

RMRC Genotyping Protocol-PCR

No.	RMRC13264 RMRC13265 RMRC13266 RMRC13279	Strain Name	NOD-Tg(Lck-Ptpn22)ASytwu NOD-Tg(Lck-Ptpn22)DSytwu NOD-Tg(Lck-Ptpn22)CSytwu NOD-Tg(Lck-Ptpn22)CSytwu
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A. Primer

	Primer name	Sequence
1	P1	ATCAGGATGGTTCTCCTCCA
2	P2	CAAAGGCCAGCTGGTGCAGACGAT

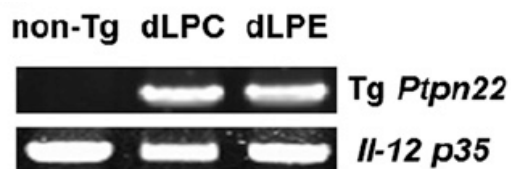
B. Reaction Conditions

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	35
dNTP	2.5	mM	4	μl	3	59	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	853	distal <i>Lck</i> promoter 3'-end to transgenic <i>Ptpn22</i> central region

D. Gel Photo



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