

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	69572
MMRRC Strain Name	129S1.Cg- <i>Rbn2</i> ^{tm1Rmz} /RmzMmnc
Gene Name(s)	fibrillin 2
Breeding Protocol(s)	Backcross
Protocol Date	7/12/24

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

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 55C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq

Bands: WT: No Band MUTANT: ~600bp

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

Fbn2(69572)F: GGG GAA CTT CCT GAC TAG GG

Fbn2(69572)R: CCA AAT CCA TTC CTC CTT GA

Note from the donating investigator: Homozygous mice are born with contractures of carpal, metacarpal, and phalangeal joints in the forelimbs (disappear within a few days)



of post-natal life) and can be designated as knockout mice with this feature alone. Homozygous mice also display bilateral syndactyly, which is easily observable when the mice are of weaning age.

To genotype wild-type or heterozygous mice use the PCR protocol. Heterozygous mice will show a band but no contracture or syndactyly; while wild-type mice will show no banding, contracture or syndactyly.