

## Genotyping of Peroxisome Proliferator Activated Receptor-gamma Floxed (PPAR $\gamma$ /f) Mice by PCR

**Purpose:** To identify PPAR $\gamma$ /f mouse gene from C57BL/6 wild type mouse PPAR $\gamma$  gene.

### Gene Information:

The PPAR $\gamma$  gene is located on the chromosome number 6 at location 115,387,692 -115,456,020 (68,329bp) and is of 1769 bps in transcript length. It contains 7 exons encoding a protein of 505 amino acids (ENSMUSTO0000000450 Mouse ensembl ID) (genbank: AY071819). The floxed mice possess *loxP* sites on either side of exons 1 and 2 of the targeted gene.

### Primers:

**PPAR-g-F1** (intron 3) **5' - TGTAATGGAAGGGCAAAGG- 3'** (position: 20236-20255)

**PPAR-g-R1** (intron 3) **5' - TGGCTTCCAGTGCATAAGTT- 3'** (position: 20419-20401)

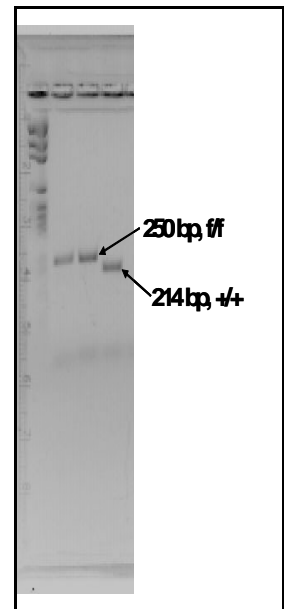
### PCR:

Reaction

1. Genomic DNA (1  $\mu$ l)
2. Promega PCR Master Mix (2x; 10  $\mu$ l)
3. Primers (100 pmol; 1.0  $\mu$ l each)
4. Taq polymerase (0.1  $\mu$ l)
5. PCR water (final reaction volume - 20  $\mu$ l)

Program

1. 1 cycle - 94°C 3 min
2. 35 cycles - 94°C for 30 sec,  
60°C for 1 min,  
72°C for 1 min
3. 1 cycle - 72°C 2 min
4. Hold at 4°C.



### Expected bands on TBE agarose gel electrophoresis:

Resolve DNA bands on a 2% agarose gel.

Wild-type band = 214 bp

Floxed band = 250 bp

### Reference:

He W. *et al.*, Adipose- specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. PNAS 2003: 100(26) 15712-15717.

File name: Z:\Protocols and forms\MOUSE\PCR protocols\mouse PPAR-g PCR.wpd

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Person updating: DR