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Genotyping protocol for lysophosphatidic acid receptor 1 (Lpar1) (MGI ID: 108429) tm1d mice. (Protocol edited by Evangelos Dioletis on 6-11-2015)

The Lpar1 tm1d mouse was derived by crossing Lpar1 cKO mice [B6-Lpar1$^{tm1c(EUCOMM)Wtsi}$/Flmg] with a Cre driver strain.
The offspring were further mated with C57BL6 mice in order to remove Cre recombinase from the genetic background.

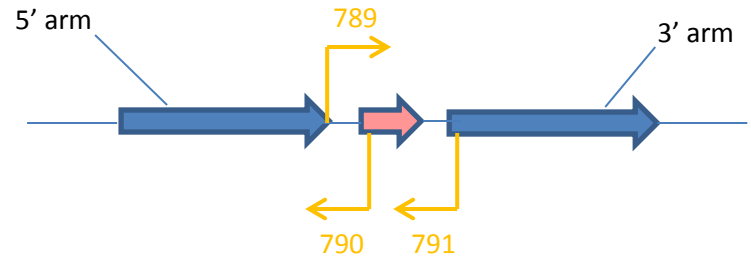
Genotyping primers

Fwd primer (789): 5'- GGA TGC TAT TCT GGG GAT GA -3' (20 bp, T_m=58.4°C)
Rev primer_1 (790): 5'- ATA CCC AAT GCA GCC AAA AA -3' (20 bp, T_m=54.3°C)
Rev primer_2 (791): 5'- TCA TGG ACA CTT GGA CTA ATG AA -3' (23 bp, T_m=59.3°C)

The current PCR protocol can discriminate between homo- and heterozygous knockout mice.

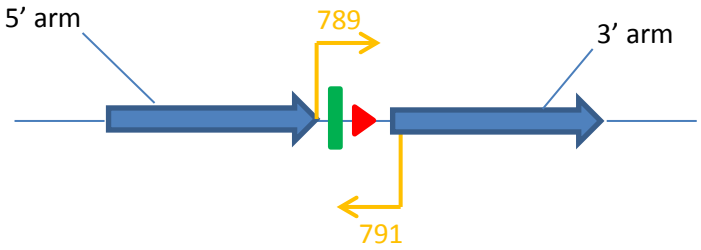
A schematic representation of the genotyping strategy along with the primer binding sites and the expected PCR products is shown below:

Genotyping of Lpar1 tm1d mice



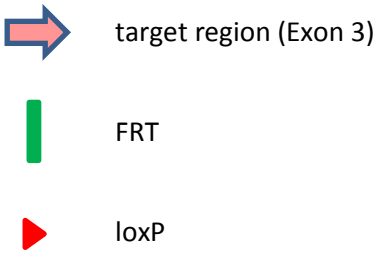
WT

789-790:	325 bp
789-791:	1,879 bp



tm1d

789-791:	564 bp



Please note that the expected PCR products for the WT and defloxed (knockout) alleles are 325 bp and 564 bp long respectively.

Typical pipetting scheme for a single reaction (in µl):

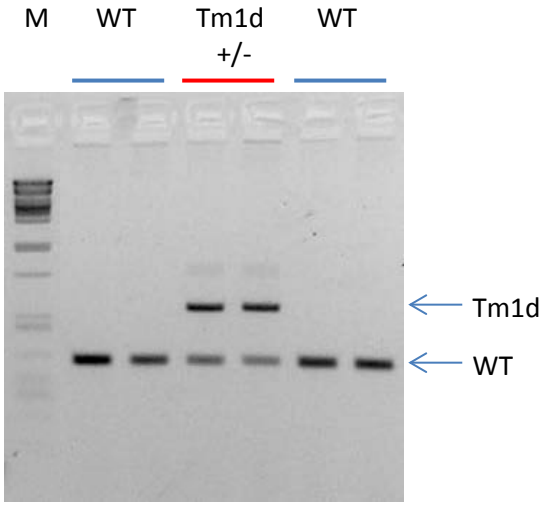
ddH_2O	10.4
10x buffer*	2
$MgCl_2$ (25mM)	1.2
dNTPs (2.5 mM each)	2
Fwd primer (5 µM)	1 (789)
Rev primer 1 (5 µM)	1 (790)
Rev primer 2 (5 µM)	1 (791)
DNA template (50-100 ng/µl)	1
Taq DNA polymerase	0.4
Total volume (µl)	20

*10x PCR Buffer: 500 mM KCl, 100mM Tris-HCl (pH 9.0 at 25°C), 1% Triton X-100.

Thermocycler conditions:

95°C for 5 min
95°C for 30 s
60.7°C for 30 s } x35
72°C for 45 s
72°C for 5 min

Example of genotyping LPAR1 tm1d mice.



2% agarose gel electrophoresis in 1x TBE
35 min at 120V (6.7V/cm).
M: Marker (*Pst*I-cut lambda DNA)

***Pst*I-cut lambda DNA**

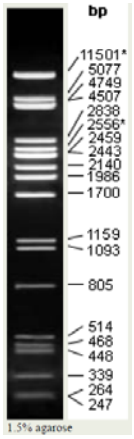


Figure taken from: http://www.taq-dna.com/phage-lambda-dna-psti-digest-ready-to-use-_144.html