國家實驗研究院
NARLabs 國家實驗動物中心 National Laboratory Animal Center

RMRC Genotyping Protocol-PCR

No.	RMRC13436	Strain Name	C57BL/6-*Wwox*^{em2Wdcw}/Narl

A. Primer

	Primer name	Sequence
1	Wwox 5F	5’-GGTATGACTTTGTGGGTGTTGGC-3’
2	Wwox 3B	5’-GGTTGGAGGCAGATAGACATCTTG-3’

B. Genomic extraction :

☐ Phenol-Chloroform extraction □ commercial extraction kit _____

■Lysis buffer #ABP-PP-MT01500/ Allele □ other _____

C. Reaction Conditions

Step 1. PCR

Component	Concentration		Volume		Step	Tempera -ture, °C	Time,	Number of Cycles
H_2O	--		12.2	µl	1	98	2min	1
PCR buffer	5X Primer STARG bf		4	µl	2	98	10s’	30
	dNTPs		1.6	µl	3	60	15s’	
	polymerase		0.4	µl	4	68	3min	
Primer 1	20	µM(pmol/µl)	0.4	µl	5	68	10min	1
Primer 2	20	µM(pmol/µl)	0.4	µl	6	20	∞	
DNA	--		1	µl				
Total			20	µl				

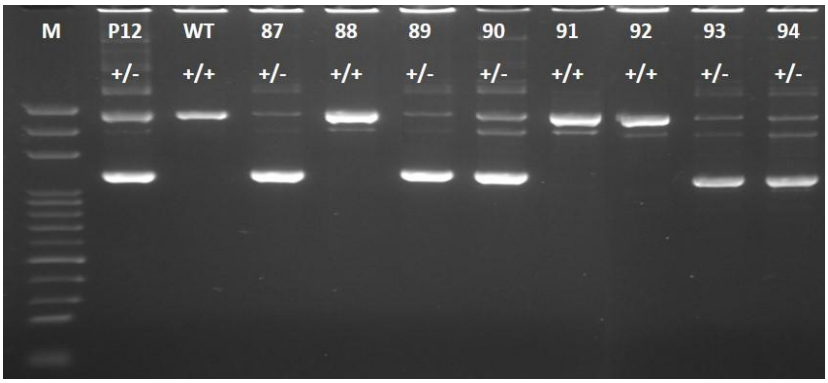
The information of polymerase, including company name and catalog number：

TAK R050B 1,000 Units/PK PRIMESTAR GXL DNA POLYMERASE 試劑

PCR Product

	Primer set	Product size	Target region
1	Wwox 5F/ Wwox 3B	2601 bp	Wild type allele
2	Wwox 5F/ Wwox 3B	≤1187 bp	Knockout allele

D. Gel Photo



*1.5% TBE gel