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Genotyping protocol for lysophosphatidic acid receptor 1 (LPAR1) (MGI ID: 108429) lacZ reporter mice.

Protocol edited by Evangelos Dioletis on 22-10-2015.
Information presented here applies to the lacZ-tagged null allele (Lpar1$^{tm1b(EUCOMM)Wtsi}$), which was derived from the Lpar1$^{tm1a(EUCOMM)Wtsi}$ allele via Cre-mediated recombination .

Genotyping primers

Fwd primer (727): 5'- CGGTCGCTACCATTACCAGT -3' (20 bp, T_m=60.5°C)
Rev primer (728): 5'- ACTGATGGCGAGCTCAGACC -3' (20 bp, T_m=62.5°C)

Primers 727 and 728 bind mutant gDNA only.
Expected PCR product length: 380 bp

The current protocol does not discriminate between homo- and heterozygous mice.

Typical pipetting scheme for single reactions (in µl):

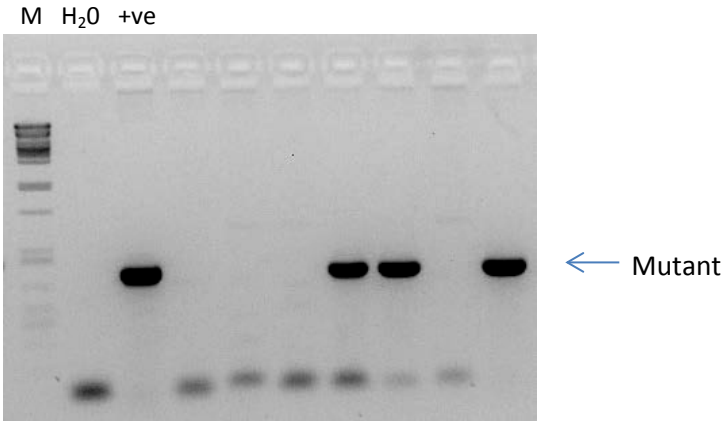
ddH_2O	12.4
10x buffer*	2
$MgCl_2$ (25mM)	1.2
dNTPs (2.5 mM each)	1
Fwd primer (5 µM)	1 (727)
Rev primer 1 (5 µM)	1 (728)
DNA template (50-100 ng/µl)	1
Taq DNA polymerase	0.4
Total volume (µl)	20

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

Thermocycler conditions:

94°C for 5 min
94°C for 30 s
58°C for 30 s } x35
72°C for 45 s
72°C for 5 min

Example of genotyping LPAR1 lacZ reporter (Lpar1$^{tm1b(EUCOMM)Wtsi}$) mice.



2% agarose gel electrophoresis in 1x TBE
35 min at 120V (6.7V/cm).
Controls
M : Marker (*Pst*I-cut lambda DNA)
H_2O : water instead of gDNA template (-ve control)
+ve : gDNA from LPAR1 tm1b mouse (+ve control)

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

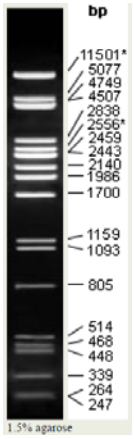


Figure taken from: http://www.taq-dna.com/phage-lambda-dna-psti-digest-ready-to-use-_144.html