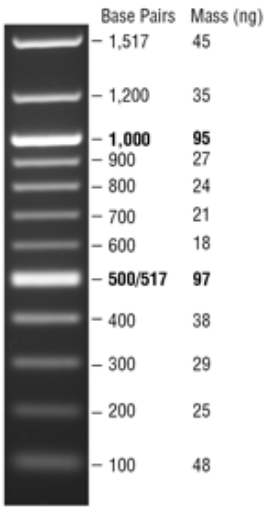
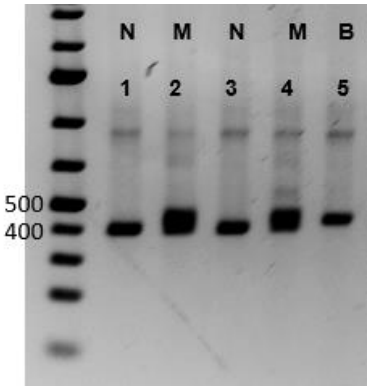
Spp1^{floxed} **Mouse Genotyping Protocol**



N = non-targeted, WT product only
M = monoallelic, or heterozygous LoxP
B = biallelic, or homozygous LoxP



Note that this single PCR reaction can differentiate between WT, heterozygous, and homozygous floxed Spp1. After the new colony is established, there is no need to perform all three PCR reactions (3', 5', and full length) for routine genotyping.

	Sequence (5'->3')	Length
Spp1 3' Fwd1	TTGGAAAATCCTGCAGGCTGACTT	20 bp
Spp1 5' Rev1	GTTGGAAGACAGGCAGCCCCAC	21 bp
PCR product length	410 bp = no LoxP, 444 bp = LoxP	

Any polymerase can be used for routine PCR. The VAPRase polymerase in the example PCR reaction shown below is prepared by the Vanderbilt Antibody and Protein Resource and can be purchased through the Molecular Cell Biology Resource Core.

PCR reaction (50 ul)

10x VAPRase buffer	5 ul
10 mM dTNPS	1 ul
10 uM 3' Spp1 Fwd primer	1 ul
10 uM 3' Spp1 Rev primer	1 ul
VAPRase HF polymerase	1 ul
Nuclease free water	39 ul
DNA tail lysate	2 ul

PCR program

1. 98ºC, 30 sec
2. 98ºC, 10 sec
3. 68ºC, 20 sec
4. 72ºC, 25 sec
5. Go to 2, 35 X
6. 72ºC, 2 min
7. 12ºC, forever