

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11389
MMRRC Strain Name	B6;129S2- <i>Mycn</i> ^{tm1Par} /Mmnc
Gene Name(s)	V-myc cyclotomatosis viral related oncogene, neuroblastoma derived (<i>Mycn</i>)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	2/24/14

MMRRC #11389 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: 60°C for 30 sec

Step 4: 72°C for 45 sec

Step 5: 32x from step 2 to step 4

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.

Com-F (11389): TGTGGGGAGCTTGCAGAAGAGATT

WT-R (11389): CATCTCCGTAGCCCAATTCGAAGG

Mut-R (11389): AGAGCAGCCGATTGTCTGTTGTGC

Bands expected:

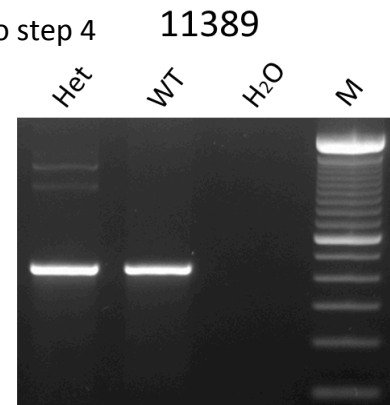
WT: [Com-F (11389) + WT-R (11389)]: 434 bp

MUTANT: 1.3-kb (longer incubation times and/or use of rapid polymerase may amplify a ~1.3 kb band from the KO allele.)

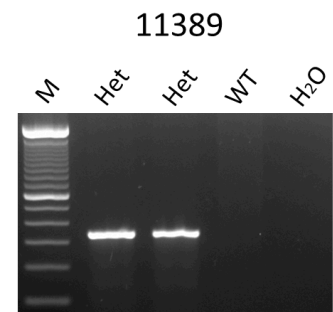
MUTANT: [Com-F (11389) + Mut-R (11389)]: 356 bp

WT: no band

Run on 2% agarose gel in TAE.



Primers: Com-F (11389) + WT-R (11389)
M: 100 bp DNA ladder (Invitrogen)



Primers: Com-F (11389) + Mut-R (11389)
M: 100 bp DNA ladder (Invitrogen)