

## Genotyping of Angiotensin type 1a (AT1a) receptor<sup>flox</sup> by PCR

**Purpose:** To identify presence of AT1a receptor floxed gene.

**Chromosomal Location:** AT1a receptor (Agtr1a = Jackson's nomenclature) is located on Chromosome 13 (cM 16).

**Strategy for floxing:** Targeting exon 3, which contains the entire coding region (1.9 kb)  
Third loxP site is placed on 5' side of exon 3  
LoxP flanked Neo is placed on 3' side of exon 3 (distance between loxP sites >1.5 kb)  
Distance between single loxP and loxP flanked Neo = ~2.5 kb  
Target region is 2.3 kb and includes exon 3

**Primers:** Primer sets A5 (CACCTCATTCAAAGTATCACAC) and N1 (TGCGAGGCCAGAGGCCACTTGTGTAGC)  
A5 = 5' to short homology arm, outside region used to create targeting construct  
N1 = inside neo cassette

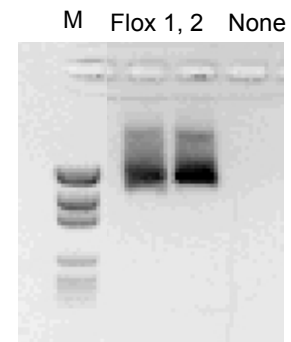
### PCR:

Reaction                    8.1 µl sterile H<sub>2</sub>O  
                                  10 µl buffer(2X; Promega)  
                                  0.3 µl of each primer (concentration = 1 µl/100 pmol)  
                                  0.3 µl Taq polymerase  
                                  1 µl DNA

Program:                    1 cycle = 94°C - 2 min  
  
                                  35 cycles = 94°C - 20 sec  
    62°C - 1 min  
    72°C - 2 min

                                  1 cycle = 72°C - 2 min

**Product size:** Floxed AT1a receptor (A5/N1) = 1.8 kb  
Wild type = no band



**Reference:** InGenious

Z:\AAA PPG\Rateri Core\PCR protocols\at1a receptor flox pcr.wpd