

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11651
MMRRC Strain Name	B6;129S5- <i>Rab6b</i> ^{Gt(Betageo)300Lex} /Mmnc
Gene Name(s)	RAB6B, member RAS oncogene family (Rab6b)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	6/26/14

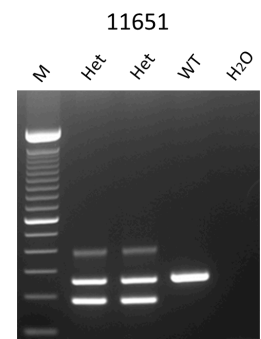
MMRRC #11651 PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

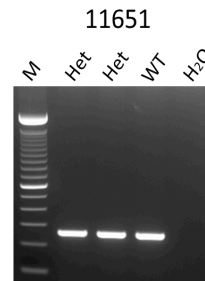
Taq: Apex and Chromataq 5X Buffer

Thermal Cycler:

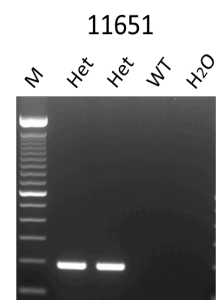
- Step 1: 94°C for 5 min
- Step 2: 94°C for 15 sec
- Step 3: 65° for 30 sec; Decrease 1°C /cycle
- Step 4: 72°C for 40 sec
- Step 5: go to step 2, 10 cycles
- Step 6: 94°C for 15 sec
- Step 7: 55°C for 30 sec
- Step 8: 72°C for 40 sec
- Step 9: go to step 6 to 8, 30 cycles
- Step 10: 72°C for 7 min



11651
M Het Het WT H₂O
Primers: NIH (11651) 5' + TM-3' (11651) + NIH (11651) LTR
M: 100 bp DNA ladder (Invitrogen)



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Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

NIH (11651) LTR: AAATGGCGTTACTTAAGCTAGCTTGC

NIH (11651) 5': TAGAGTGACCTTGAAGGCAGAGC

TM-3' (11651): CCTGCCCTGTCCCTTTCTTGT

Bands expected: WT [NIH (11651) 5' + TM-3' (11651)]: 261 bp

Mutant [NIH (11651) LTR + TM-3' (11651)]: 190 bp

~400-bp faint band appeared if the three-primer reaction was applied.

Run on 1% agarose gel in TAE.