

## RMRC Genotyping Protocol-PCR

No.	RMRC13366	Strain Name	C57BL/6J- <i>Fut8<sup>em1.1Rbya</sup></i> /Narl
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### A. Primer

	Primer name	Sequence
1	Fut8 5VF2	5'- ATCTCTCAACCATGTGGCTG -3'
2	Fut8 5VR2	5'- CCTTCATAGCAACTCTAAGG -3'
3	Fut8 3VF1	5'- ACATGAAACAGGTCCAATCTCCT-3'
4	Fut8 3VR1	5'- ACTTTCCTACAGTTAAACAGGCA-3'

### B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H <sub>2</sub> O	--	18.75 $\mu$ l	1	95	300	1
PCR buffer	10x	2.5 $\mu$ l	2	95	30	35
dNTP	10 mM	2.0 $\mu$ l	3	58	30	
Primer 1	10 $\mu$ M( $\rho$ mol/ $\mu$ l)	0.5 $\mu$ l	4	72	30	
Primer 2	10 $\mu$ M( $\rho$ mol/ $\mu$ l)	0.5 $\mu$ l	5	72	420	1
DNA	--	0.5 $\mu$ l	6	4	$\infty$	-
Taq	5 U/ $\mu$ l	0.25 $\mu$ l	7			
Mg <sup>2+</sup>	- mM	- $\mu$ l	8			
Total		25 $\mu$ l	9			

### C. PCR Product

	Primer set	Product size	Target region
1	Fut8 5VF2 Fut8 5VR2	217 bp (WT) 257 bp (floxed)	5' LoxP site
2	Fut8 3VF1 Fut8 3VR1	437 bp (WT) 477 bp (floxed)	3' LoxP site

### D. Gel Photo

