

RMRC Genotyping Protocol-PCR

No.	RMRC13156	Strain Name	C3H.B6- Pstpip2 ^{Y180C} /Sbc
-----	-----------	-------------	---------------------------------------

A. Primer

	Primer name	Sequence
1	olMR0016	5'—GTCAGTCGAGTGCACAGTTT—3'
2	olMR0015	5'—CAAATGTTGCTTGTCTGGTG—3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		7.2	μl	1	95	2'	1
PCR buffer	10x			μl	2	95	30"	35
dNTP	0.2	mM		μl	3	56.8	30"	
Primer 1	1	μM(μmol/μl)	0.4	μl	4	72	30"	
Primer 2	1	μM(μmol/μl)	0.4	μl	5	72	5'	1
DNA	--		2	μl	6			
Taq Master Mix Red	0.025	U/μl	10	μl	7			
Mg ²⁺	--	mM	--	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	olMR0016	olMR0015	123–400 bp	Digest the gel fragment for the sequencing
2			123–400 bp	Digest the gel fragment for the sequencing

D. Sequencing:

