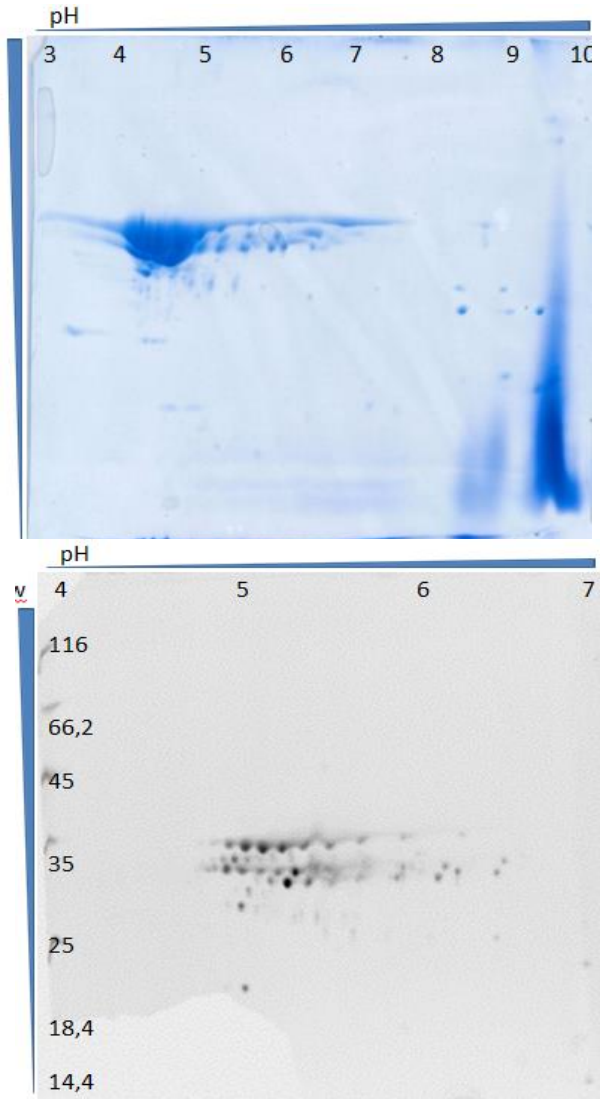
- 19 milk samples from Lipizzan mares / 19 blood samples
- PCR amplification of DNA to determine the intron 1 polymorphisms
- RT PCR amplification of cDNA fragments from the region of two known weak exons



- The evaluation of DNA fragments sizing and quantification by the Agilent 2100 Bioanalyser was performed

- 2-DE coupling IEF and SDS-PAGE (Görg, 2007)





