

RMRC Genotyping Protocol-PCR

No.	RMRC13252	Strain Name	<i>B6;129-Supt4a^{tm1Thc}</i>
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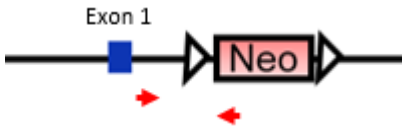
A. Primer

	Primer name	Sequence
1	Supt4hLNL5 1 F	5'-AGTTCTCGGCATAACTGAGCACAAG-3'
2	Supt4hLNL5 1 R	5'-CTAGCTTGGCTGGACGTAAACTCCT-3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		15.3	μl	1	95	180	
PCR buffer	10x		2	μl	2	95	30	
dNTP	2.5	mM	0.7	μl	3	58	20	
Primer 1	10	μM(μmol/μl)	0.4	μl	4	72	25	To 2 Step 35 cycles
Primer 2	10	μM(μmol/μl)	0.4	μl	5	72	300	
DNA	--		1	μl	6			
Taq	5	U/μl	0.2	μl	7			
Mg ²⁺		mM		μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	Supt4hLNL5 1 F	Supt4hLNL5 1 R	366 bp	 agttctcggcataactgagcacaagttgtctccccgaca gacgttcggtgaacttgctgtggtggactggaaagaca ctaggagccagagcagccgagagagtagcaggagag ggtacctgaccccaaagaggctagaaaatgtgaagct gaattagggacgcgagcaaaaagggtatgaggggtggag ttttaggaacagtttgagaggagagggtgtgggtgtat agaaaacgaagaggttggtttgttgaaatattaaggcg ttgccgatcatattcaataacccttaataacttcgtataa tgtatgctatacgaagtattaggtctgaagaggagtttac gtccagccaagctag

D. Gel Photo

