

RMRC Genotyping Protocol-PCR

No.	RMRC13171	Strain Name	NOD-Tg(Itgax-Aire)ESytwu
-----	-----------	-------------	--------------------------

A. Primer

	Primer name	Sequence
1	AGTP-F	TTCCATCCCTCCCCAGTGA
2	AGTP-R	GAGAGGGTTAGGGATAGGCT

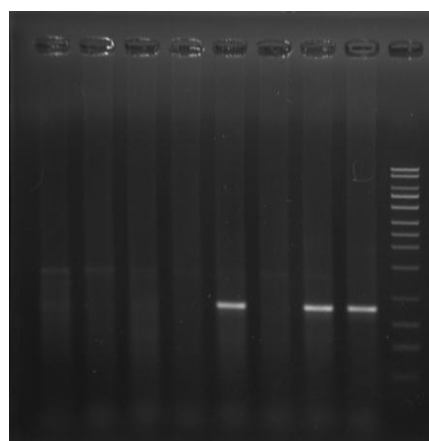
B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		7	μl	1	95°C	180	1
PCR buffer	2x (150 mM Tris-HCl pH 8.5, 40 mM (NH ₄) ₂ SO ₄ , 3.0 mM MgCl ₂ , 0.2% Tween, 0.4 mM dNTPs, 0.05 units/μL Ampliqon Taq DNA polymerase)		10	μl	2-4	95°C	45	35
						60°C	60	
						72°C	90	
dNTP		mM	x	μl	5	72°C	300	1
Primer 1	10	μM(μmol/μl)	1	μl				
Primer 2	10	μM(μmol/μl)	1	μl				
DNA	50 ng/ul		1	μl				
Taq		U/μl	X	μl				
Mg ²⁺		mM	x	μl				
Total			20	μl				

C. PCR Product

	Primer set		Product size	Target region
1	AGTP-F	AGTP-R	854 bp	
2			bp	

D. Gel Photo



854 bp