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.	RMRC13435	Strain Name	C57BL/6-*Wwox*^{em1Wdcw}/Narl

A. Primer

	Primer name	Sequence
1	Wwox 3F	5’-CCAGGGAAGTCTTGGAAACCTC-3’
2	Wwox 3B	5’-GGTTGGAGGCAGATAGACATCTTG-3’
3	Wwox 5F	5’-GGTATGACTTTGTGGGTGTTGGC-3’
4	Wwox 5B	5’-AGGGGGGAAAGGTTCTGATGAG-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	1min	
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				

PCR product by Set I digested with Psi I (2hr.37℃)

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

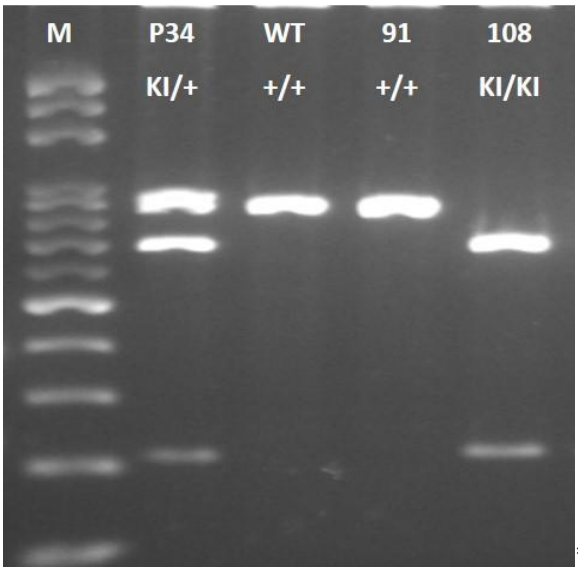
TAK R050B 1,000 Units/PK PRIMESTAR GXL DNA POLYMERASE 試劑

PCR Product

	Primer set	Product size	Target region
1	Wwox 3F/ Wwox 3B	890 bp	Wild type allele

2	Wwox 3F/ Wwox 3B	915 bp	Floxed allele
PCR product by Set I digested with Psi I			
1		890 bp	Wild type allele
2		212 + 703 bp	Floxed allele

D. Gel Photo



*2.5% TBE gel

Step 2.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	1min	
Primer 3	20	µM(pmol/µl)	0.4	µl	5	68	10min	
Primer 4	20	µM(pmol/µl)	0.4	µl	6	20	∞	1
DNA	--		1	µl				
Total			20	µl				

PCR product by Set I digested with Psi I (2hr.37℃)

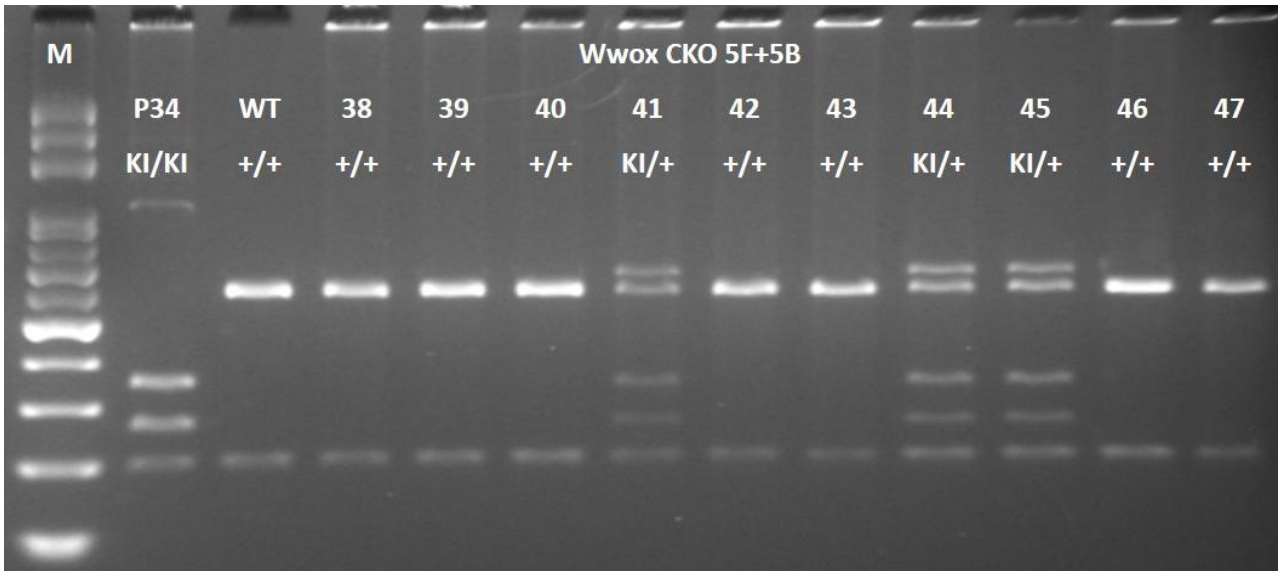
The information of Taq, including company name and catalog number：

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

PCR Product

	Primer set	Product size	Target region
1	Wwox 5F/ Wwox 5B	815 bp	Wild type allele
2	Wwox 5F/ Wwox 5B	839 bp	Floxed allele
PCR product by Set I digested with Psi I			
1		204 + 611 bp	Wild type allele
2		204 + 277 + 358 bp	Floxed allele

E. Gel Photo



*2.5% TBE gel