

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36711
MMRRC Strain Name	B6;129S7- <i>Nobox^{tm1Rajk}</i> /Mmnc
Gene Name(s)	NOBOX oogenesis homeobox; targeted mutation 1, Aleksandar Rajkovic (<i>Nobox^{tm1Rajk}</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	8/5/13

MMRRC #36711 PCR Reaction

	<u>1X</u>
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

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 62°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 5: Step 2 to 4; Cycles: 30
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

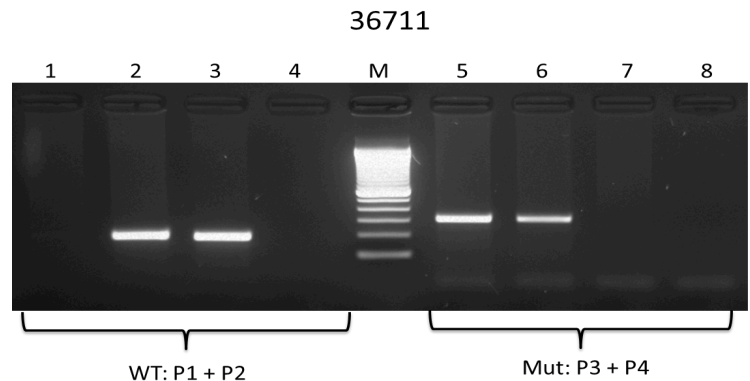
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

P1 (36711): GCC AGT CCA TAG CCT CTC AC
 P2 (36711): CGG GTC CTC ACT AAG CAA AA
 P3 (36711): CTT GGA CGT TGG GGT CTC TA
 P4 (36711): GCA GTG TTG GCT GTA TTT TCC

Bands expected:

WT (primers P1 + P2) 200 bp
 MUTANT (primers P3 + P4) 300 bp

Run on 2% agarose gel in TAE



Lane 1, 5: Hom; Lane 2, 6: Het; Lane 3, 7: WT; Lane 4, 8: H₂O;
 M: 50 bp DNA ladder Invitrogen