

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	15231
MMRRC Strain Name	B6.129S4(FVB)- <i>F2rl3</i> ^{tm1.1Cgh} /Mmnc
Gene Name(s)	coagulation factor II receptor-like 3 (<i>F2rl3</i>)
Breeding Protocol(s)	Intra-Strain breeding Backcross to C57BL/6J
Protocol Date	10/201/13

MMRRC# 15231 PCR Reaction

NOTE: Run two, 2-primer reactions: (Par4 & WT R) and (Par4 & KO R) **RUN SEPARATELY.**

Denaturing: 95°C for 60 sec
 Annealing Temp: 62°C for 90 sec
 Extension: 72°C for 90 sec
 Cycles: 30

Taq: **Apex and Chromataq Buffer**

Bands: WT: 600bp
 MUTANT: 380bp

	1X
DNA	0.5
10X Buffer	2.5
10mM dNTPs	1.0
20 uM Primer 1 & 2	2.0
Taq	1.0
ddH ₂ O	17.5
25mM MgCl ₂	1.5

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

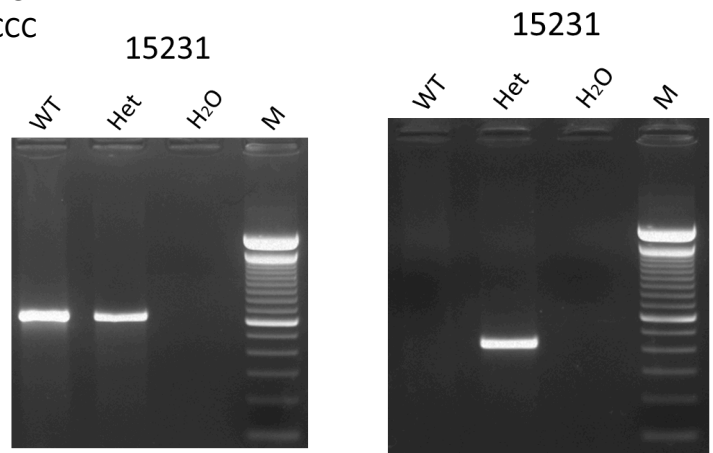
PAR4 Forward: CAGATGTTTCCTGGGCTGGGTG

lacZ Wt reverse: ATTGTGGGTGCCTCAGTGTCCC

KO reverse:(LACZ5'R):

CAGGGTTTTCCAGTCACGACG

Run on 2% agarose gel in TAE.



Primers: Par4 & WT R

Primers: Par4 & KO R

M: 100 bp DNA ladder (Invitrogen)

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