Material and methods

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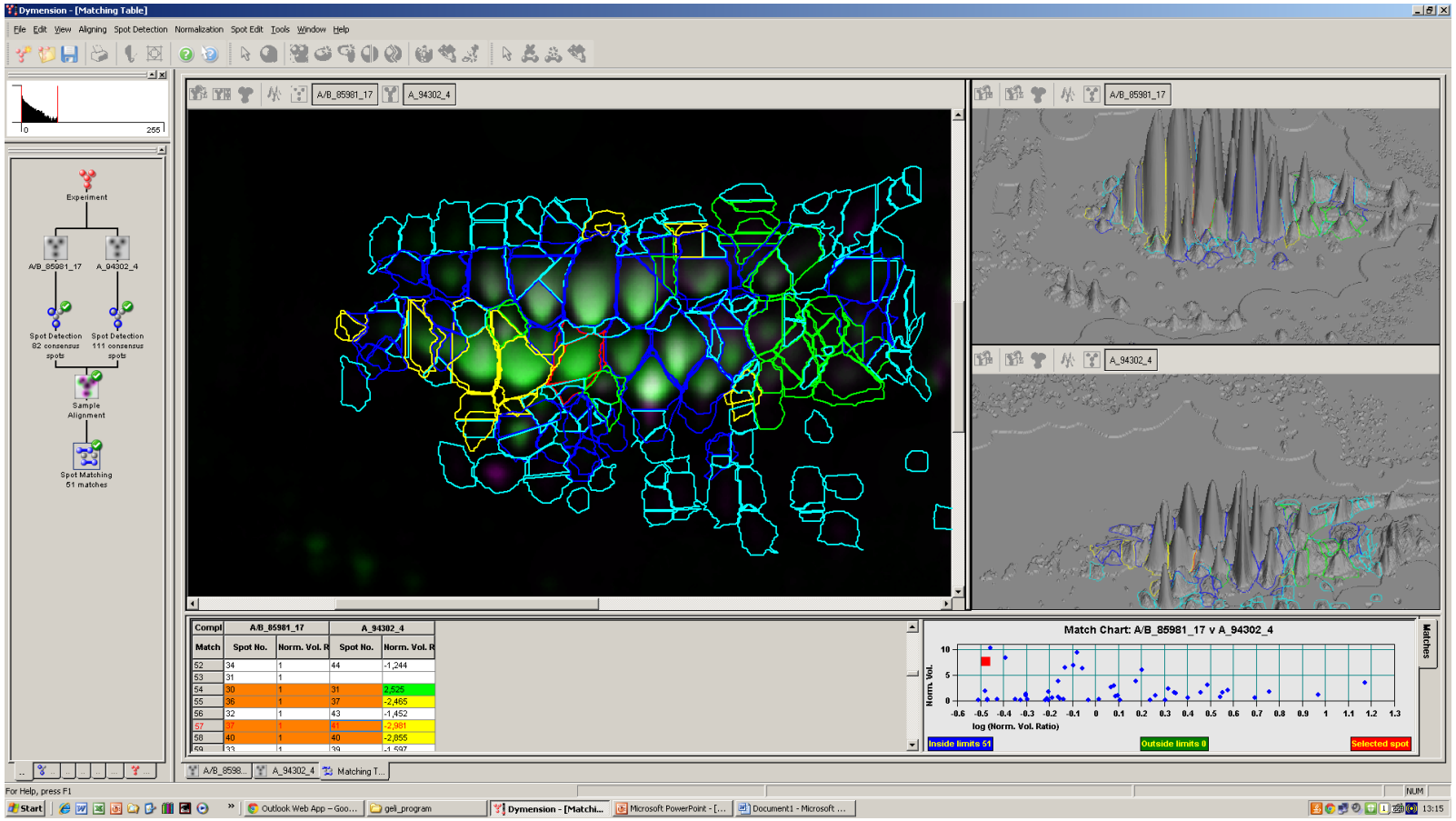
- Gel staining with Sypro Ruby Protein Stain
- 2-DE protein gel images were analyzed using image processing 2-D Dymension programe (Syngene)



- Protein identification according Edman sequencing

Material and methods

We confirmed two SNPs in intron 1 of β-CN gene and their connection with 3 different splicing patterns of weak exons (Lenasi et al., 2006)

Polymorphic site	Single nucleotide polymorphism		
Intron 1: 119	TGA**A**GAA	TGA**A**/**T**GAA	TGA**T**GAA
Intron 1: 269	AAA**T**GTC	AAA**T**/**C**GTC	AAA**C**GTC

Haplotype A-T — **14 mares** — **(1)**

Haplotype A/T-T/C — **4 mares** — **(2)**

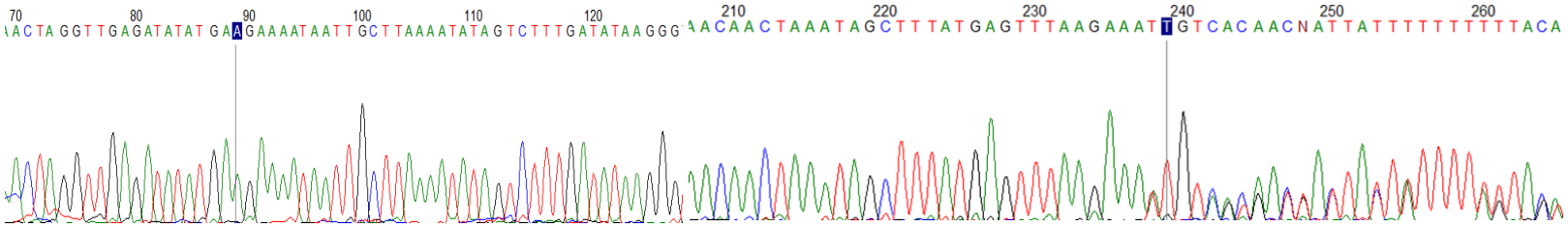
Haplotype T-C — **1 mare** — **(3)**

Splicing pattern:

