

RMRC Genotyping Protocol-PCR

No.	RMRC13276	Strain Name	NOD-Tg (Lck-Mgat5)ASytwu
	RMRC 13277		NOD-Tg (Lck-Mgat5)BSytwu
	RMRC 13278		NOD-Tg (Lck-Mgat5)DSytwu

A. Primer

	Primer name	Sequence
1	P1	GACATCCCAGCATCCTTACG
2	P2	CAAAGGCCAGCTGGTGCAGACGAT

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		12.2	μl	1	94	180	1
PCR buffer	10x		2	μl	2	94	50	30
dNTP	2.5	mM	4	μl	3	60	30	
Primer 1	20	μM(μmol/μl)	0.25	μl	4	72	30	
Primer 2	20	μM(μmol/μl)	0.25	μl	5	72	600	1
DNA	(100 ng/μl)		0.5	μl	6			
Taq	5	U/μl	0.2	μl	7			
Mg ²⁺	50	mM	0.6	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	P1	P2	1063	distal <i>Lck</i> promoter 3'-end to transgenic <i>Mgat5</i> central region

D. Gel Photo

