

RMRC Genotyping Protocol-PCR

No.	RMRC13367	Strain Name	C57BL/6J- <i>Fut8</i> ^{em1.2Rbya} /Narl
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A. Primer

	Primer name	Sequence
1	Fut8 5VF2	5'- ATCTCTCAACCATGTGGCTG -3'
2	Fut8 5VR2	5'- CCTTCATAGCAACTCTAAGG -3'
3	Fut8 3VR1	5'- ACTTCCCTACAGTTAAACAGGCA-3'

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		18.75	μl	1	95	300	1
PCR buffer	10x		2.5	μl	2	95	30	35
dNTP	10	mM	2.0	μl	3	58	30	
Primer 1	10	μM(ρmol/μl)	0.5	μl	4	72	30	
Primer 2	10	μM(ρmol/μl)	0.5	μl	5	72	420	1
DNA	--		0.5	μl	6	4	∞	-
Taq	5	U/μl	0.25	μl	7			
Mg ²⁺	-	mM	-	μl	8			
Total			25	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	Fut8 5VF2	Fut8 5VR2	217 bp (WT)	5'LoxP site
2	Fut8 5VF2	Fut8 3VR1	1430 bp (WT) 418 bp (deletion)	5'LoxP site+3'LoxP site

D. Gel Photo

