

Genotyping of Angiotensin type 1a (AT1a) receptor^{flox} by PCR (First Lox-P site)

Purpose: To identify presence of AT1a receptor Lox-P gene so flox/flox, flox/+ and +/+ mice may be identified.

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

Strategy for PCR screen: Primer 3-2 is upstream from first Lox-P site
Primer 3-263 is downstream from first Lox-P site

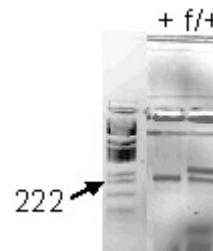
Primers: Primer sets AT1aff 3-2 = TGTTGCATCTACATCCTG
AT1aff 3-263 = TCTAAAGAAACCTCATGAAC

PCR:

Reaction 6.9 µl sterile H₂O
*10 µl buffer(2X; Promega)
1 µl of each primer (concentration = 1 µl/100 pmol)
*0.1 µl Taq polymerase (5u/µl; Promega)
1 µl DNA

*Note: If you are using Promega GoTaq Green Master Mix, you do not have to add Taq polymerase

Program: 1 cycle = 95°C - 4 min
35 cycles = 95°C - 1 min
53°C - 1 min
72°C - 1 min
1 cycle = 72°C - 5 min



Product size: Flox allele AT1a receptor = 262 bp
Wild type allele = 202 bp

Reference: InGenious

C:\Documents and Settings\cltrim2\Local Settings\Temporary Internet Files\Content.Outlook\BL0T20WV\Agtr1a receptor 1st lox-p pcr.wpd