

# PRNP genotyping in sheep with scrapie disease – cases from Poland

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## Background

Scrapie is one of the fatal, prion diseases and belongs to the group of transmissible spongiform encephalopathies (TSEs). Scrapie affects sheep and goats and active surveillance for this disease is obligatory in Poland. There are two scrapie types: classical and atypical (NOR98). They differ in aetiology and genetics. It has been shown, that ovine *PRNP* gene polymorphism influences the scrapie susceptibility. Alleles coding alanine (codon 136) and arginine (codons 154 and 171) are associated with classical scrapie resistance, allele coding valine (codon 136) is associated with classical scrapie susceptibility and allele coding phenylalanine (codon 141) is associated with increased atypical scrapie susceptibility. On the basis of the *PRNP* genotype, classical scrapie risk classes were established (from NSP1 to NSP5, with the increasing risk of classical scrapie occurrence). The aim of the study was to confirm the differences in genetic background of atypical and classical scrapie in sheep scrapie cases diagnosed in Poland. In years 2009–2023, 124 scrapie cases were identified in Poland (fig. 1). Among them, 87 were Polish origin (84 NOR98 in sheep, 2 NOR98 in goats, 1 classical scrapie case in goat). Goat cases were not included in this study.

## Material and Methods

Since 2009, we have sequenced the *PRNP* gene coding region of all sheep with scrapie diagnosed in Poland (Western-Blot method): 18 cases with classical scrapie and 103 cases of atypical scrapie. All classical scrapie individuals were imported to Polish slaughterhouses. We have never found the classical scrapie among sheep of Polish origin.

We used BigDye® Terminator v3.1 Cycle Sequencing Kit (*Applied Biosystems*) and capillary electrophoresis (3130xl and 3500xl Genetic Analyzers) to perform the analysis. The data was analysed in BioEdit Sequence Alignment Editor. For one individual we couldn't obtain the complete genotype due to low DNA quality. It was excluded from the study. The codons 136, 141, 154 and 171 were also genotyped using TaqMan Assays and StepOne Plus Real-time PCR System (*Applied Biosystems*) with method accredited according to ISO 17025:2018 and tested in EURL-TSE genotyping schemes.

## Results and Discussion

We found 11 different genotypes in atypical scrapie group (AS) and 10 in classical scrapie group (CS) (fig. 2). The most frequent genotypes in atypical and classical scrapie sheep were A<sub>136</sub>L<sub>141</sub>R<sub>154</sub>R<sub>171</sub>/ALHQ and ALRQ/ALRQ, respectively. Allele VLRQ, connected with the highest susceptibility to classical scrapie, was found in 5 classical scrapie sheep and in one atypical scrapie case (AFRQ/VLRQ) (fig. 3). Allele ALRR, associated with classical scrapie resistance, had frequency 15,69% (AS; 16 sheep) and 22,22% (CS; 1 sheep). It has resulted in classical scrapie risk groups frequencies (fig. 4). Allele AFRQ, connected with atypical scrapie, was observed in 30,39% AS sheep and in 11,1% CS sheep, what significantly exceeds this allele frequency observed in healthy sheep (2–6% according to different authors). We have also discovered other polymorphisms in *PRNP* gene: M112T, H143R, N176K, R211Q, E224K and three silent mutation in codons R231R, L237L, L253L (fig. 5). The different sheep origin (country and breed) may partially explain the *PRNP* gene variability.

The atypical scrapie cases were found among different Polish sheep breeds (e.g. Polish Merino, Polish Mountain Sheep, Wrzosówka, Pomorska sheep, Polish Lowland sheep, Romanov sheep, Świniarka, Suffolk, Wielkopolska). Therefore, *PRNP* genotypes should be monitored regularly in sheep breeds with atypical scrapie occurrence. Our study confirmed the differences in atypical and classical scrapie genetic background.

## Conclusions

Polish sheep breeds are free from classical scrapie.

Our study confirmed the differences in atypical and classical scrapie genetic background.

