

Genotyping of Angiotensin II Type 1a (AT1a) Receptor Deficient Mice by PCR

Purpose: To identify the AT1a receptor deficient mouse gene from wild type gene.

Gene Information: The AT1a receptor gene has two introns and three exons. The third exon contains the open reading frame. It encodes for a protein that is 359 amino acids and a calculated molecular weight of 40,855 Da. The neocassette that disrupts this gene is inserted at an EcoRI splice site and spans bps 110 - 635, the neocassette removes approximately 0.5 kb and inserts approximately 1 kb of neo gene. This screen utilizes two primers located on the outsides of the EcoRI site.

Primers:

#1	5' - AAATGGCCCTTAACTCTTCTACTG- 3'
#2	5' - ATTAGGAAAGGGAACAGGAAGC - 3'

PCR:

Reaction

1. 7.3 μ l H₂O
2. 10 μ l Master Mix (Promega)
3. 0.3 μ l of each primer
4. 2 μ l genomic DNA
5. 0.1 μ l Taq polymerase

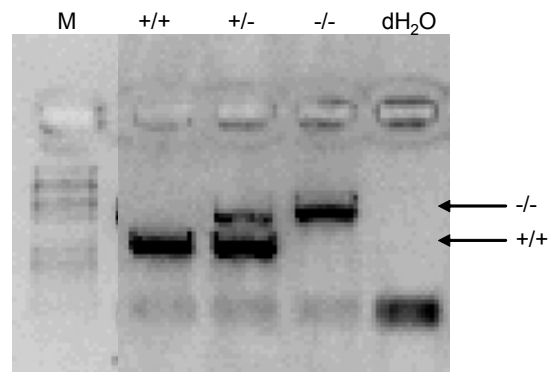
Program

1.Cycle 1: (1x)	Step 1:	94.0 °C for 02:00
2.Cycle 2: (40x)	Step 1:	94.0 °C for 00:45
	Step 2:	54.0 °C for 00:45
	Step 3:	72.0 °C for 01:30
3.Cycle 3: (1x)	Step 1:	72.0 °C for 04:00
	Step 2:	4.0 °C for HOLD

Expected Bands on TBE Agarose Gel

Electrophoresis:

AT1a r+/+ 650 bp
AT1a r +/- 650 bp and ~1.1 kb
AT1a r -/- ~1.1 kb



Screening of Double Deficient Mice:

This strategy may be used to detect the AT1a receptor gene in double deficient or knockout mice, and the primers are specific for the AT1a receptor gene and not neomycin cassette.

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

Updated: 11-21-06