

RMRC Genotyping Protocol-PCR

No.	RMRC13334	Strain Name	C57BL/6N- <i>Fbxo40</i> ^{mlaNarl} /Narl
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A. Primers

No.	Primer name	Sequence
1	Fbxo40LF	GCCGTTTGATAAACGCACCCAAGTT
2	Fbxo40LR	CAGAACCTTCCAGTTGAACTCCCTT

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	5.9 μl	1	94	80	1
Primer 1	20 μM (pmol/μl)	0.3 μl	2	94	15	35
Primer 2	20 μM (pmol/μl)	0.3 μl	3	60	15	35
gDNA	50-250 ng/μl	1 μl	4	68	30	35
OneTaq*	2X Master Mix	7.5 μl	5	68	60	1
Total		15 μl				

*M0488 OneTaq Hot Start Quick-Load 2X Master Mix with Standard Buffer from New England Biolabs.

C. PCR Product

No.	Primer set		Product size	Target region
1	Fbxo40LF	Fbxo40LR	200 bp	Wildtype
2	Fbxo40LF	Fbxo40LR	300 bp	Mutant

D. Gel Photo

