

RMRC Genotyping Protocol-PCR

No.	RMRC13258	Strain Name	NOD-Tg(INS-anti-Ctla4scFv)Sytwu
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A. Primer

	Primer name	Sequence
1	pINS I HIP3'-Fw (P1)	5'-GGATCCTGAGAACTTCAGG-3'
2	pINS-anti-CTLA-4-Rw (P2)	5'-CAGGTCCTGACTCCTGAAGC-3'

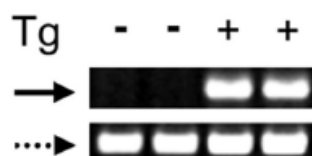
B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--			μl	1	94	120	1
PCR buffer	10x		2	μl	2	94	60	30-35
dNTP	10	mM	0.5	μl	3	59	60	
Primer 1	10	μM(ρmol/μl)	1	μl	4	72	90	
Primer 2	10	μM(ρmol/μl)	1	μl	5	72	600	1
DNA	100ng			μl	6			
Taq	0.025	U/μl	0.2	μl	7			
Mg ²⁺	20	mM	1	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set	Product size	Target region
	pINS I HIP3'-Fw (P1)	1127 bp	
	pINS-anti-CTLA-4-Rw (P2)		

D. Gel Photo



Genotyping of tail genomic DNA by PCR to test for the existence of the transgene is indicated by the solid arrow and the internal control of *IL-12 p35* is indicated by the dashed arrow