

## Genotyping of Matrix Metalloprotenase 2 Deficient (MMP-2<sup>-/-</sup>) Mice by PCR

**Purpose:** To identify the MMP2 deficient mouse gene from wild type gene.

**Gene Information:** The MMP-2 gene is located on the 8 chromosome. In MMP-2 <sup>-/-</sup> mice, the exon 1 of MMP-2 gene is exchanged to reverse pgk-neo gene. The antisense primers of neo gene and exon 2 of MMP-2 gene can bind to the mutant allele and amplificatae. The null PCR fragment is 1.1 Kb length. The wild type primers bind to exon 1 of MMP-2 gene. The wild type PCR fragment is 120 bp length.

**Primers:** For mutant allele  
GelAEx2.A2:TGTATGTGATCTGGTTCTTG  
NeoSE.A1:TGCAAAGCGCATGCTCCAGA  
For wild allele  
GelAEx1.S2: CAACGATGGAGGCACGAGTG  
GelAEx1.A1: GCCGGGGA ACTTGATGATGG

### PCR:

**Reaction**

1. Genomic DNA (2µl)
2. Promega PCR Master Mix (2x; 10 µl)
3. Primers (100 pmol; 0.1 µl each for Ex1.S2, Ex1.A1 and .2 µl each for Ex2.A2 and NeoSE.A1)
4. Taq (0.3µl)
5. PCR water (8.1 µl) - final reaction volume = 20 µl

**Program**

1. 35 cycles- 94 °C for 1min, 57 °C for 1min, 72 °C for 1 min
2. 1 cycle - 72 °C for 7 min
3. Hold at 4 °C

### Expected bands on TBE agarose gel electrophoresis:

MMP2 +/+ = 120 bp  
MMP2 <sup>-/-</sup> = 1.1Kb

### Screening double KO mice:

This strategy may be used to detect the MMP-2 gene in double null or knockout mice, as one of the null primers are specific for the MMP-2 gene.

### Reference:

Takeshi Itoh, Shigeyoshi Itohara, et al. Unaltered secretion of B-amyloid precursor protein in gelatinase A (MMP-2)-deficient mice. J. Bio. Chem. 1997: 272; 22389-92

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