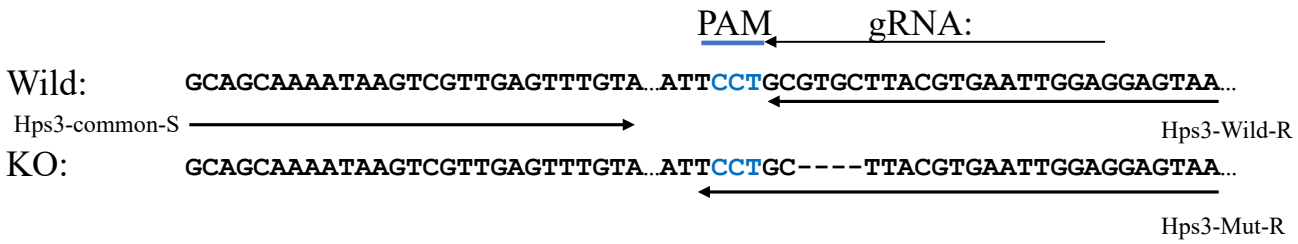
Genotyping of Hps3-KO mice

1. Positions of genome deletion and genotyping primers

The knockout allele has 4-bp deletion in exon 3 of the Hps3 gene.



2. Our protocol of Genotyping PCRs for Hps3-KO mice

This section describes our method we are routinely using for genotyping Hps3-KO mice.

2.1) Primer sequences:

Primer1: Hps3-common-S

- Sequence: 5'- GCAGCAAAATAAGTCGTTGAGTTTGTA -3' (27-mer)

Primer2: Hps3-Wild-R

- Sequence: 5'- TTACTCCTCCAATTCACGTAAGCACGC -3' (27-mer)

Primer3: Hps3-Mut-R

- Sequence: 5'- TTACTCCTCCAATTCACGTAAGCAGGA -3' (27-mer)

2.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (µL)	Tube 2 (µL)
Water	8	8
Primer1 (Hps3-common-S, 10 µM)	0.5	0.5
Primer2 (Hps3-Wild-R, 10 µM)	0.5	-
Primer3 (Hps3-Mut-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).

2.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	30 cycles*
60 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	Once
4 °C	∞	

*: Please note that false positive bands may be amplified when more cycles are employed (e.g., 35 cycles) for genotyping PCRs.

2.4) Product size:

Primers 1 and 2: approx. 120 bp for wild-type alleles
Primers 1 and 3: approx. 120 bp for KO alleles

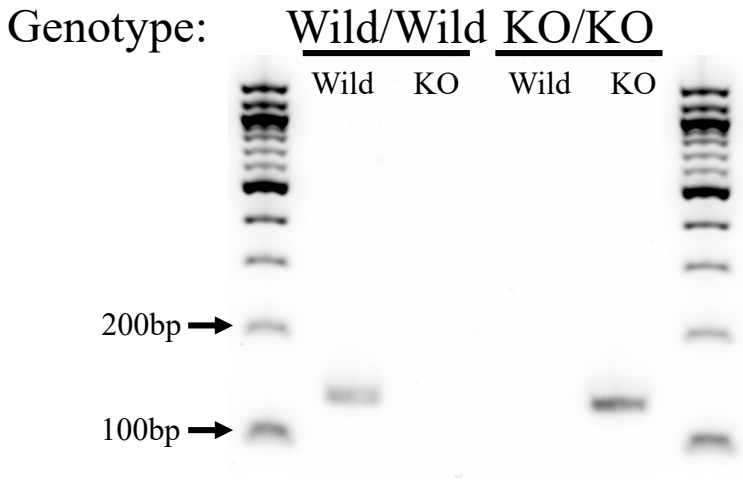


Figure 1. Electropherogram of PCR products from mice homozygous for wild and KO alleles with E-gel EX 2% (ThermoFisher) and a 100-bp ladder (NEB).

2.5) Reference(s)

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