Genotyping of Pex11a KO mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping Pex11a KO mice.

1.1) Primer sequences:

- Primer1: 5'-A1
  - Sequence: 5'- TGT CCC ATC TGC AGC AAG ATC TC -3' (23-mer)
- Primer2: 3'-LAN1
  - Sequence: 5'- CCA GAG GCC ACT TGT GTA GC -3' (20-mer)
- Primer3: 5'-W
  - Sequence: 5'-ATT CAT CAC CAT GTC ATT GTC ATC AT -3' (26-mer)

1.2) Reaction mixture:

	Tube 1(μL)	Tube 2(μL)
Water	8	8
Primer1 (A1,10 μM)	0.5	0.5
Primer2 (LAN1,10 μM)	0.5	
Primer3 (W,10 μM)		0.5
Taq polymerase (U/μL)	10	10
DNA extracted from tail (purified)	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 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:

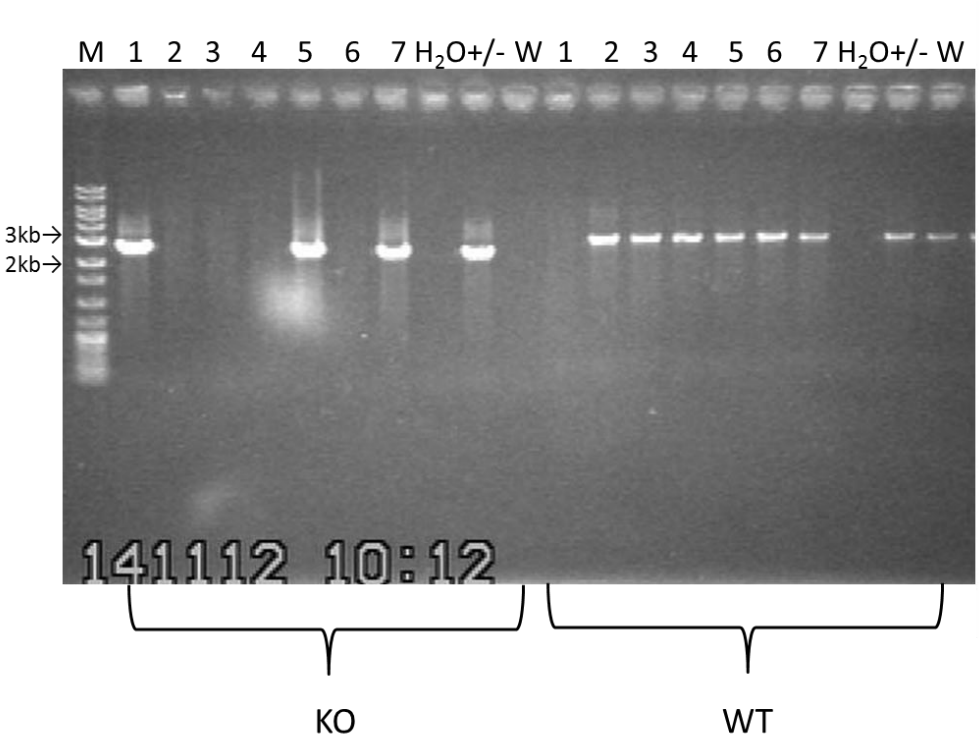
95 °C	15 min	Enzyme activation and first denature
94 °C	20 sec	35 cycles
62 °C	1 min	
72 °C	2.5 min	
72 °C	5 min	once
4 °C	∞	

Thermal cycler:Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: approx. 2.7 kb for KO alleles

Primers 1 and 3: approx. 3.4kb for WT alleles



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