

Summary of ACE2-KO mice (nbio388)

An entire coding sequence of the *Ace2* gene on X chromosome in C57BL/6Ncr mice was removed by CRISPR/Cas9 system using TAKE method [1] with Cas9 proteins (Integrated DNA Technologies (IDT)) and two guide RNAs (gRNAs, IDT) targeting 5'- and 3'-UTRs of the gene. Positions of guide RNAs, primers, and the removed sequence are shown in Figure 2.

1. Whole-genome sequence of *Ace2* gene

The whole mouse *Ace2* gene sequence can be retrieved from Ensembl database (<https://www.ensembl.org>).

2. Guide RNA sequences

- ♦ *Ace2*-gRNA-5UTR-#1: TGGATGGGATCTTGGCGCAC /GGG (1903 - 1922)
- ♦ *Ace2*-gRNA-3UTR-#1: TTGGCTTCGGTGTGGCATCT /GGG (49195 - 49214)

(PAM sequences are shown after slashes.)

3. Genotyping PCR

a. Primers

- ♦ *Ace2ko*-S1811: TTTTCTTCTCTTCTCAGTGCCC
- ♦ *Ace2ko*-R2049: GACAGGTCTTCAGCTTCCTGAT
- ♦ *Ace2ko*-S49135: GGGTGATATGGTTGGGTAAC TG
- ♦ *Ace2ko*-R49423: GGTCTCTACCTGATGTCTGGCT

b. For detecting wild alleles:

- For detecting the presence of unmodified 5'-UTR

Detect 239 bp bands by PCRs with primers *Ace2ko*-S1811 and *Ace2ko*-R2049.

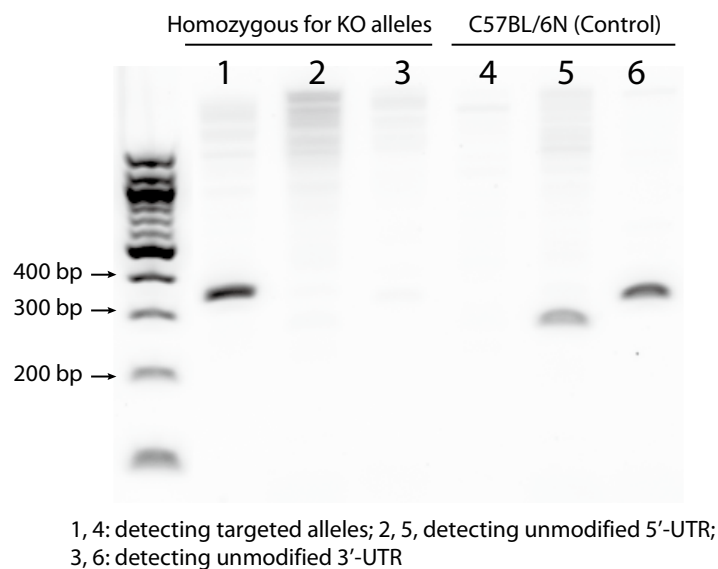
- For detecting the presence of unmodified 3'-UTR

Detect 289 bp bands by PCRs with primers *Ace2ko*-S49135 and *Ace2ko*-R49423.

c. For detecting targeted alleles

Detect ~320 bp bands by PCRs with primers *Ace2ko*-S1811 and *Ace2ko*-R49423.

Figure 1: PCR examples



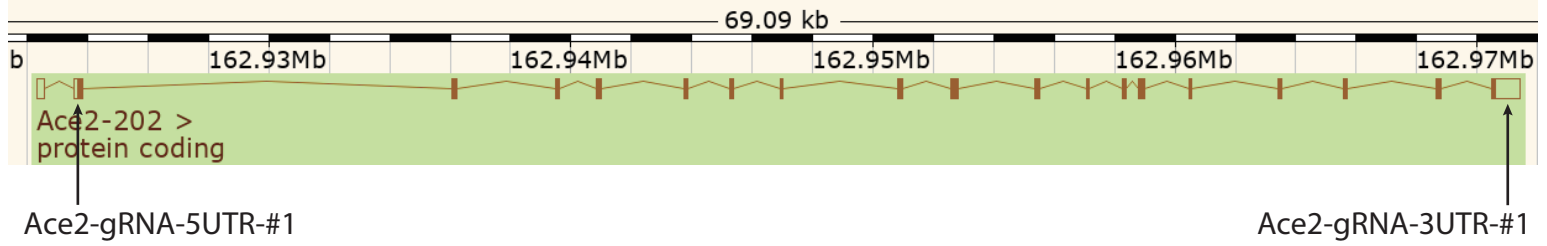
4. References

1. Kaneko T, Mashimo T. Simple Genome Editing of Rodent Intact Embryos by Electroporation. PLoS One. 2015; 10: e0142755.

Figure 2.

