Routine genotyping of the *Gars*^{201R}: EMMA No. 87/88

PCR:

Primers were resuspended in a dilution of 10μM.

Gars exon 5 primer sequence:

Forward: **CACGTGCTTGCTCTAGCAAGA**

Reverse: **GTCTACCACTGAACACAGTCC**

25μl reactions contained 15-30ng of DNA, 10μM of forward and reverse primers, 22μl Megamix Blue PCR Master Mix (Microzone).

PCR conditions:

Stage	**Temperature (°C)**	**Time**	**Number of Cycles**
Initial denaturation	94	15 mins	1
Denaturation	94	30 seconds	34
Annealing	60	30 seconds	
Extension	72	30 seconds	
Final Extension	72	5 minutes	1

Restriction enzyme digestion:

The *Gars*^{201R} mutant gene changed a restriction site for the enzymes HaeII and HhaI. HaeII and HhaI restriction enzymes used were purchased from NEW England Biolabs (NEB). Restriction digest of PCR product were carried out according to the manufacturer's instructions. At least 1U enzyme/μg of DNA was used for a minimum of 1hr.
A protocol was designed for routine genotyping by PCR followed by RFLP analysis. The above PCR primers lie within intron 4 and intron 5 respectively, and thus span exon 5 of *Gars.* They amplify a 422 bp product. This amplicon was digested with HhaI to give fragments of 422bp (no restriction site, wildtype C3H and C57BL/6 *Gars* loci) and of 169bp and 253bp (*Gars*^{201R} mutant gene).