

Werner's Transgenic Genotyping Protocol

Description:

Mice containing the human Werner's cDNA (including those with point mutations such as K577M) can be genotyped with this protocol. Amplified product should be approximately **0.4 KB**.

Primers:

A-wrn 5' - ACT GGC AAG GAT CAA ACA GAG -3'

P-wrn 5' - CTG GTG ACT GTA CCA TGA TAC -3'

Reaction Mix (For one reaction):

H2O	13.8ul
10X PCR Buffer	1.5ul
25mM MgCl ₂	4.0ul
25uM A-wrn 5'Primer	1.0ul
25uM P-wrn 3'Primer	1.0ul
2mM dNTP	2.5ul
Taq Polymerase	0.2ul

*Hot start: Add 1ul 10X Buffer and 1ul DNA to PCR tube. Overlay with 30ul mineral oil. Start reaction. At pause add 23ul reaction mix to tube, under oil. Continue reaction.

Thermocycler Settings:

100C for 5min

72C Pause

94C for 5 min

cycle 35 times

94C for 30 sec

60C for 30 sec

72C for 30 sec

72C for 7 min

4C forever

Electrophoresis:

Run on 2% Gel