

## Genotyping of Angiotensinogen<sup>fllox</sup> ( $\Delta$ floxed allele) by PCR

**Purpose:** To identify the presence of the angiotensinogen (agn) floxed gene (Lox P1 site and  $\Delta$ floxed allele).

**Chromosomal Location:** Angiotensinogen is located on Chromosome 8.

**Floxed gene information:** Targeting exon 2

Third loxP site is placed on 5' side of exon 2

LoxP flanked Neo is placed on 3' side of exon 2 (distance between loxP sites >1.68 kb)

Distance between single loxP and loxP flanked Neo = ~3.4 kb

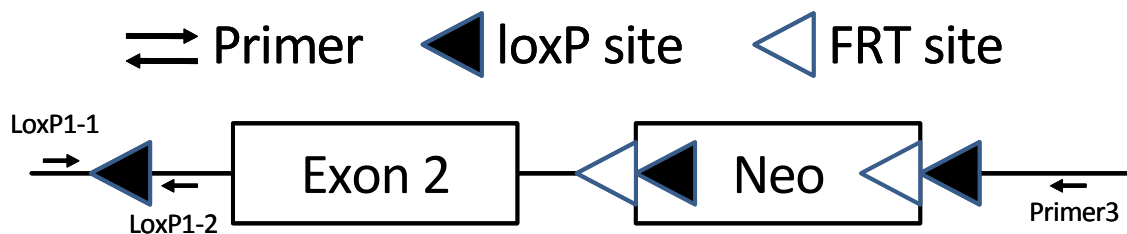
Target region is 1.67 kb and includes exon 2

**Primers:** Primer sets  
LoxP 1-1 = AAC CTT GTC TGG AGT GGG  
LoxP 1-2 = TCA GAG ATC CGT GGG AAC  
Primer 3 = TCC ACC TCA CAG GTA GCA ATG

Primer LoxP1-1 is upstream, LoxP1-2 is downstream from first Lox-P site, and Primer3 is downstream of the Neo cassette.

The location is based on the sequence with LoxP sites, above or in the target region containing LoxP1 site.

LoxP 1-1 and LoxP 1-2 detect a floxed allele (250 bp); LoxP 1-1 and Primer 3 detect either WT allele (188 bp) or  $\Delta$ floxed allele (362 bp)



**PCR:** Reaction  
3.5  $\mu$ l sterile H<sub>2</sub>O  
10  $\mu$ l buffer(2X green; Cat#M712C, Promega)  
Primers (concentration =10 pmol/1  $\mu$ l): LoxP 1-1: 2.5  $\mu$ l; LoxP 1-2: 1.5  $\mu$ l;  
Primer3: 1.5  $\mu$ l)  
1  $\mu$ l DNA

**Program:**

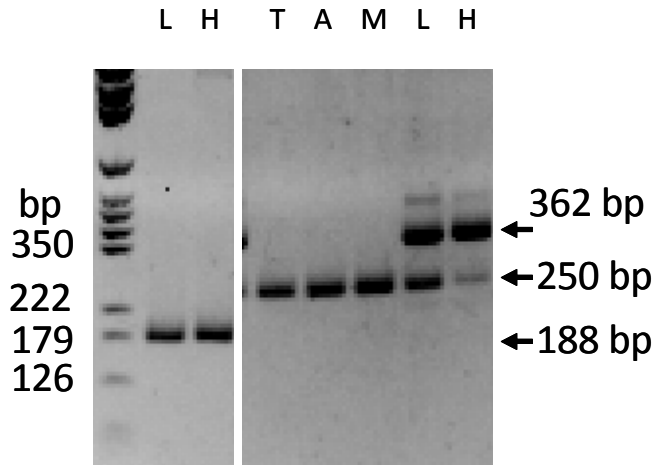
1. 1 cycle - 94°C for 5 min
2. 35 cycles - 94°C for 45 sec  
60°C for 1 min  
72°C for 1 min
3. 1 cycle - 72°C 6 min
4. Hold at 4°C.

**Amplicon size:**

Agtn  $\Delta$ floxed allele = 362 bp (primers: LoxP1-1 + Primer3)

Agtn floxed allele = 250 bp (primers: LoxP1-1 + LoxP1-2)

Wild type allele = 188 bp (primers: LoxP1-1 + Primer3)



**Figure.** A PCR example of angiotensinogen (agtn) floxed alleles. DNAs isolated from an agtn $\Delta$ /f x Cre $^{+}/0$  mouse (Albumin-Cre) and an agtn wild type mouse. T: tail; A: aorta; M: peritoneal macrophages; L: liver; H: hepatocytes.

**Reference:** InGenious

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