

RMRC Genotyping Protocol-PCR

No.	RMRC13429	Strain Name	C57BL/6- <i>Phex</i> ^{Hyp} /Narl
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A. Primer

	Primer name	Sequence
1	Phex09_F	CAGAATGGATTATGCTCGGC
2	Phex09_R	TTTCAAAGCAATTTTCACCGTAC

B. Reaction Conditions

Component	Concentration	Volume	Step	Temp.	Time
H ₂ O	-	8.8 µl	1	95°C	5 min
Primer 1	10 µM	0.4 µl	2	95°C	30 sec
Primer 2	10 µM	0.4 µl	3	60°C	30 sec
Taq master mix	2X	10 µl	4	72°C	40 sec
gDNA	50~250 ng/ul	0.4 µl	5	Go to 2 for 35 cycles	
Total		20 µl	6	72°C	5 min

C. PCR Product

	Primer set		Product size	Target region
1	Phex09_F	Phex09_R	333 bp	PCRproduct 需進行定序，須具備符合以下序列片段： MT： AGAG(142,349)_(142,347)AAGA (WT：CGAGAG <u>G</u> AAGAA)

D. Gel Photo