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Genotyping protocol for lysophosphatidic acid receptor 1 (LPAR1) (MGI ID: 108429) cKO mice. (Protocol edited by Evangelos Dioletis on 15-10-2015)

The LPAR1 cKO mouse was derived by crossing the knockout-first allele [B6-Lpar1^{tm1a(EUCOMM)Wtsi}/Flmg] with a FLP deleter (flipper) [B6-Gt(ROSA)26Sor^{tm1(FLP1)Dymj}]. The offspring were further mated with C57BL/6 mice in order to remove FLP recombinase from the genetic background.

Genotyping primers

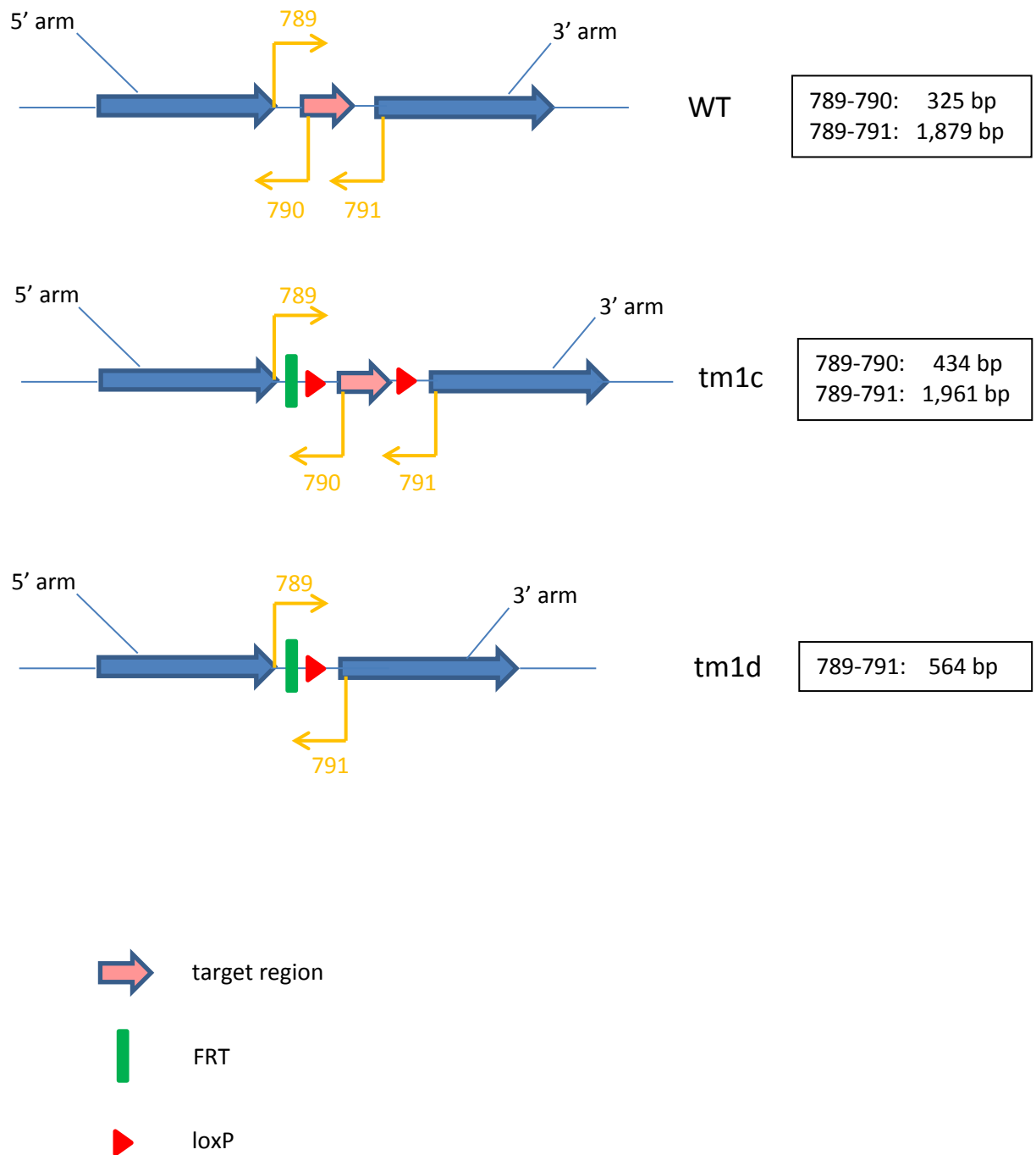
Fwd primer (789): 5'- GGA TGC TAT TCT GGG GAT GA -3' (20 bp, T_m=58.4°C)
Rev primer_1 (790): 5'- ATA CCC AAT GCA GCC AAA AA -3' (20 bp, T_m=54.3°C)
Rev primer_2 (791): 5'- TCA TGG ACA CTT GGA CTA ATG AA -3' (23 bp, T_m=59.3°C)

The current PCR protocol can discriminate between homo- and heterozygous floxed (tm1c) mice. Furthermore, following mating with the appropriate Cre mouse, it is also possible to identify defloxed mice (tm1d) both in the homo- and heterozygous state.

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

Note that the expected PCR products for the WT, floxed and defloxed alleles are 325 bp, 434 bp and 564 bp long respectively.

Genotyping of LPAR1 cKO mice



Typical pipetting scheme for a single reaction (in μ l):

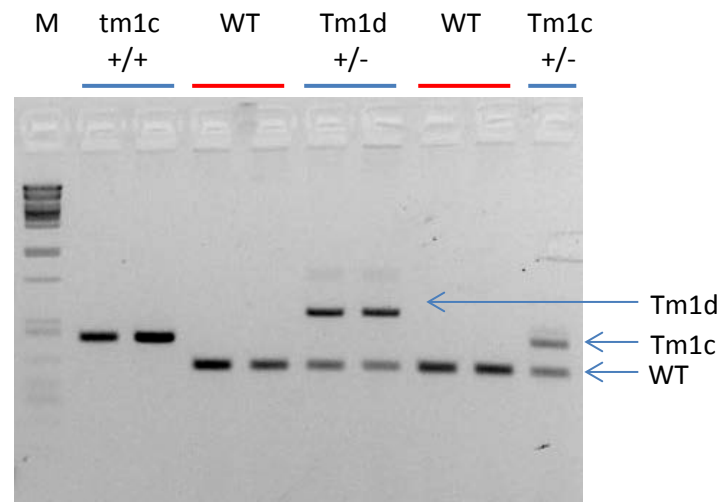
ddH ₂ O	10.4
10x buffer*	2
MgCl ₂ (25mM)	1.2
dNTPs (2.5 mM each)	2
Fwd primer (5 μ M)	1 (789)
Rev primer 1 (5 μ M)	1 (790)
Rev primer 2 (5 μ M)	1 (791)
DNA template (50-100 ng/ μ l)	1
Taq DNA polymerase	0.4
Total volume (μl)	20

*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 floxed (tm1c) and defloxed (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**

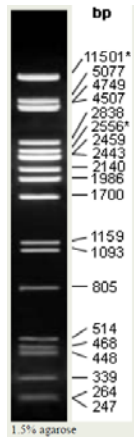


Figure taken from: <http://www.taq-dna.com/phage-lambda-dna-psti-digest-ready-to-use-144.html>