Genotyping PCR for SIK3 KO mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping SIK3 KO mice.

1.1) Primer sequences:

- Primer1:a
  - Sequence: 5'- GCT ACC AAC TTG GTT ACA GTT GCT -3' (24-mer)
- Primer2: b2
  - Sequence: 5'- CGA GGG TCA GCA GCA ACT TCT-3' (21-mer)
- Primer3: c2
  - Sequence: 5'- GAG ACT AGT GAG ACG TGC TAC T-3' (22-mer)

1.2) Reaction mixture:

	Tube (µL)
Water	8
Primer1 (a,10 µM)	0.5
Primer2 (b2,10 µM)	0.25
Primer3 (c2,10 µM)	0.25
Taq polymerase	10
DNA extracted from tail (diluted 500 times)	1
total	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 conditions:

94 °C	2 min	Enzyme activation and first denature
94 °C	30 sec	40 cycles
60 °C	30 sec	
72 °C	40 sec	
72 °C	2.5 min	once
4 °C	∞	

Thermal cycler: Veriti (Life technologies) with 0.2mL tubes.

1.4) Product size:

250 bp for wild-type alleles
450 bp for KO alleles

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