

RMRC Genotyping Protocol-PCR

種原編號	RMRC13062	品系名稱	C57BL/6- <i>Il7</i> ^{m1.Jy}
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A. Primer

	Primer name	Sequence
1	IL-7 MT	5'-GAA AAC ATG TAT GTG ATG ATA CAA AGA-3'
2	IL-7 WMT	5'-CAA ATG TCA AAG AGC GTC TCT TGC TTC-3'
3	IL-7 WT	5'-GAA AAC ATG TAT GTG ATG ATA CAA AGG-3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		16	μl	1	94	300	1
PCR buffer	10x		2.5	μl	2	94	30	5
dNTP	2.5	mM	2	μl	3	63	60	
Primer 1	20	μM(μmol/μl)	0.5	μl	4	72	40	
Primer 2	20	μM(μmol/μl)	0.5	μl	5	94	30	5
DNA	--		2	μl	6	61	60	
Taq	5	U/μl	0.2	μl	7	72	40	
DMSO	-	mM	1.25	μl	8	94	30	5
Total	25	μl			9	59	60	
					10	72	40	
					11	94	30	20
					12	57	60	
					13	72	40	

C. PCR Product

	Primer set		Product size	Target region
1	IL-7 MT	IL-7 WMT	500 bp	For IL-7 mutant
2	IL-7 WT	IL-7 WMT	500 bp	For IL-7 wildtype

D. Gel Photo

