

RMRC Genotyping Protocol-PCR

No.	RMRC23005	Strain Name	SD-Lep ^{em1Narl} /Narl
-----	-----------	-------------	---------------------------------

A. Primer

	Primer name	Sequence
1-1	rLeptin_12210F	CCTTGAAGAGGGGATTCTCACC
1-2	rLeptin_12664B	CAGCCACCAGAGGACAAACTTG
2-1	rLeptin_9974F	CCTATGTTCAAGCTGTGCCTATCC
2-2	rLeptin_14342B	TTCCTTGTCACATCCTGTTCCG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		10.9	μl	1	94	3'	1
PCR buffer	10x		1.5	μl	2	94	30''	35
dNTP	10	mM	0.3	μl	3	60	30''	35
Primer 1	10	μM(ρmol/μl)	0.25	μl	4	72	30''	35
Primer 2	10	μM(ρmol/μl)	0.25	μl	5	72	3'	1
gDNA	50~250 ng/ul		1	μl	6			
Taq	5	u/μl	0.3	μl	7			
Formamide	1	mM	0.5	μl	8			
Total			15	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	rLeptin_12210F	rLeptin_12664B	455 bp	Wildtype
2	rLeptin_9974F	rLeptin_14342B	1836 bp	Mutation(deletion)

D. Gel Photo