With exons 5 and 8
Full length variant

Alernative variants

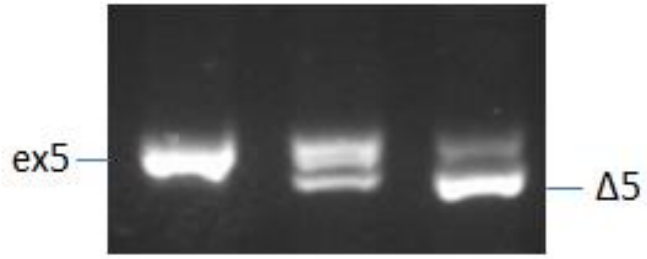
Skipped exons 5 and 8
Short variant



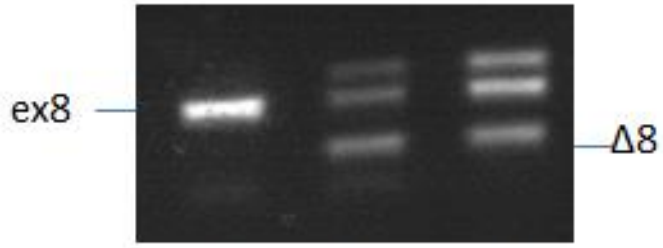
Results

cDNA fragments quantity

β-CN exon 5

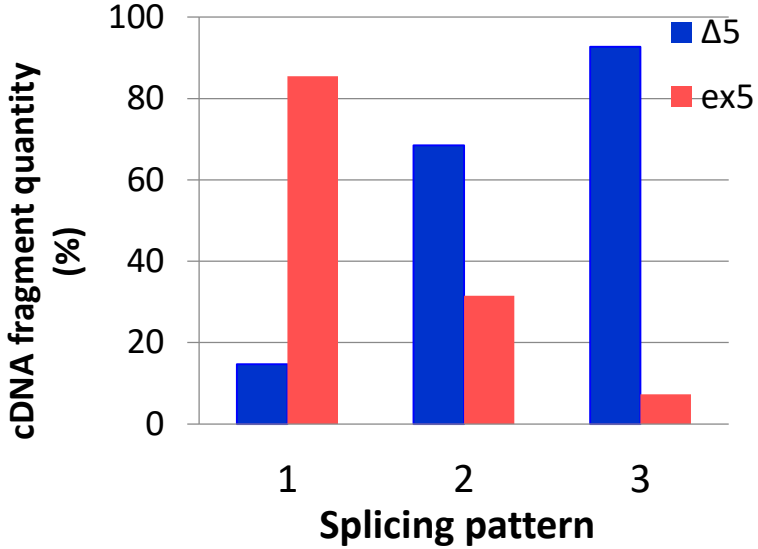


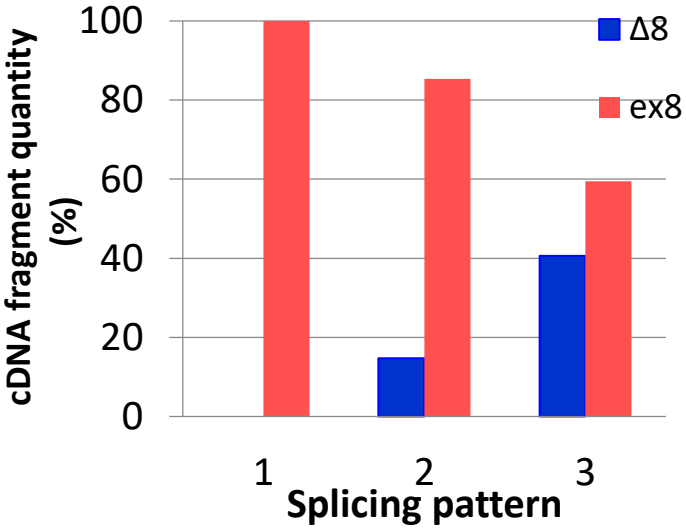
β-CN exon 8



Splicing pattern:	(1)	(2)	(3)
Δ5(%)	14,7	68,5	92,7
ex5(%)	85,5	31,5	7,3

	(1)	(2)	(3)
Δ8(%)	0	14,7	40,6
ex8(%)	100	85,3	59,4

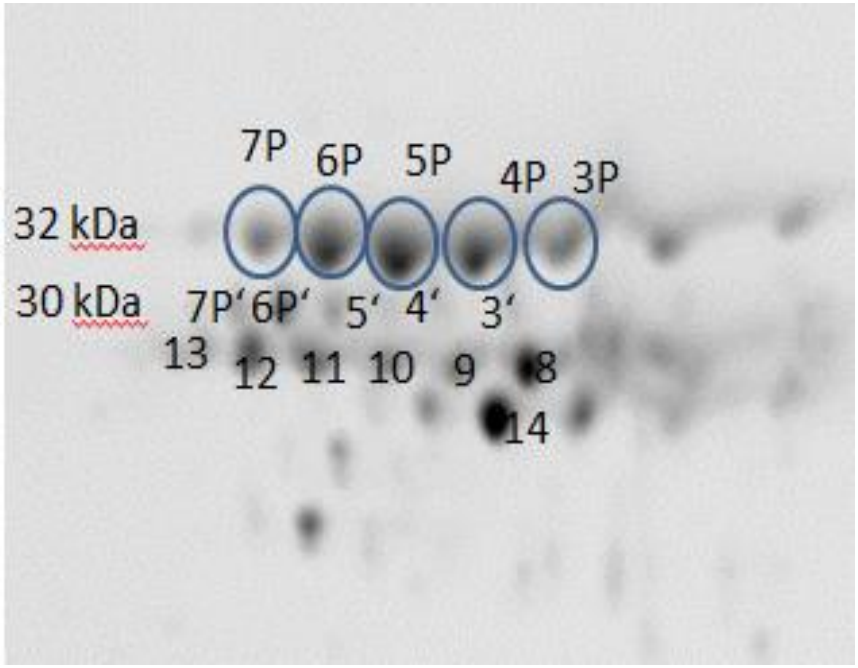




Two β-CN variants were recognized in 2-DE gels

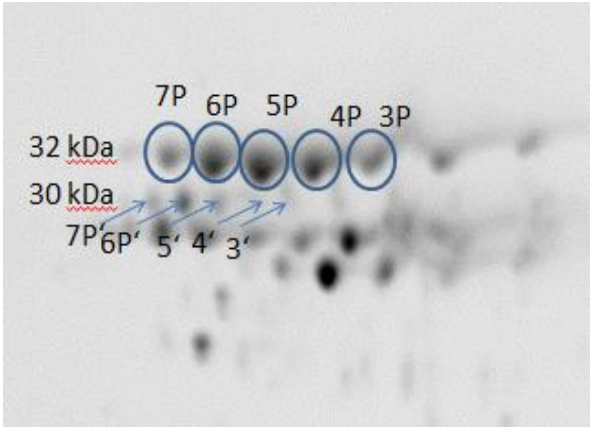
- full length β-CN
- β-CN$^{\Delta 5}$ without exon 5

…..and their phosphorylated variants

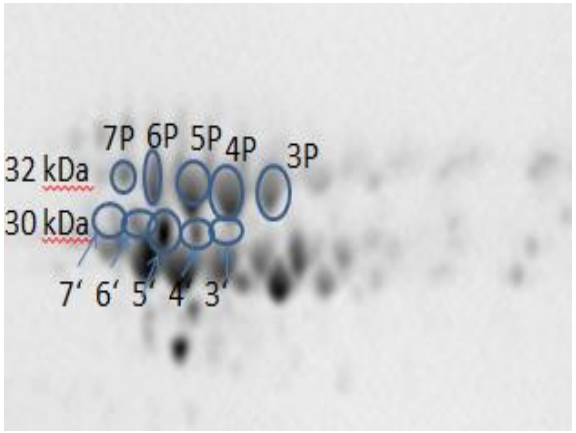


- Spots 3P, 4P, 5P, 6P in 7P represented phosphorylated variants of β-CN
- Spots 3‘, 4‘, 5‘ 6P‘ in 7P‘represented phosphorylated variants of β-CN$^{\Delta 5}$
- Spots from 8 to 14 represented phosphorylated variants of α_{S1}-CN

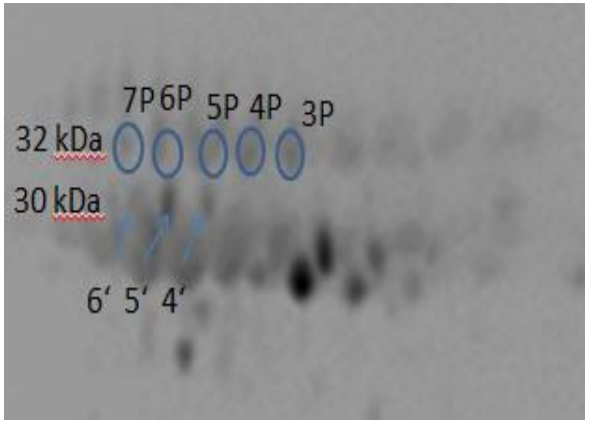
Is it possible to interpret the genotype of the mare on the basis of 2-DE casein profile?



