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Towards
the veterinary
diagnostics
of the
future

Main topic: Animal Health

Developement of a novel and efficient equine SNP genotyping method and its utility in studying the prevalence of genetic diseases, coat color and various traits of interest for equine industry

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Next Generation Sequencing (NGS) have enabled the identification of molecular markers widely used to detect causal mutations in horses for genetic disorders or traits as coat color. The aim of this study was to develop and evaluate a SNP custom panel as a potential useful tool for equine genotyping. This panel was designed with support from Thermo Fisher Scientific, Waltham, Ma, USA and included 108 targets (33 genetic disorder markers, 40 coat color markers and 35 gait traits). For all targets, samples of known genotype were analyzed (no mutation, heterozygoty or homozygote mutation) with the AgriSeq targeted GBS (Genotyping by sequencing) solution. This method utilizes a highly efficient multiplexed PCR chemistry where hundreds of markers can be targeted and uniformly amplified in a single reaction.

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Briefly, after extraction of DNA from horse hair or blood, sample DNAs were normalized and a multiplexed PCR was performed with the custom primer panel. Librairies were prepared according to the AgriSeqTM HTS library kit and sequenced on the Ion GeneStudioTM S5 system. Genotyping was then carried out automatically by Torrent Variant Caller (TVC) on the Torrent Suite Software (TS).

For the 108 targets, sample results obtained with the custom panel were compared with the expected genotypes for which results were provided from reference laboratories or obtained after Sanger sequencing analysis. All results were consistent except for 2 markers for which a redesign was needed. Indeed, the custom panel could be if necessary amended or updated if other molecular markers of interest are recently highlighted.

This equine SNP genotyping method based on AgriSEQ technology resulted a cost-effective strategy to prevent the disease risk, to study the prevalence of traits and to improve the knowledge about horse's performance and abilities. This efficient tool could thus be easily used in veterinary laboratories for large-scale routine genetic analyses.