

RMRC Genotyping Protocol-PCR

No.	RMRC13205	Strain Name	NOD-Tg(INS-anti-Tnfrsf9scFv)Sytwu
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A. Primer

	Primer name	Sequence
1	pIns I HIP 3' F	GGATCCTGAGAACTTCAGG
2	anti-4-1BB BW	AGAGACAGTGCAGGTGAGGGA

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		16.65	μl	1	94	180	1
PCR buffer	10x		2.5	μl	2	94	50	35
dNTP	2.5	mM	2	μl	3	58	50	
Primer 1	20	μM(μmol/μl)	0.5	μl	4	72	80	
Primer 2	20	μM(μmol/μl)	0.5	μl	5	72	600	1
DNA	(100 ng/μl)		2	μl	6			
Taq	5	U/μl	0.1	μl	7			
Mg ²⁺	50	mM	0.75	μl	8			
Total			25	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	pIns I HIP 3' F	anti-4-1BB BW	1225 bp	
2			bp	

D. Gel Photo

