

08.05.10 MS
10.08.13 MLS

Genotyping Protocol: **MMRRC 130**

Strain Characteristics: Random integration of human WRN cDNA containing the K577M mutation

Assay Type: PCR - cannot distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat#69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Primer Information:

- 1) Name: A-wrn Sequence: 5'-ACT GGC AAG GAT CAA ACA GAG-3'
- 2) Name: P-wrn Sequence: 5'-CTG GTG ACT GTA CCA TGA TAC-3'

Primer location: *Homo sapiens* Werner syndrome (WRN) coding region

Assay Name: Werners Transgene PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
A-wrn	IDT	25µM	0.3
P-wrn	IDT	25µM	0.3
sterile water	Fisher		5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 5 minutes
- 2) 94°C 30 seconds
- 3) 60°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7minutes
- 7) 4°C hold until refrigerate product

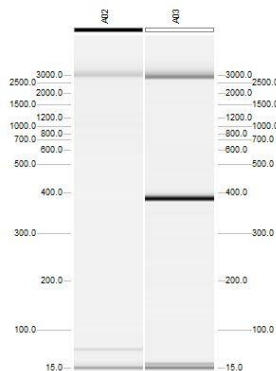
Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Expected products:

Transgene positive: 388 bp
Transgene negative: no product

Example gel:



Lane A02 displays a transgene negative sample (no product)
Lane A03 displays a transgene positive sample (388bp product)

*Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected