

Genotyping of Angiotensin II Receptor type 2 Deficient (AT2^{-/-}) Mice by PCR

Purpose: To identify the AT2 receptor deficient mouse gene from wild type gene.

Gene Information: The AT2 gene is located on the X chromosome and is 1089 nucleotides (nt) in length. It contains 3 exons encoding a protein of 363 amino acids with the entire coding region in the third exon. The neomycin-resistance expression cassette disrupts the third exon at Pst I - Kpn I sites, located at 166 and 602 base pairs (bp) respectively. The primers chosen for the PCR screen anneal at 145 nt (upstream of the neo cassette) and 644 nt (downstream of the neo cassette). The wild type PCR fragment is 500 nt and null fragment is 500 nt + neo cassette length, approximately 1.1 Kb.

Primers:

1. AT2 anti-sense primer 5' - GGGATTCCTTCTTTGAGAC - 3'
2. AT2 sense primer 5' - GTAAGAATTTGGAGTTGCTG - 3'

PCR:

Reaction	<ol style="list-style-type: none">1. Genomic DNA (3 µl)2. Promega PCR Master Mix (2x; 10 µl)3. Primers (100 pmol; 0.3 µl each)4. Taq polymerase (0.1 µl)5. PCR water (final reaction volume - 20 µl)
Program	<ol style="list-style-type: none">1. 35 cycles - 95°C for 1 min, 56°C for 1 min, 72°C for 2 min2. 1 cycle - 72°C 5 min3. Hold at 4°C.

Expected bands on TBE agarose gel electrophoresis:

AT2 receptor +/+ = 500 bp
AT2 receptor -/- = 1.1 kb

Screening of Double Deficient Mice:

This strategy may be used to detect the AT2 alleles in double null or knockout mice, as the primers are specific for the AT2 gene and not the neomycin cassette.

Reference

Ichiki, T. *et al.*, Effects on blood pressure and exploratory behavior of mice lacking angiotensin II type-2 receptor. *Nature* 1995: (377) 748-750.

File name: Z:\AAA PPG\Rateri Core\PCR protocols\at2PCR.wpd

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