

## PCR Screening for Osteopetrotic mutation

### Principle:

The Osteopetrotic gene is located on the mouse chromosome 3. The op mutation is a spontaneous frameshift mutation (a thymidine insertion 262 base pairs from ATG initiation site) that generates a stop codon 21 base pairs downstream in the MCSF (macrophage colony stimulating factor) gene. The PCR product contains 158 base pairs bands for both wild type and op mutant. To detect the op mutation, the PCR product is digested with MBO II, a restriction endonuclease that produces an ambiguous single base extension in the wild type fragment, generating two fragments, 136 and 22 base pairs. The wild type fragment is detected at 158 bp band, the op mutated at 136 bp and the heterozygous will have both 158 and 136 bands.

### Primers:

MCSF-MBO II-F: sense: 5'GCT ACC TAA AGA AGG CCT TTC T-3'  
MCSF-MBOII-R: antisense: 5' CTT GTT CTG CTC ATA GTC C- 3'

### PCR:

<u>Reaction</u>	1. Genomic DNA (7: 1)
	2. Promega PCR Mg free buffer (10X; 2: 1)
	3. Primers (100pmol/: 1; 0.5: 1 each)
	4. Promega 25 mM MgCl <sub>2</sub> 1.6: 1
	5. 10 mM DNTPs (Invitrogen) 0.4: 1
	6. Taq polymerase 0.5: 1
	7. PCR water (7.5: 1; to a final total reaction volume of 20: 1)
<u>Program</u>	1. 35 cycles- 95°C for 30 sec., 55°C for 30 sec., 72°C for 30 sec.
	2. 1 cycle - 72°C for 2 min.
	3. Hold at 15°C for 4.

### Expected bands on TBE agarose gel electrophoresis:

Wild-type band = 158 bp long.

Knock-out band = 158 bp long.

### DNA digestion with MBO II:

1. PCR product (4: 1)
2. Promega buffer ( B10X; 2: 1)
3. BSA (100X; 0.2: 1)
4. Restriction enzyme(MBO II 8U/: ; 0.5: 1)
5. PCR water(13.3 : 1; to a final volume of 20: 1)

### Reference:

The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating gene;Nature,(6274):442-4, May, 1990

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