

RMRC Genotyping Protocol-PCR

種原編號	RMRC13069	品系名稱	C57BL/6-Tg(CD3ε)F14CD3-3Abgn
------	-----------	------	------------------------------

A. Primer

	Primer name	Sequence
1	Enhancer sense	AGAGGACGGTGGCGGTGGAA
2	Enhancer antisense	AGCCCAAGGCTGAGGGGGAG
3	Exon 6 sense	AAGTCTCCATCTCTGGAACCACAGTAA
4	Exon 6 antisense	TCTGGTTTGCTTCCTCTGGGGT

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		14.1	μl	1	94	300	1
PCR buffer	10x		2	μl	2	94	30	
dNTP	10	mM	0.4	μl		55	30	
Primer 1	10	μM(μmol/μl)	0.4	μl		72	45	35
Primer 2	10	μM(μmol/μl)	0.4	μl	3	72	420	1
DNA	5ng/ul~10ng/ul		2	μl				
Taq	5	U/μl	0.1	μl				
Mg ²⁺	50	mM	0.6	μl				
Total			20	μl				

C. PCR Product

	Primer set		Product size	Target region
1	Enhancer sense	Enhancer antisense	476 bp	hCD3ε Enhancer
2	Exon 6 sense	Exon 6 antisense	208 bp	hCD3ε Exon 6

D. Gel Photo