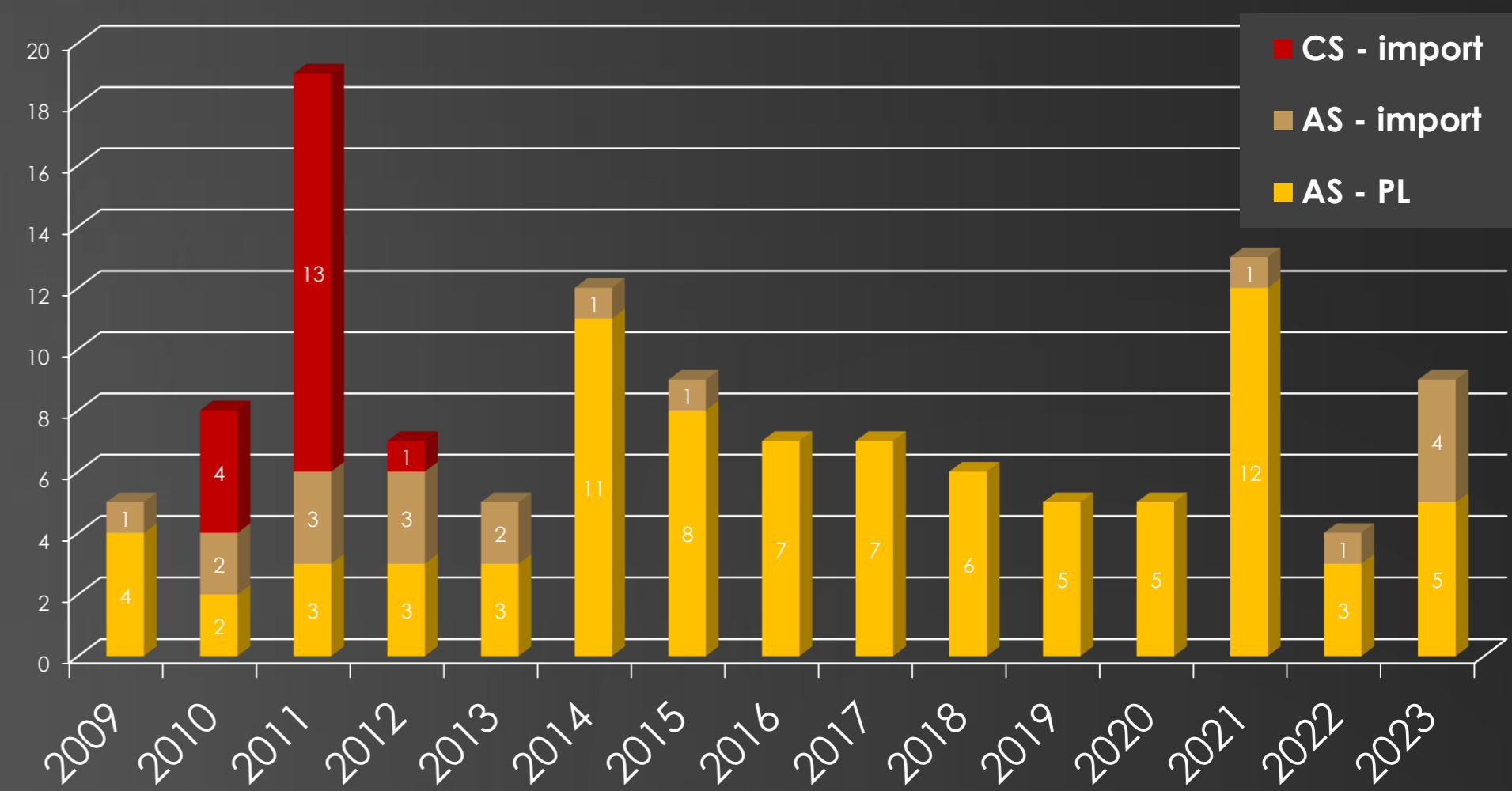


Figure 1. Number of diagnosed scrapie cases genotyped in Poland; CS – classical scrapie AS – atypical scrapie cases; import – sheep from import; PL – Polish sheep.

