

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	43604
MMRRC Strain Name	B6J.129P2(Cg)- <i>Nras</i> ^{tm1.1Nesh} Tg(Tyr-cre/ERT2)13Bos/Mmnc
Gene Name(s)	neuroblastoma ras oncogene, tyrosinase, cre tamoxifen-dependent recombinase
Breeding Protocol(s)	Sib-mating
Protocol Date	08/30/2019

PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

3, 2 Primer Reactions

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 30sec

Step 3: 60C(Nras) or 57C(Tyr-Cre), 30sec

Step 4: 72C, 30sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex and Chromataq 5X Buffer

Bands: MUT: 371bp (Nras1 P1+P3), ~180bp (Tyr-Cre 1+IVSR)

WT: 487bp (Nras1 P2+P3), No Band (Tyr-Cre 1+IVSR)

Primer sequences 5' to 3':

Nras(43604)P1 GCAAGAGGCCCGGCAGTACCTA

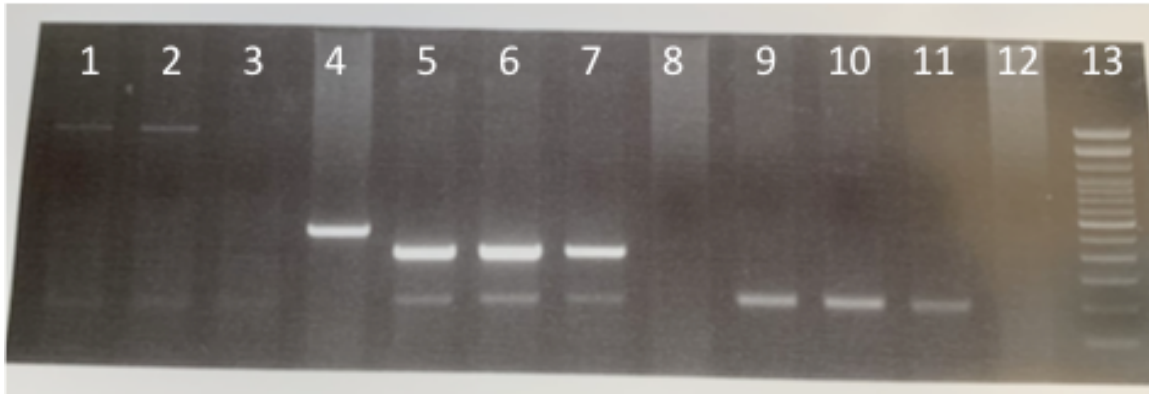
Nras(43604)P2 AGACGCGGAGACTTGGCGAGC

Nras(43604)P3 GCTGGATCGTCAAGGCGCTTTTCC

TyrCre(43604)1 TGATAGTCACTCCAGGGGTTG

TyrCre(43604)IVSR CGA TCC GGA GCT TTT TG

Run on 2.0% agarose gel in TAE.



Nras-tm1 Wild-type Reaction

1. Homozygous Sample
2. Homozygous Sample
3. Homozygous Sample
4. Wild-type Control

Nras-tm1 Mutant Reaction

5. Homozygous Sample
6. Homozygous Sample
7. Homozygous Sample
8. Wild-type Control

Tyr-Cre Reaction

9. Tyr-Cre Positive Sample
10. Tyr-Cre Positive Sample
11. Tyr-Cre Positive Sample
12. Wild-type Control

13. 100bp Marker (Invitrogen)