Genotyping protocol

General information:

Strain name	tatdn1 floxed

Primers:

Name	Sequence	Primer type
TatdloxP_Fw	CTACTCTTAAAGACAGCCTTCC	
TatdNeo_Fw	GGTGGATGTGGAATGTGTGC	
TatdNeo_Rv	CTTTATTTCTCAGATGTGTGGGC	

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
wt		
mutant		

Reaction mix:

ddH_2O	18,5	µl
PCR Buffer 15mM $MgCl_2$	2,5	µl
dNTPs 2,5mM	2,5	µl
Primer 100µM	0,125	µl
Primer 100µM	0,125	µl
Taq Polymerase 5U/µl	0,250	µl
DNA	1	µl
Final volume	25	µl

PCR program:

95	°C	5	min	
95	°C	30	sec	X35
60	°C	30	sec	
72	°C	30	sec	
72	°C	10	min	

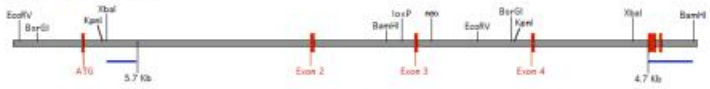
Expected fragment size:

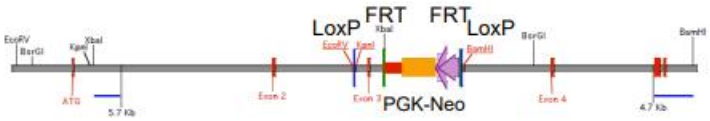
LoxP -/-	270 bp
LoxP +/+ Neo +/+	441 bp
LoxP -/- Neo -/-	400 bp

Comments/Additonal information:

Tatdn1 floxed mice genotyping

Wild type allele

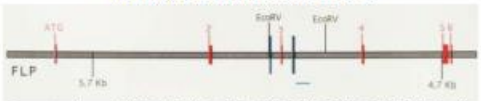




Targeted allele

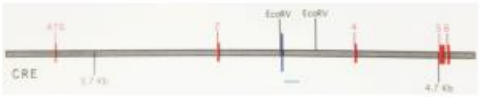
Tatd loxP$^{+/+}$ *Neo*$^{+/+}$ x *ROSA FLPe*

Conditional allele after FLPe



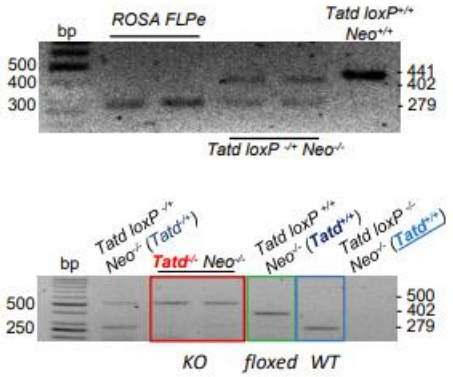
Tatd loxP$^{+/+}$ *Neo*$^{-/-}$ x *CAG-Cre*

Conditional allele after Cre (*Tatd KO*)



Genotyping: FWD1 (5'Tatd) FWD2(PGK) REV (3'Tatd)

Allele	Primer sequence		PCR conditions			PCR product	
			T^{a} (°C)	Time	Cycles	Genotype	size (bp)
Tatd loxP neo	Fwd	CTACTCTTAAAGACAGCCTTCC	95	3 min	1	loxP$^{+/+}$ neo$^{+/+}$	441
	Fwd	GGTGGATGTGGAATGTGTGC	95	30 sec		loxP$^{+/+}$ neo$^{-/-}$	400
	Rev	CTTTATTTCTCAGATGTGTGGGC	60	30 sec	35	loxP$^{-/-}$	270
			72	45 sec			
			72	2 min	1		
CAG cre	Fwd	AGGTTCGTTCACTCATGGA	95	2 min	1	Cre$^{-/-}$	-
	Rev	TCGACCAGTTTAGTTACCC	95	1 min		Cre$^{+/+}$	250
			55	1 min	30		
			72	1 min			
			72	10 min	1		
Tatd	Fwd	GGGCTCCAGGTTCAGTGAGAG	95	3 min	1	*Tatd*$^{+/+}$	270
	Rev	CTTTATTTCTCAGATGTGTGGGC	95	1 min		*Tatd*$^{-/-}$	500
	Fwd	CTACTCTTAAAGACAGCCTTCC	60	1 min	35		
			72	1 min			
			72	10 min	1		
	Fwd	ACGAACCTGGTCGAAATCGTGCG	94	2 min	1	Cre$^{-/-}$	-
	Rev	CGGTCGATGCAACGAGTGATGAG	94	45 sec		Cre$^{+/-}$	350


bp
ROSA FLPe
Tatd loxP+/+
Neo+/+
500
400
300
441
402
279
Tatd loxP -/+ Neo-/-
bp
Tatd loxP -/+
Neo-/- (Tatd-/+)
Tatd-/- Neo-/-
Tatd loxP +/+
Neo-/- (Tatd+/+)
Tatd loxP -/-
Neo-/- (Tatd+/+)
500
250
500
402
279
KO
floxed
WT