

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30023
MMRRC Strain Name	B6.129X1- <i>Hras1</i> ^{tm1Esn} /Mmnc
Gene Name(s)	Harvey rat sarcoma virus oncogene 1 (<i>Hras1</i>)
Breeding Protocol(s)	Backcross
Protocol Date	8/25/14

MMRRC #30023 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 45 sec

Step 3: 62°C for 45 sec

Step 4: 72°C for 60 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: MUTANT: 336bp WT: 434bp

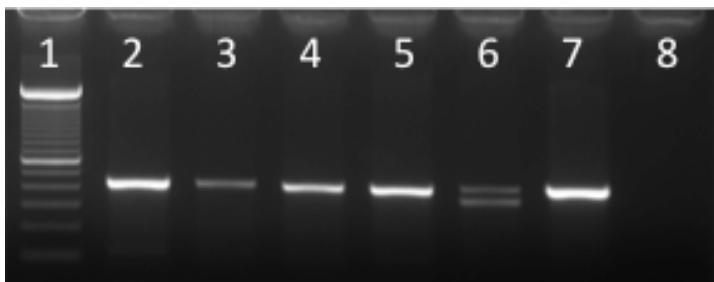
Primer sequences 5' to 3':

Hras(30023)WT: ACC TGC CAA TGA GAA GCA CAC TTA GCC

Hras(30023)Com: ATA GTT GTA GGT TGC ACC CAC ATG CCG

Hras(30023)Mut: CTA CCG GTG GAT GTG GAA TGT GTG CGA

Run on 2% agarose gel.



1. 100bp Ladder
2. Wild-Type Sample
3. Wild-Type Sample
4. Wild-Type Sample
5. Wild-Type Sample
6. Heterozygous Sample
7. Wild-Type Control
8. Negative Control