Chromosomal map and guide-RNA targets of Ace2 gene

Retrieved from <https://www.ensembl.org>



5'-UTR of Ace2 gene

1750	1760	1770	1780	1790	1800
CTCCAGGATT	AACTTCATAT	TGGTCCAGCA	GCTTGTTTAC	TGTTCTCTTC	TGTTTCTTCT
1810	1820	1830	1840	1850	1860
Ace2-S1811					
TCTGCTTTTT	TTTTCTTCTC	TTCTCAGTGC	CCAACCCAAG	TTCAAAGGCT	GATGAGAGAG
1870	1880	1890	1900	1910	1920
Ace2-gRNA-5UTR-#1					
AAAACTCAT	GAAGAGATT	TACTCTAGGG	AAAGTTGCTC	AGTGGATGGG	ATCTTGGCGC
1930	1940	1950	1960	1970	1980
Ace2-gRNA-5UTR-#1					
ACGGGAAAAG	ATGTCCAGCT	CCTCCTGGCT	CCTTCTCAGC	CTTGTGTCTG	TTACTACTGC
1990	2000	2010	2020	2030	2040
TCAGTCCCTC	ACCGAGGAAA	ATGCCAAGAC	ATTTTAAAC	AACTTTAATC	AGGAAGCTGA
Ace2-R2049					
2050	2060	2070	2080	2090	2100
AGACCTGTCT	TATCAAAGTT	CACCTTGCTC	TTGGAATTAT	AATACTAACA	TTACTGAAGA
Ace2-R2049					
2110	2120	2130	2140	2150	2160
AAATGCCCAA	AAGATGTAA	GTTCTTGAGG	CTACCCAGGG	GGTTATTGAT	TGCTTCTTAA

Exons are marked in yellow.
Coding sequences in exons are shown in Red.

3'-UTR of Ace2 gene

48730	48740	48750	48760	48770	48780
TACCATCACT	TTAATAAGGA	GACTTTGTTF	TATTTGACAG	GAAAAATGAA	ACAAAAAGAG
48790	48800	48810	48820	48830	48840
AAGAGAACC	TTATGACTCG	ATGGACATTG	GAAAAGGAGA	AAGCAATGCA	GGATTCCAAA
48850	48860	48870	48880	48890	48900
ACAGTGATGA	TGCTCAGACT	TCCTTTTAGC	AAAGCACTTG	TCATCTCTCT	GTATGTAAT
48910	48920	48930	48940	48950	48960
GCTAACTTCA	TAGTACACAA	AATATGAGAG	TATACACATG	TCATTAGCTA	TCAAACATAT
48970	48980	48990	49000	49010	49020
GATCTGTTC	GTAACGTTG	TCCAAAGAGC	ATCAGACTTG	AGTGGACATC	TTCAGTACA
49030	49040	49050	49060	49070	49080
TTGCTTTTCA	TATTTATTTT	TGCCTAAGGA	TTTGACATCT	CTTCTGTTTA	TTAATAGAGA
49090	49100	49110	49120	49130	49140
Ace2-S49135					
TGTTTATCTT	AGCATAAAG	AGGAAAATGT	GCCTTTGGCC	TCACAGTCTA	TCCAGGGTGA
49150	49160	49170	49180	49190	49200
Ace2-S49135					
TATGTTGGG	TAACTGGAGT	TAGAAGATGA	GATGATGTCT	CTTGGGGGCA	AGTGTGGGCT
49210	49220	49230	49240	49250	49260
Ace2-gRNA-3UTR-#1					
TCGGTGTGGC	ATCTGGGCTG	TGAAGTGGTG	GGACTGTTGA	GTTGAGAAT	GGTGCTCGCT
49270	49280	49290	49300	49310	49320
GGTCACTTGA	ATCCAAGTGT	GACGTCATGC	TCTGTGGCTT	CTGCCTTCAC	ACTTCTCACT
49330	49340	49350	49360	49370	49380
TCAAGTACTG	TAGGAATTTG	TTACAGTAA	TACTTGAAAT	GGACTGTCCC	TTCTTTGGAG
49390	49400	49410	49420	49430	49440
GTGCAGTTCA	ACGGAGAAA	AACCCAGACA	TCAGGTAGAG	ACCATGACCT	TTTCTCTTCC
Ace2-R49423					
49450	49460	49470	49480	49490	49500
AAACTTGATC	AACATCTCTC	TAACAAGACA	CAGCTAGCAC	AGGAAACTCC	ACGAACCCAG
49510	49520	49530	49540	49550	49560
AGCATGCCTG	TCAGAAACTA	CTTCCATTAT	TCTCCATTG	TGGAGTAAGG	GAAAATTCCA
49570	49580	49590	49600	49610	49620
GATGAATGCT	CGATCTGTGA	GATGGGTGCC	CAGTCTCTGA	AATTGTTTGT	ATTTTCTCAC
49630	49640	49650	49660	49670	49680
AGGGTCTGAG	CAATGGTGAA	CACAAAGCCG	ACCTCAATAA	ATACTTATTA	GATTTAGACA
49690	49700	49710	49720	49730	49740
CTCCTCTGAT	CTTATGTGTG	CTTATGTGTC	TGTTATTTCT	GGCTCTCATT	GTATGGACAA