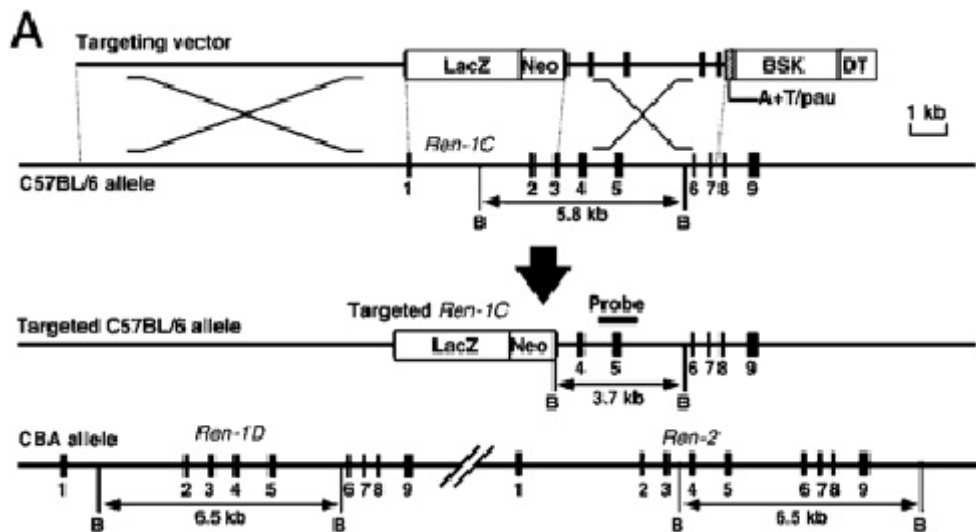


## Genotyping of Renin Deficient (Renin 1c<sup>-/-</sup>) Mice by PCR

**Purpose:** To identify the renin deficient mouse gene (renin 1c<sup>-/-</sup>) from the C57BL/6 wild type mouse renin gene (renin 1c<sup>+/+</sup>).

**Gene Information:** The mouse Renin gene is located on chromosome 1. It contains 9 exons and 8 introns encoding a protein of 402 amino acids (GenBank: AC068906). In renin<sup>-/-</sup> mice, the renin gene has been disrupted by insertion of a neomycin (Neo) cassette sequence in replacement of exon 1-3, including ATG start codon. The cloning vector used in this deficient mouse is pMC1neo (GenBank no: U43611).

**Target disruption of renin gene:**



### Primers:

**Renin-F1** (exon 1) 5'-ACTCTTGTTGCTCTGGAGTCCTT-3' (position: 89067-89089)

**Renin-R1** (intron 1) 5'-AGTAGTAGAAGGGGGAGTTGTGG-3' (position: 89331-89309)

**NEO-F** (neo gene) 5'-GCTACCCGTGATATTGCTGAA-3' (position: 1410-1430)

**Renin-R2** (intron 3) 5'-CTTCCCAAAGCTGGCTTATTC-3' (position: 93412-93392)

### PCR:

- Reaction
1. Genomic DNA (1  $\mu$ l)
  2. \*Promega PCR Master Mix (2x; 10  $\mu$ l)
  3. Primers (10 pmol/ $\mu$ l; 0.2  $\mu$ l each)
  4. \*Taq polymerase (0.1  $\mu$ l)
  5. PCR water (8.1  $\mu$ l)
- Total reaction volume is 20  $\mu$ l

\*Note: If you are using Promega GoTaq Green Master Mix, you do not have to add Taq polymerase

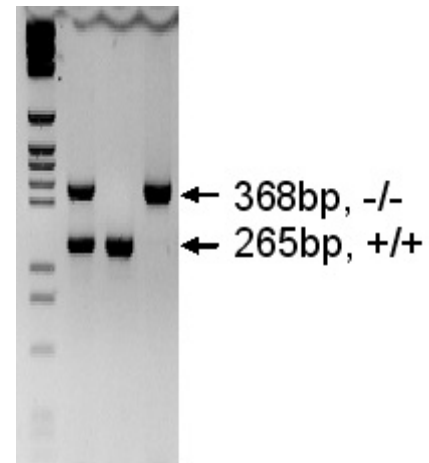
Program 1. 1 cycle - 94°C for 5 min

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.

### Expected Bands on TBE Agarose Gel

**Electrophoresis:** Resolve DNA bands on a 1.5% agarose gel.

renin +/+	265 bp
renin +/-	265 bp and 386 bp
renin -/-	386 bp



### Screening Compound Deficient Mice:

This strategy will detect the renin alleles in compound deficient mice, as the primers are designed for the renin gene and the neomycin cassette.

### Reference:

Yanai K et al. JBC 2000; 275(1): 5-8

File name: C:\Documents and Settings\cltrim2\Local Settings\Temporary Internet Files\Content.Outlook\BL0T20WV\renin genotyping.wpd

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Person prepared: Hong Lu

Daugherty Lab

Cardiovascular research Center

University of Kentucky