

Genotyping Protocol

PI: Antonis Hatzopoulos

Mouse line: Grem2KO mix or Grem2KO 129Sv

Genotyping Primers:

Primer 1 For: CTGTGCAGCAGAGAAAGCTG

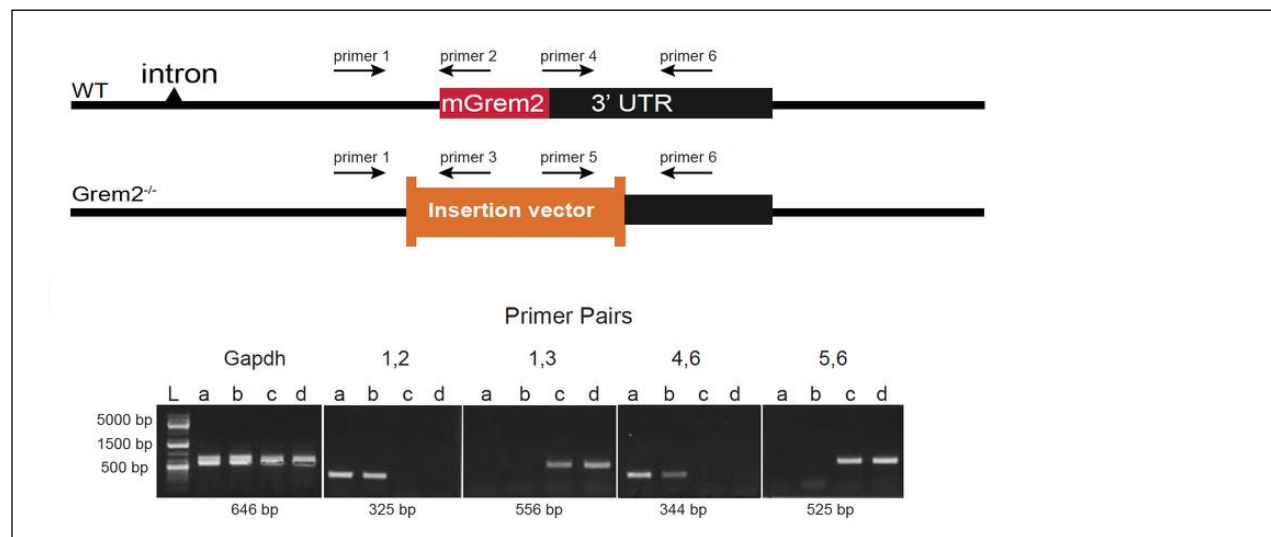
Primer 2 Rev: TGGCAATGTACCTCATCTCA

Primer 3 Rev: CTGTCCATCTGCACGAGACT

Primer 4 For: TCTGGTACCCACGAGGACAAGC

Primer 5 For: GTCTGAGTAGGTGTCATTCTA

Primer 6 Rev: CACAGATCACTCGATGCTCT



PCR Protocol:

There are 6 primers to genotype the Grem2 KO line that form 4 primer pairs.

Primer #1 pairs with primer #2 to amplify the WT allele, and with primer #3 to amplify the KO allele at the 5' end of the gene locus.

Primer #6 pairs with primer #4 to amplify the WT allele, and with primer #5 to amplify the KO allele at the 3' end of the gene locus, as outlined in the image above.

H ₂ O	6 µl
10 uM primer (forward)	1 µl
10 uM primer (reverse)	1 µl
2X KAPA polymerase mix	10 µl
Tail DNA	2 µl
Total	20 µl