

PCR of Angiotensin-Converting Enzyme 2

Purpose: To identify the ACE2 gene and Neo gene-first step for screening ACE2 genotypes of ACE2 genetic disrupted mice.

Gene Information:

The mouse ACE2 gene is located on chromosome X. It contains 18 exons and 17 introns encoding a protein of 798 amino acids (GenBank: AB053181), or 805 amino acids (ENSMUST00000073973).

Target disruption of ACE2 gene:

In ACE2^{-/-} mice, the ACE2 gene has been disrupted by insertion of a neomycin (Neo) cassette sequence in replacement of at least exon 9 (information from Dr. Coffman's lab at Duke University). The cloning vector used in this deficient mouse is pMC1neo (GenBank no: U43611).

Primers:

The sequences of the following 2 primer pairs were provided by Coffman's lab.

Neo

Neo-833F: 5' GCA GGA TCT CCT GTC ATC TCA CC 3'

Neo-1023R: 5' GAT GCT CTT CGT CCA GAT CAT CC 3'

amplicon length: 191 bp; detect ACE2 knockout band

ACE2-intron 8 and exon 9

ACE2-9F1: 5' GGG CCA GAG TAT CTG CCC AG 3'

ACE2-9R1: 5' GCA GGA TCT CCT GTC ATC TCA CC 3'

amplicon length: 380 bp; detect ACE2 wild type band, but not detectable in ACE2^{-/-} mice

PCR:

Reaction	1.	Genomic DNA (1 µl): used renin ^{-/-} DNA sample # 748
	2.	Promega PCR Master Mix with green dye (2x; 10 µl; Cat#M712C)
	3.	Primers (10 pmol/µl; 0.5 µl each)
	4.	PCR water (8 µl)
		Total reaction volume is 20 µl

Program	1.	1 cycle - 94 °C for 5 min
	2.	35 cycles - 94 °C for 45 sec 60 °C for 1 min 72 °C for 1 min
	3.	1 cycle - 72 °C 6 min
	4.	Hold at 4 °C.

Notes: This protocol will give false positives if the mice are ACE2^{-/-} x LDLR ^{-/-}.

Expected Bands on TBE Agarose Gel Electrophoresis:

Resolve DNA bands on a 2 % agarose gel.

ACE2 +/+	380 bp
ACE2 +/-	380 bp and 191 bp
ACE2 -/-	191 bp

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