

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36709
MMRRC Strain Name	B6;129S7- <i>Sohlh1</i> ^{tm1Rajk} /Mmnc
Gene Name(s)	spermatogenesis and oogenesis specific basic helix-loop-helix 1 (<i>Sohlh1</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	9/7/13

MMRRC #36709 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 50°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 5: Step 2 to 4; Cycles: 29 for Mut Rxn (G3 + HPRT2) and 25 for WT Rxn (G1 + RefG2)
 Step 6: 72°C for 7 min

	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: Denville Taq and Chromataq Buffer

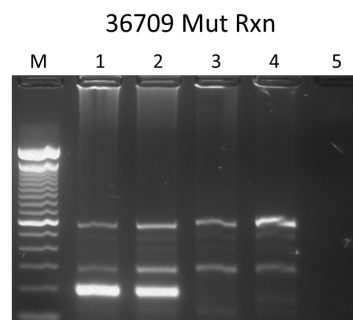
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

G1 (36709): GAG TCT CTG GCA TTA CGG GAT
 RefG2 (36709): CTG AGT CTC AGG CTG AGG AG
 G3 (36709): CTG GAG CCC AAG AAG ACA AG
 HPRT2 (36709): GCA GTG TTG GCT GTA TTT TCC

Bands expected: WT (primers G1 + RefG2): 328 bp
 MUTANT (primers G3 + HPRT2): 220 bp

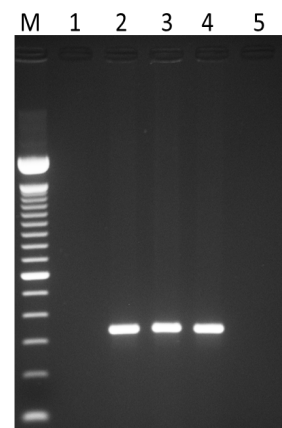
[Note: there is potential for two non-specific amplification bands (~290 bp and ~590 bp)].

Run on 2% agarose gel in TAE.



Mut, primers G3 + HPRT2
 Lane 1: Hom; Lane 2: Het; Lane 3, 4: WT; Lane 5: H₂O;
 M: 100 bp DNA ladder (Invitrogen)

36709 WT Rxn



WT, primers G1 + RefG2
 Lane 1: Hom; Lane 2: Het; Lane 3, 4: WT; Lane 5: H₂O;
 M: 100 bp DNA ladder (Invitrogen)