

Genotyping of Matrix Metalloprotenase-9 (MMP9) Deficient Mice by PCR

Purpose: To identify MMP9 deficient mouse gene from the wild type mouse gene.

Gene Information: The MMP9 gene contains 13 exons and 12 introns, and is 8190 nucleotides (nt) in length . The neomycin cassette is located in part of exon 2 and all of intron 2. The primers chosen for the PCR screen anneal at 146 nt (upstream of the neo cassette) and 420 nt (downstream of the neocassette). The wild-type PCR fragment is ~500 nt and the null fragment is ~500 nt + neo cassette length, approximately 1.6 kb.

Primers: MMP9-146 5'-ACTTGTACCGCTATGGTTACAC
MMP9-420 5'-GGCGTCATCGATCATGTC

PCR:

Reaction

1. Genomic DNA (1.0 μ l)
2. Invitrogen 5x Buffer J (4 μ l)
3. Primers (100 pmol; 0.35 μ l each)
4. Taq polymerase (0.3 μ l)
5. Invitrogen 10 mM dNTP Mix (2 μ l)
5. PCR water (12 μ l)

Note: For this PCR to work you must not batch more than three samples at a time.

Program

1. 1 cycle - 94°C for 2 min
2. 35 cycles - 94°C for 1 min, 56.2°C for 2 min, 72°C for 3 min
- 3 1 cycle - 72°C 7 min
- 4 Hold at 4°C.

Expected Bands on 1% TBE Agarose Gel Electrophoresis:

Wild-type band = ~500 bp

Null or knockout = ~1.6 kb

Screening Double Deficient Mice:

This strategy may be used to detect the MMP9 alleles in double null or knockout mice, as the primers are specific for the MMP9 gene and not the neomycin cassette.

Reference:

Masure *et al.* 1993. European J. Biochemistry, 218:129-141

File name: Z:\AAA PPG\Rateri Core\PCR protocols\MMP9 PCR screen.wpd

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Person updating: DR