

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	204
MMRRC Strain Name	B6.129X1- <i>Wrn</i> ^{tm1Lgu} /Mmnc
Gene Name(s)	Werner syndrome homolog (<i>Wrn</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	6/10/11

MMRRC# 204 PCR Reaction

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	1.0
10 uM Primer F/R	1/1
Taq	0.5
ddH ₂ O	12.5
25mM MgCl ₂	2.0

NOTE: Run 2 reactions for each sample (P1+P2 for wild-type) and (P2+P3 for mutant).

Thermal Cycler:

94°C for 60 sec
 55°C for 60 sec
 72°C for 30 sec
 35x

Taq: Apex and Chromataq 5X Buffer

Bands: MUTANT: 300bp
 WT: 213bp

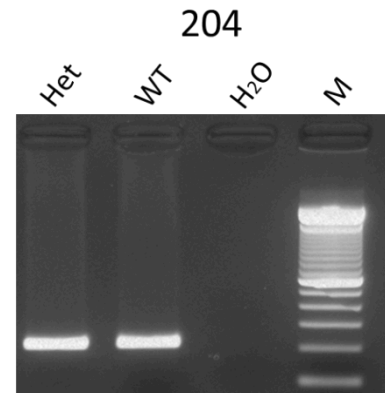
Primer sequences 5' to 3':

P1(204): caataaccaatggaattctaagc

P2(204): tcaaattttatcccaacccttag

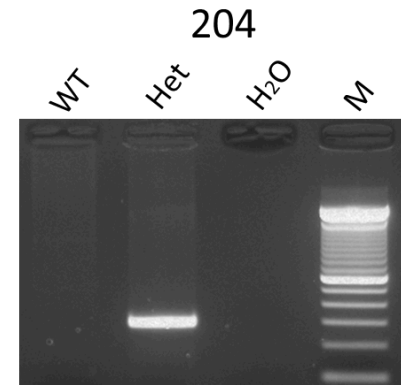
P3(204): gcctgcagctggcgccatc

Run on 2.0% agarose gel in TAE.



Primers: P1 + P2

M: 100 bp DNA ladder (Invitrogen)



Primers: P2 + P3

M: 100 bp DNA ladder (Invitrogen)