

RMRC Genotyping Protocol-PCR

種原編號	RMRC13061	品系名稱	C57BL/6- <i>Lep</i> ^{145E} /Hcj
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A. Primer

	Primer name	Sequence
1	Leptin-E3F1	CTATGGCTGGTTCTTCACGG
2	obLEP-ASPR	GCAGCCTGCTCAAAGCCT
3	noLEP-ASPF	CTACTCCACAGAGGTGGT
4	LEP-ASPR	GGCTCTCTTCTCCTGAAG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O			14.9	μl	1	95	300	1
PCR buffer	10x		2.5	μl	2	95	30	35
dNTP	2	mM	2.5	μl	3	73	30	
Leptin-E3F1	10	μM(μmol/μl)	1.6	μl	4	68	30	
obLEP-ASPR	10	μM(μmol/μl)	0.4	μl	5	68	300	1
noLEP-ASPF	10	μM(μmol/μl)	0.8		6			
LEP-ASPR	10	μM(μmol/μl)	0.8		7			
DNA	--		1	μl	8			
Taq	5	U/μl	0.5		9			
Mg ²⁺	-	mM		μl				
Total			25	μl				

C. PCR Product

	Primer set		Product size	Target region
1	Leptin-E3F1	obLEP-ASPR	578 bp	Obese
2	noLEP-ASPF	Lep-ASPR	170 bp	Normal
3	Leptin-E3F1	Lep-ASPR	709 bp	Normal

D. Gel Photo

