Genotyping of Hps5-KO mice

1. Our protocol of genotyping PCRs for Hps5-KO mice

This section describes our method we are routinely using for genotyping Hps5-KO mice.

1.1) Primer sequences:

Primer1: **HSP5-E2-S**

- Sequence: 5'- CAACCGTCATCTTTCTGGCTAAATG -3' (25-mer)

Primer2: **HSP5-E2-R**

- Sequence: 5'- CCACAAAACAAGGAACTAATGCAGG -3' (25-mer)

Primer3: **Hsp5-E7-R**

- Sequence: 5'- GGTAGAATCAAATCTTGACCAGCCC -3' (25-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (μL)	Tube 2 (μL)
Water	8	8
Primer1 (**HSP5-E2-S**, 10 μM)	0.5	0.5
Primer2 (**HSP5-E2-R**, 10 μM)	0.5	-
Primer3 (**Hsp5-E7-R**, 10 μM)	-	0.5
Taq polymerase (U/μL)	10	10
DNA extracted from tail (diluted 200 times)	1	1
total	20	20

Taq polymerase: HotStarTaq Master Mix Kit (Qiagen). The enzyme is a chemically modified Taq polymerase for hot start PCR and needs 15-min incubation at 95 °C for activation. Master Mix contains components such as an enzyme, dNTP, Mg at 2 x concentration. Please see Qiagen's website for details (http://www1.qiagen.com/Products/Pcr/HotStarTaqSystem/HotStarTaqMasterMix.aspx).

1.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	35 cycles
55 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	once
4 °C	∞	

1.4) Product size:

Primers 1 and 2: approx. 238 bp for wild-type alleles
Primers 1 and 3: approx. 220 bp for KO alleles

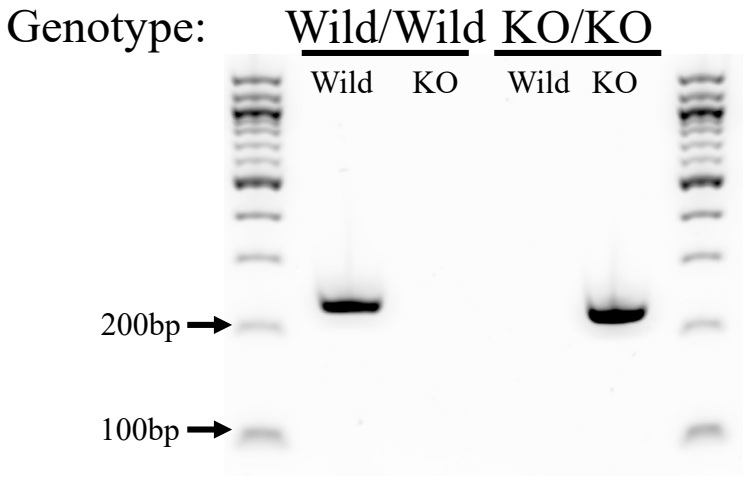

Figure 1. Electropherogram of PCR products from mice homozygous for wild and KO alleles with E-gel EX 2% (ThermoFisher) and a 100-bp ladder (NEB).

1.5) Reference(s)

Nbio_ID = 337; Dec. 24, 2019 (Tue)