

## H/M Genotyping Protocol: MMRRC Line 130

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

**Assay Type:** PCR - can not distinguish heterozygous animals from homozygous animals

**DNA Extraction:** DNA from tail snips was extracted using Qiagen's DNeasy kit. 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: ACT GGC AAG GAT CAA ACA GAG  
2) Name: P-wrn                      Sequence: CTG GTG ACT GTA CCA TGA TAC

Primer location:            A-wrn = Homo sapiens Werner syndrome (WRN) mRNA  
   P-wrn = Homo sapiens Werner syndrome (WRN) mRNA

### PCR Master Mix Components:

#### Master Mix:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
A-wrn	IDT	25µM	0.3
P-wrn	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

### PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template

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 product: 388 bp