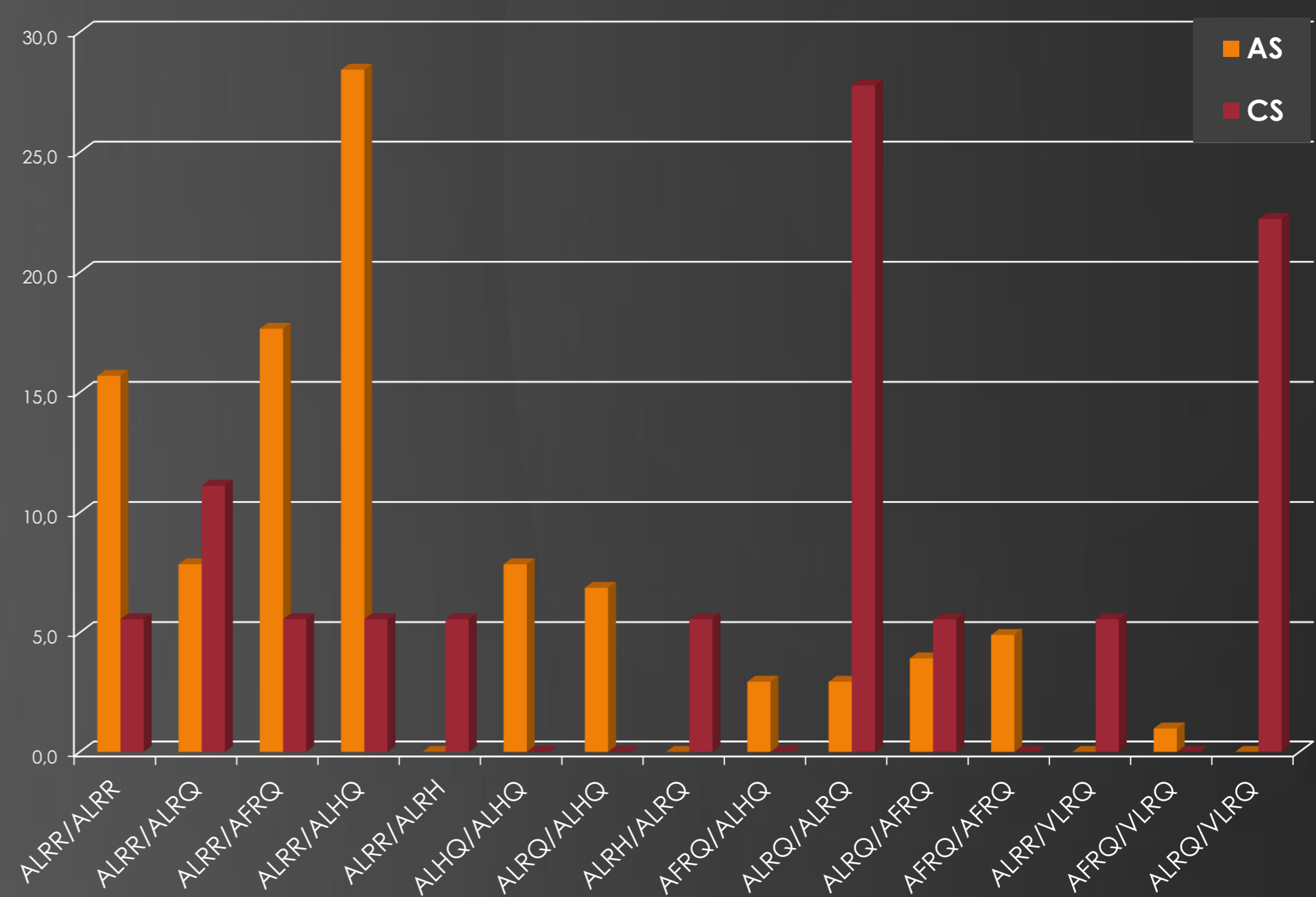


Figure 2. The *PRNP* genotype frequencies [%] in the atypical (AS) and classical (CS) scrapie groups.

