

RMRC Genotyping Protocol-PCR

No.	RMRC13147	Strain Name	C57BL/6-Nme6<tm1LjN6>
-----	-----------	-------------	-----------------------

A. Primer

	Primer name	Sequence	
1	Check WT primer	F:GTACTCAACATCTGGACCCTAACAC	R:ACTGACATCTAAGGTTGTCCTCTTG
2	Check KO primer	F:CTGCATTCTAGTTGTGGTTTGTCCA	R:GCTGTCAATCTCTCCATGTGGATAAC

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		11	μl	1	95	300	1
PCR buffer	5x		4	μl	2	95	30	35
dNTP	2.5	mM	1.7	μl	3	55	30	35
Primer 1	10	μM(μmol/μl)	1	μl	4	72	30	35
Primer 2	10	μM(μmol/μl)	1	μl	5	72	120	1
DNA	--		1	μl	6	7	∞	1
Taq	0.4μl of enzyme per 20 μl reaction		0.3	μl	7			
Mg ²⁺	No add	mM	0	μl	8			
Total			20	μl	9			

C. PCR Product

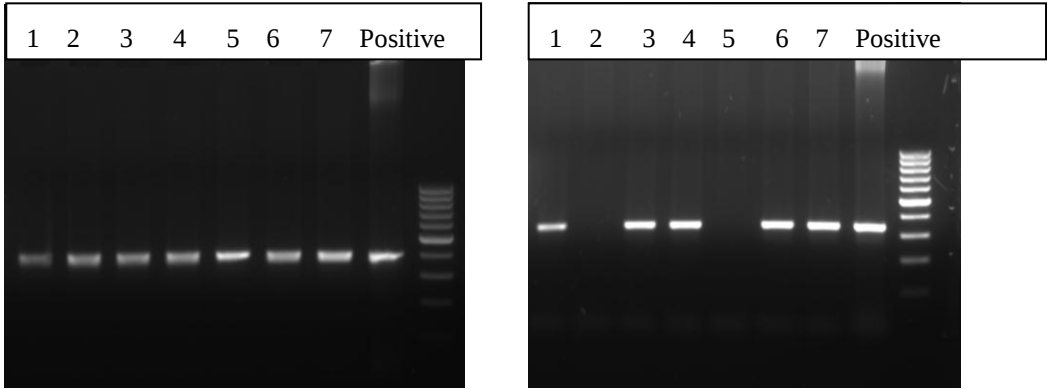
	Primer set	Product size	Target region	
1	WT-F	WT-R	376 bp	CTCTTCAAGACCTCCCTCCTGATGATGCCTCCTTGAGGAAGGACACCT TCTCAAGTTAACACGGCCCTGCGAAAGTGTGGTCAGCTGCCCTTAACA CATGAGCGTAGGTGGCGGGGTACTCAACATCTGGACCCTAACACTCCT CAGCAGCACCGACACTTCAGCGATCCTTTGGATAGCTTGATTGATTCT CATCTCTGGGGTGGTTGGTTCTCCAGGCTGTTTCATCAGCAGATTCTGA GCAACAAGTTCCTCATTGTACGAACGAGGGAAGTGCAGTGGAAGCTG GAGGACTGCCGGAGGTTTTACCGAGAGCATGAAGGTGAGGCTGGTGG TCTTCGTTACGGTGTAGACATGAGCACACACGTGTGCATGGTGTGCAT ATGTGTGGTGTGTGTGTGTGTGCGTGCGCATGTATGTGGTGCACATGC ATATATATGTGAAGTGTACAGGTGTACATGTACAGGCAAGAGGACAAC

				CTTAGATGTCAGT
2	KO-F	KO-R	276 bp	CTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGT CTGGATCCGGGGGTACCGCGTCGAGAAGTTCCTATTCCGAAGTTCCTA TTCTCTAGAAAGTATAGGAACTTCGTCGAGATAACTTCGTATAGCATA ATTATACGAAGTTATGTCTGAGATATCTAGACCCAGCTTTCTTGTACAAA GTGGTTGATATCTCTATAGTCGCAGTAGGCGGAAGGACGTCTTGGGGC CAGTGAGTTATCCACATGGAGAGATTGACAGC

D. Gel Photo

WT primer

KO primer



以上述結果得知 1、3、4、6、7 號老鼠是有帶有 KO gene 的 Heterologous Mice