

## Genotyping of Angiotensinogen<sup>lox</sup> (Lox P1) by PCR

**Purpose:** To identify the presence of the angiotensinogen (agtn) floxed gene (Lox P1 site).

**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 LoxP1-1 is upstream, and LoxP1-2 is downstream from first Lox-P site

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

### PCR:

Reaction                    4 µl sterile H<sub>2</sub>O  
                                  10 µl buffer(2X green; Cat#M712C, Promega)  
                                  2.5 µl of each primer (concentration = 1 µl/10 pmol)  
                                  1 µl DNA

### Program:

1. 1 cycle - 94°C for 5 min
2. 35 cycles - 94°C for 45 sec  
                  56°C for 1 min  
                  72°C for 1 min
3. 1 cycle - 72°C 6 min
4. Hold at 4°C.

### Amplicon size:

Flox allele agtn = 250 bp

Wild type allele = 188 bp

### Reference:

 InGenious

Primer information provided by Debra L. Rateri

Protocol prepared: Hong Lu

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