

RMRC Genotyping Protocol-PCR

No.	RMRC13267	Strain Name	NOD-Tg(dLck-Maf)ASytwu
No.	RMRC13268	Strain Name	NOD-Tg(dLck-Maf)BSytwu
No.	RMRC13269	Strain Name	NOD-Tg(dLck-Maf ^{K33R})ESytwu
No.	RMRC13270	Strain Name	NOD-Tg(dLck-Maf ^{K33R})JSytwu

A. Primer

	Primer name	Sequence
1	dLck (F)	ATAGC ACGGC TGGAG TTGAG TGC
2	WT-c-Maf (R)	TCGGT CTCCA CCGGT TCCTT TT
3	K33R-c-Maf (R)	TCGGT CTCCA CCGGT TCCTT TC

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		13	μl	1	94	300	1
PCR buffer	10x		2	μl	2	94	30	32
dNTP	10	mM	2	μl	3	60	30	32
Primer 1	10	μM(μmol/μl)	0.5	μl	4	72	60	32
Primer 2	10	μM(μmol/μl)	0.5	μl	5	72	600	1
DNA	--		50ng	μl	6	25	300	1
Taq	10	U/μl	1	μl	7			
DMSO		mM	1	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	dLck (F)	WT-c-Maf (R)	865 bp	
2	dLck (F)	K33R-c-Maf (R)	865 bp	

D. Gel Photo