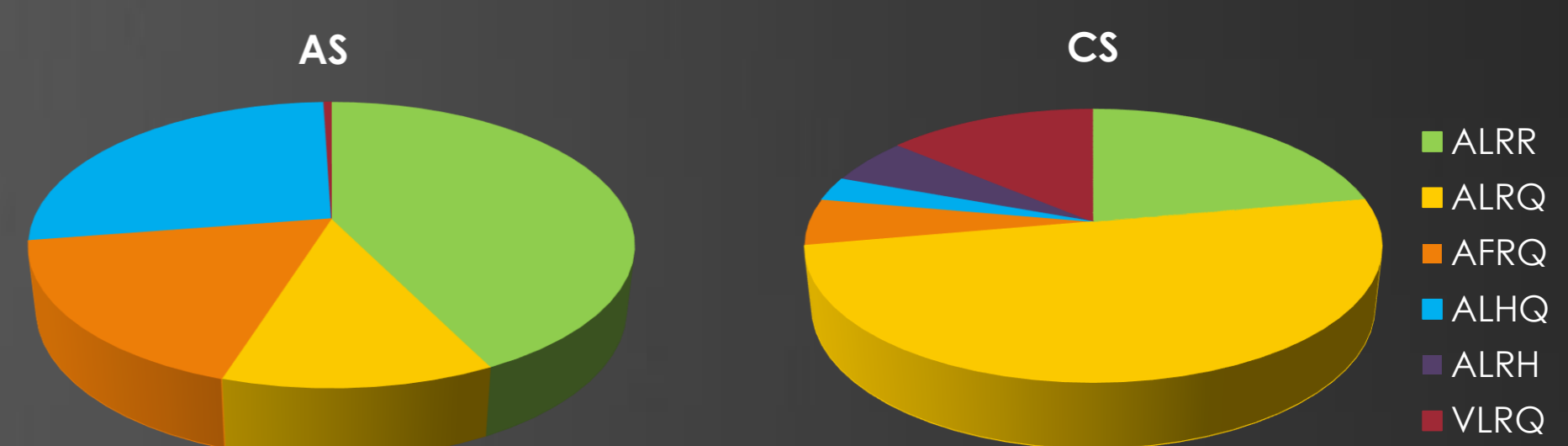


Figure 3. The *PRNP* allele frequencies [%] in the atypical (AS) and classical (CS) scrapie groups.

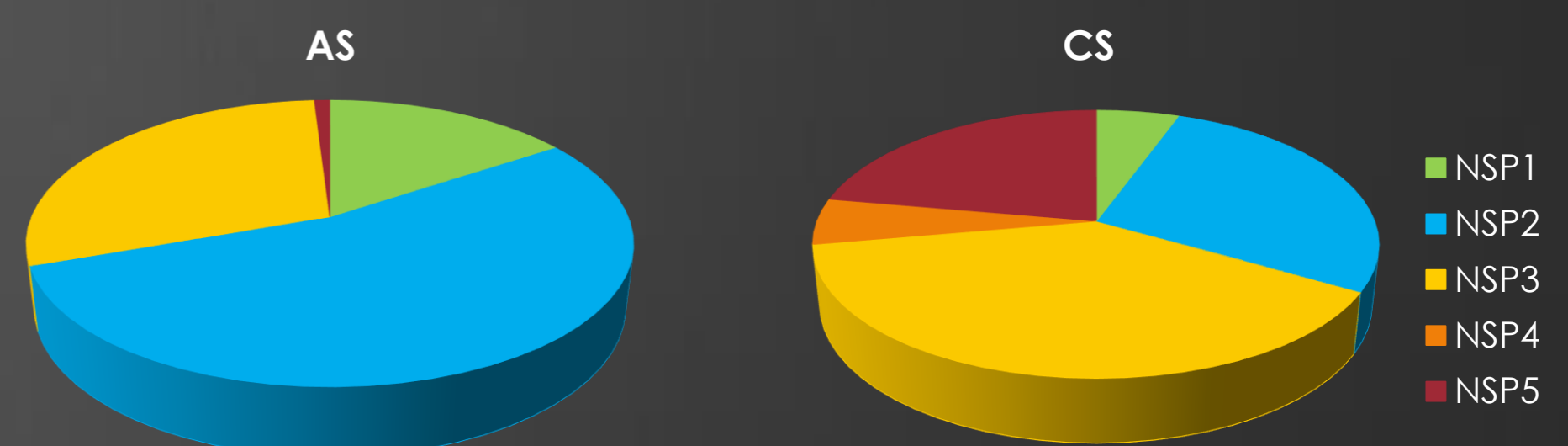


Figure 4. The frequency [%] of animals belonging to classical scrapie risk groups; AS – atypical scrapie; CS – classical scrapie.

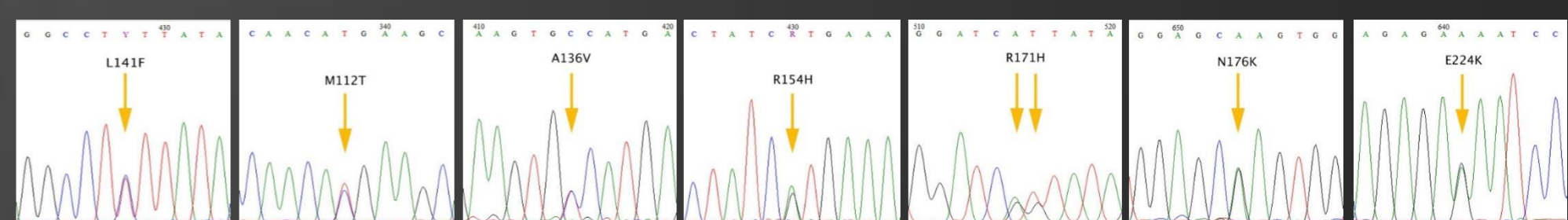


Figure 5. Fragments of chromatograms from different *PRNP* gene polymorphic sites.



Pomorska breed



Wrzosowka breed



Romanowska breed