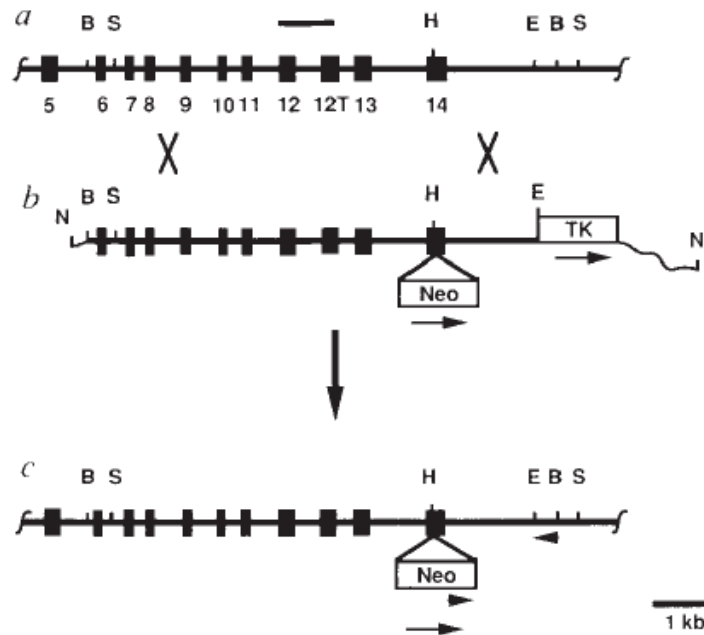


## Genotyping of ACE Deficient (ACE -/-) Mice by PCR

**Purpose:** To identify the ACE deficient mouse gene (ACE -/-) from the C57BL/6 wild type mouse ACE gene (ACE +/+) .

**Gene Information:** The mouse ACE gene is located on chromosome 11. It contains 25 exons and 24 introns encoding a protein of 1312 amino acids (Ensembl: ENSMUSG00000020681). In ACE-/- mice, the ACE gene is disrupted by insertion of a neomycin (Neo) cassette sequence in replacement of exon 14. The cloning vector used in this deficient mouse is pPNTneo (BAC cloning vector; GenBank No:AY192024).

### Target disruption of ACE gene:



### Primer information (obtained from Jackson Website):

**oIMR0723** (intron 13) 5'-AGTGGAGGGTATTTGTCAGGGC-3'

**oIMR0722** (exon 14) 5'-TAATTCCTTGGGAGGCAGCACT-3'

**oIMR0724** (neo gene) 5'-TAAAGCGCATGCTCCAGACTGC-3'

### PCR:

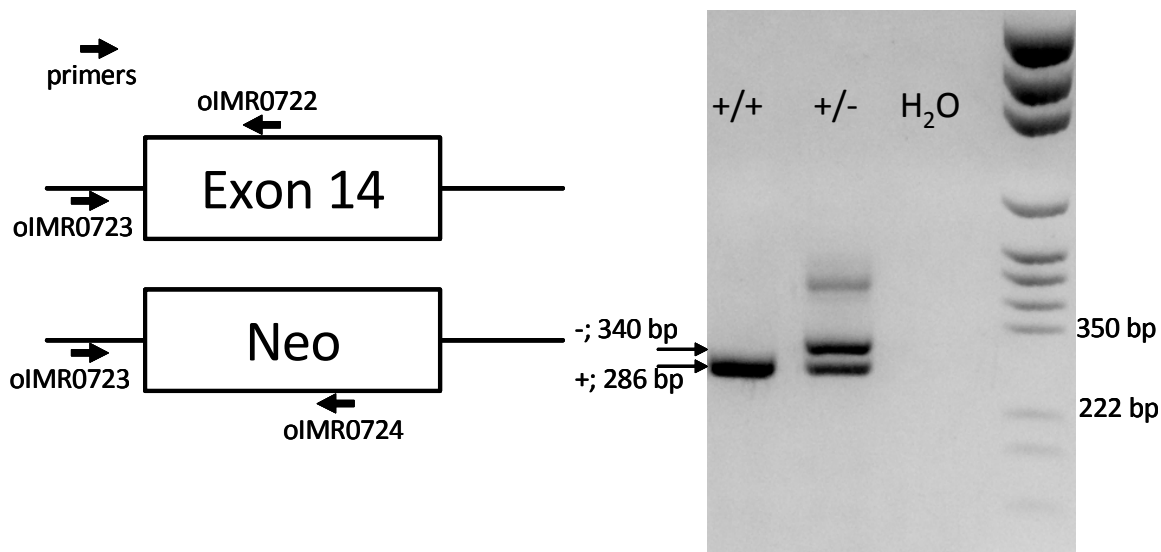
#### Reaction

1. Genomic DNA (1  $\mu$ l)
2. \*Promega PCR GoTaq Green Master Mix Cat# M712C (2x; 10  $\mu$ l)
3. Primers (10 pmol/ $\mu$ l): oIMR0723: 1 $\mu$ l; oIMR0722: 0.8 $\mu$ l; oIMR0724: 0.8 $\mu$ l
4. PCR water (6.4  $\mu$ l)

Total reaction volume is 20  $\mu$ l

- 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

### Gel Image



### Expected Bands on TBE Agarose Gel Electrophoresis:

Resolve DNA bands on a 2 % agarose gel.

ACE +/+	286 bp (primers: oIMR0723 + oIMR0722)
ACE +/-	286 bp and 340 bp
ACE -/-	340 bp (primers: oIMR0723 + oIMR0724)

### Screening Compound Deficient Mice:

This strategy detects the ACE alleles in compound deficient mice (e.g. ACE -/- x LDLR -/- mice), as the primers are designed specifically for the ACE gene and the neomycin cassette.

### Reference:

Krege JH et al. Nature 375(6527):146-148.

Person prepared: Mingming Zhao

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Verified by Hong Lu on 10/14/08

Daugherty Lab

Cardiovascular research Center

University of Kentucky

File name: Z:\PCR\Genotyping Protocols\Protocols for active strains\Protocols for website\ACE genotyping.wpd