

Genotyping of Angiotensinogen by PCR

Purpose: To identify the presence or absence of the angiotensinogen (agt) gene.

Chromosomal Location: Angiotensinogen is located on Chromosome 8.

Gene disruption information: in angiotensinogen $-/-$ mice, exon 2 is deleted.

Primer information #1: obtained from Jackson's website

Primer sets #1 (4 primers)

oIMR0400 = 5' TGC ACG GGT TCT GAG GAT CCA 3'

oIMR0401 = 5' TAA AGC GCA TGC TCC AGA CT 3'

oIMR0402 = 5' GTA TAC ATC CAC CCC TTC CA 3' (position: exon 2)

oIMR0403 = 5' GGA AGT GAA CGT AGG TGT TGA A 3' (position: exon 2)

PCR product from oIMR0400 + oIMR0401 = 1.2 kb (null band)

PCR product from oIMR0402 + oIMR0403 = 748 bp (wild type band)

These primer sets do not detect LDL receptor null allele.

PCR #1:

Reaction 6.2 μ l Nuclease free H₂O
 10 μ l buffer(2X green; Cat#M712C, Promega)
 0.7 μ l of each primer (concentration = 1 μ l/10 pmol)
 1 μ l DNA

Program:

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

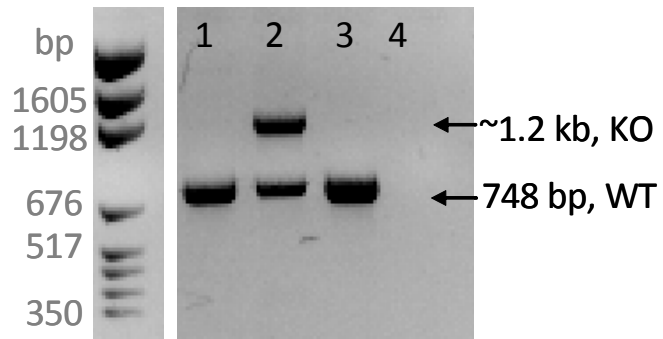


Figure 1. Examples of Agt genotyping. 1: +/+; 2: +/-; 3: +/+ (LDLR $-/-$); 4: H₂O

Primer information #2: obtained from Jackson's website

Primer sets #2 (4 primers)

oIMR0013 = 5' CTT GGG TGG AGA
GGC TAT TC 3'

oIMR0014 = 5' AGG TGA GAT GAC
AGG AGA TC 3'

oIMR0015 = 5' CAA ATG TTG CTT
GTC TGG TG 3' (position:?)

oIMR0016 = 5' GTC AGT CGA GTG CAC AGT TT 3' (position: ?)

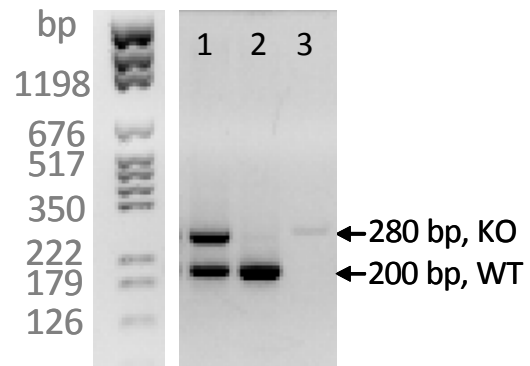


Figure 2. Examples of Agt genotyping. 1: +/-; 2: +/+; 3: H₂O

PCR product from oIMR0013 + oIMR0014 = 280 bP (null band)

PCR product from oIMR0015 + oIMR0016 ≈ 200 bp (wild type band)

PCR:

Reaction 5.8 µl Nuclease free H₂O
 10 µl buffer(2X green; Cat#M712C, Promega)
 0.8 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.

Reference:

1. Kim et al. PNAS 1995; 92 (7): 2735-2739.
2. Primer information was obtained from Jackson Laboratory (PCR conditions were modified)

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