

RMRC Genotyping Protocol-PCR

No.	RMRC13400	Strain Name	B6;129- <i>Pomgnt1^{em1Sphccc}</i> /Narl
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A. Primer

	Primer name	Sequence(5'→3')
1	Pomgnt1-L120R-F	CGG GCA GCT AAC TCC CTT AT
2	Pomgnt1-L120R-R	GGC CTG GTT GAG GAC TAT GA

B. Genomic extraction :

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

C. Reaction Conditions

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, sec	Number of Cycles
H ₂ O	--		20.5	μl	1	94	180	1
PCR buffer	10x		3	μl	2	94	30	35
dNTP	10	mM	0.6	μl	3	64	35	35
Primer 1	10	μM(μmol/μl)	0.8	μl	4	72	35	35
Primer 2	10	μM(μmol/μl)	0.8	μl	5	72	300	1
DNA	--		2	μl	6			
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺	25	mM	1.8	μl	8			
Total			30	μl	9			

Enzyme digestion: BtsUI (Thermo scientific, ER0921). At 37°C for 2hr.

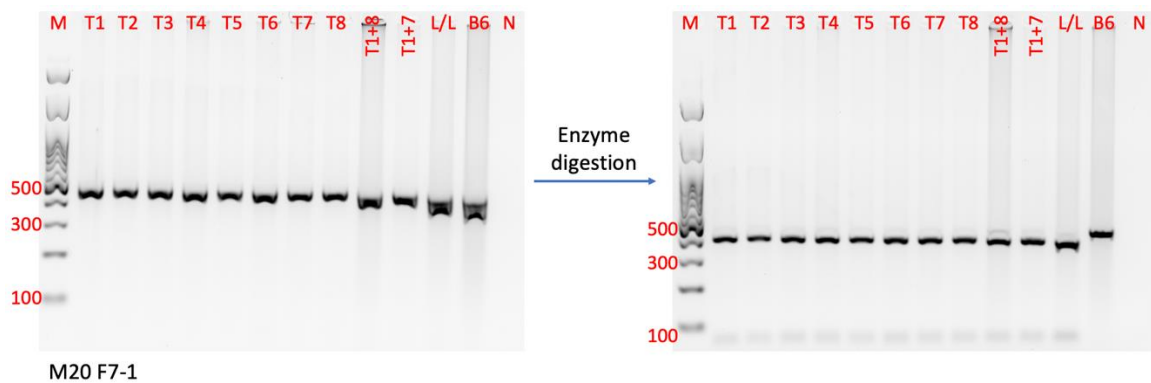
The information of Taq, including company name and catalog number : GeneDireX (MB101-0500)

D. PCR Product

	Primer set		Product size	Target region
1	Pomgnt1-L120R-F	Pomgnt1-L120R-R	432 bp	

	Enzyme	Product size	Region
1	BstUI	373 bp	

E. Gel Photo



T1	L120R/L120R
T2	L120R/L120R
T3	L120R/L120R
T4	L120R/L120R
T5	L120R/L120R
T6	L120R/L120R
T7	L120R/L120R
T8	L120R/L120R
T1+8	L120R/L120R
T1+7	L120R/L120R
L/L	L120R/L120R

B6	+/+
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