

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	71750
MMRRC Strain Name	C57BL/6J- <i>Rtel</i> ^{em1^{Khk}} /Mmnc
Gene Name(s)	regulator of telomere elongation helicase 1
Breeding Protocol(s)	Sib-mating
Protocol Date	2/25/2025

PCR Reaction

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

Note: Strain must be sequenced to determine genotype

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 57C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Band: 216bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

Rtel(71750)F: ccagtcctgtcctcataccc

Rtel(71750)R: CCACCCACCATGTTCTAGT



Sanger sequencing must be used to determine genotype. Once the PCR reaction is completed, send amplicon to be sequenced.

Primer Used: Rtel(71750)F: ccagtcctgtcctcataccc

The mutation is ATG (Met)-> AAG (Lys)

Mutation occurs at position ~130bp (AGA[T/A]GCAG)