Splicing pattern (1)
Full length β-CN
Haplotype A-T



Splicing pattern (2)
Alternative variant
Haplotype A/T-T/C

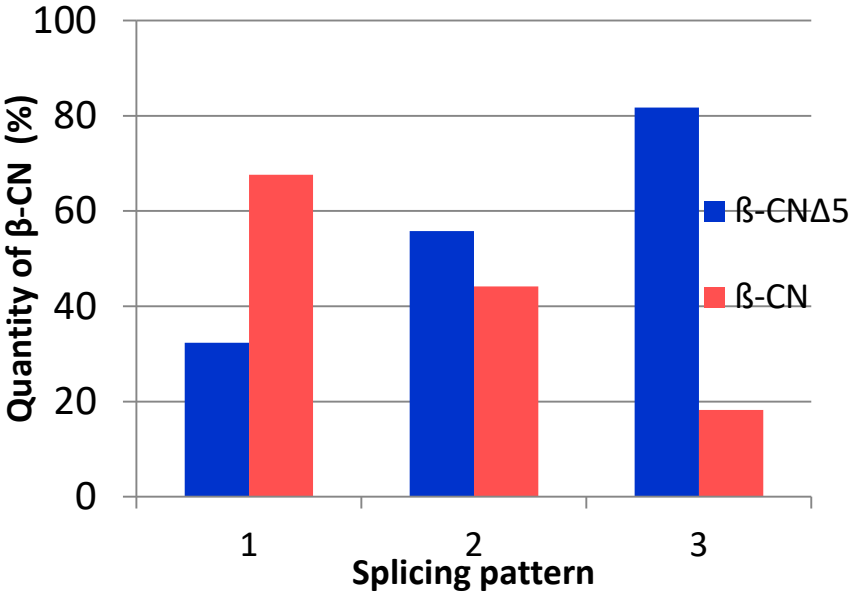


Splicing pattern (3)
Short variant β-CN
Haplotype T-C

We could interpret the splicing pattern of weak exons on the basis of the calculated quantity of individual β-CN variant

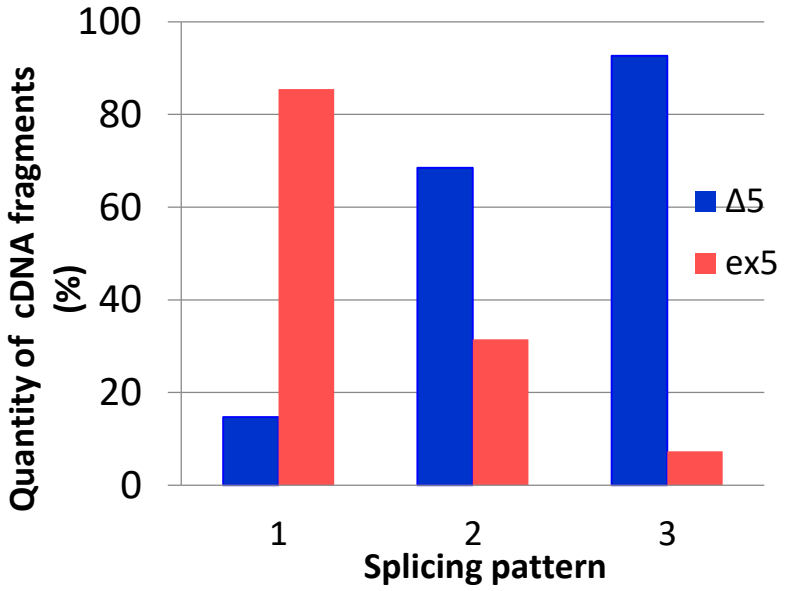
PROTEIN PROFILE:

β-CN



cDNA fragments:

cDNA of β-CN



β-CN:β-CNΔ5	2,08	0,8	0,2
	6	0,4	0,1

Conclusions

- In milk samples of Lipizzan horse prevailed full length β-CN variant.
- Full length ß-CN and short ß-CN$^{\Delta 5}$ variant can be characterized in 2-DE gel.
- If we know the ß-CN : ß-CN$^{\Delta 5}$ quantity ratio, the splicing pattern of weak exons can be presume.
- Further research has to be done.


Photo: K. Potočnik

Thank you for your attention!