Genotyping of Herp KO mice

1. Method used in our animal resource bank

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

1.1) Primer sequences:

- Primer1:
  - Sequence: `5′-CCCCTCCCCCTTTGGTTGACA-3′` (21-mer)
- Primer2:
  - Sequence: `5′-TCCAGGGGCTTAGACGCTTAC-3′` (21-mer)
- Primer3:
  - Sequence: `5′-TGGACCTGGGAGTGGACACCT-3′` (21-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (μL)	Tube 2 (μL)
Water	8.0	8.0
Primer1 (10 μM)	0.5	0.5
Primer2 (10 μM)	0.5	-
Primer3 (10 μM)	-	0.5
Taq polymerase (U/μL)	10	10
DNA extracted from tail (diluted 500 times)	1.0	1.0
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 enzyme, dNTP, Mg, etc 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	35cycles
60°C	30 sec	
72 °C	30 sec	
72 °C	5 min	once
4 °C	∞	

Thermal cycler:Veriti with 0.2mL tubes.

1.4) Product size:

Wild-type allele: 343 bp (Primers 1 and 2)

Knockout allele: 252 bp (Primers 1 and 3)