

RMRC Genotyping Protocol-PCR

No.	RMRC13137	Strain Name	FVBB6-Myo15<R2039X>/Hcj
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A. Primer

	Primer name	Sequence
1	Myo15-R2039X-F	CGTGTGTCACACTTCCCCTTGACATCGA
2	Myo15-R2039X-R	ACCTCCTAGTCAGGTCTT

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		7.9	μl	1	95	300	1
PCR buffer	10x		1.25	μl	2	95	30	35
dNTP	2	mM	1.25	μl	3	50	30	
Primer 1	10	μM(μmol/μl)	0.5	μl	4	72	30	
Primer 2	10	μM(μmol/μl)	0.5	μl	5	72	300	1
DNA	--		0.5	μl	6			
Taq	2	U/μl	0.1	μl	7			
Mg ²⁺	25	mM	0.5	μl	8			
Total			12.5	μl	9			

C. PCR Product

	Primer set	Product size	Target region
1		231bp	The PCR product contains Myo15 R2039X site.

D. RFLP

Component	Concentration		Volume		Step	Tempera-ture, °C	Time, hr	Number of Cycles
H ₂ O	--		4.95	μl	1	65	2~16hr	1
NEBuffer4	10x		1.5	μl	Myo15 C6115T (R2039X) TT(circling): 206, 25bp TC(normal): 206, 178, 28, 25bp CC(normal): 178, 28, 25bp			
BSA	1	mg/ml	1.5	μl				
TaqαI (New England BioLabs)	20000	U/ml	0.05	μl				
PCR product	--		7	μl				
Total			15	μl				

E. Gel Photo

Myo15

(Nevra et al., 2007)

FIGURE 1. D

