

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	362
MMRRC Strain Name	B6.129X1-Trp53tm1Ldo/Mmnc
Gene Name(s)	Transformation related protein 53 (Trp53)
Breeding Protocol(s)	Intra-strain mating (homozygous lethal)
Protocol Date	9/9/10

MMRRC# 362 PCR Reaction

NOTE: Sample Prep – Lysis buffer recipe

50mM Tris pH 8.8

1.0mM EDTA

0.5% Tween-20

Immediately before using, add 200ug/ml proK

1. Spin down sample in tub, add 100ul lysis buffer for ear punch, 200ul for 3-4mm tail snip.
2. Digest at 56C for twelve hours, tap down condensation, then inactivate at 80C for 10 minutes.
3. Tap spin down debris - usually 8000 rpm for 5 seconds will do it. Let samples cool to at least room temperature before doing a PCR.

Thermal Cycler:

95°C for 5 min

94°C for 45 sec

60°C for 1 min (35 cycles)

72°C for 1 min

72°C for 5 min

Taq: **Apex and Chromataq 10X Buffer**

	1X
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH ₂ O	15.5
25mM MgCl ₂	1.5

Bands: MUTANT: ~530bp, ~480bp and ~50bp

Primers are 10uM with respect to each primer (20uM total).

Primer sequences 5' to 3':

p53MF: CGG CTC TGA GTA TAC CAC CAT CCA C

p53MR: GCC TGC GTA CCT CTC TTT GCG CTC C

Set up a restriction enzyme digestion with 25uL of PCR product as shown below

Restriction Digest:

4uL Buffer

1uL Stu1

10uL ddH₂O

Add 15uL of mix to 25uL of PCR product for a total volume of 40uL.

Digest at 37C overnight (8 hours), 65C for 20 min., hold at 4C.

Load 15 uL of each sample's cut PCR product and run on 3.0% agarose gel in TAE.
(The fragments may run fast or anomalously on a 2% agarose gel.)