

RMRC Genotyping Protocol-PCR

No.	RMRC13401	Strain Name	B6;129- <i>Pomgnt1</i> ^{em2Sphccc} /Narl
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A. Primer

	Primer name	Sequence (5'-3')
1	Pomgnt1-floxed-F	TAG GAA GGA CCT GGT TGG TG
2	Pomgnt1-floxed-R	GCC TCA TCT GGA GAG GGA AT

B. Genomic extraction :

- ☐ Phenol-Chloroform extraction
 ☐ commercial extraction kit _____
- ☒ Lysis buffer Direct PCR Buffer
☐ other _____

C. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		6.4	μl	1	94	300	1
				μl	2	94	30	30
				μl	3	64	35	30
Primer 1	10	μM(ρmol/μl)	0.8	μl	4	72	120	30
Primer 2	10	μM(ρmol/μl)	0.8	μl	5	72	300	1
DNA	--		2	μl	6			
				μl	7			
amaR OnePCR			10	μl	8			
Total			20	μl	9			

Enzyme digestion: HindIII (Thermo scientific, ER0501). At 37°C for 3hr.

The information of Taq, including company name and catalog number : Simply (SM213-0250)

D. PCR Product

	Primer set		Product size	Target region
1	Pomgnt1-floxed-F	Pomgnt1-floxed-R	539 bp	

	Enzyme	Product size	Region
1	HindIII	326 bps	
2		109 bps	
3		104 bps	

E. Gel Photo

