

RMRC Genotyping Protocol-PCR

種原編號	RMRC13051	品系名稱	C57BL/6-Tg(Col2a1G649S)19Tsf
	RMRC13052		C57BL/6-Tg(Col2a1G649S)29Tsf
	RMRC13053		C57BL/6-Tg(Col2a1G1102S)Tsf
	RMRC13054		C57BL/6-Tg(Col2a1)Tsf

A. Primer

	Primer name	Sequence
1		CAAAGGTGTTCGAGGAGAC
2		CTGCACCATCTCTTCCAG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		14.1	μl	1	94	600	1
PCR buffer	10x		2	μl	2	94	30	35
dNTP	10	mM	0.5	μl	3	60	30	
Primer 1	10	μM(ρmol/μl)	0.4	μl	4	72	80	
Primer 2	10	μM(ρmol/μl)	0.4	μl	5	72	600	
DNA	--			μl	6			1
Taq	5	U/μl	0.2	μl	7			
Mg ²⁺	25	mM	2.4	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	1	2	371 bp	Transgenic
2	1	2	950 bp	Endogenous

D. Gel Photo