

***abcc4*^{ihb366/+} (AB) (CZRC catalog ID: CZ 1275)**

Nature of the mutation

Between 888 bp to 892 bp of the wild-type *abcc4* coding sequence, CATGC, is deleted in exon 7.

Sense Strand Sequence

cctccaataaccataatcacacttataattaaataaaatgtaaaatattattatacaatataaatggctcaaaaaaag
agtgcactttaaatatgctaatacacttatttttagggacggagcttcaaactctgatgaataattaaatgccttcaa
aaattatacattagactgctgaggaaatcatgtgtagtagagcataaattgggacacactaacattattgttcatactgca
atgattcatctctctctgtctcacagGAGTAAAACCGCAGCCTTGACTGACAGCCGGATACGCACTAT
GAATGAGGTGGTTTCTGGCATACTATAATTAAGATGTATGCCTGGGAGAAGCCGTTGCGCCAT
GCTGGTTAATGATGTACGAAGgtaaattacatagacttattgagtctgttgcactgctgtagcttgataggtca
gcagattgtgttactgacaagacggcatttgcctcaaggtttatggtgttacttattcaaggtgagactgaagcacag

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

Genotyping assay

Primers:

abcc4_forward: 5' cctccaataaccataatcacact 3'

abcc4_reverse: 5' ctgtgctcaagtctcaacctg 3'

PCR program:

95°C 5min
95°C 30 sec
58°C 30 sec } 30 Cycles
72°C 30 sec
72°C 8min
4°C hold

Product size: 540 bp

abcc4: 5bp deletion

>WT

TGTATGCCTGGGAGAAGCCGTTGCGCCATGCTGGTTAATGA

>abcc4

TGTATGCCTGGGAGAAGCCGTTGCGC-----TGGTTAATGA

ZKO372

Target Position: exon 1

Sense Strand Sequence

tggttgaactgaaagcaacg ccggggcctttggaggttgtttcatcaagcgttgtagtgatttactcatatcatttcgat
aaaATGTATATAAAGTCGATTGTACTAGAGGGCTTCAAGTCCTACGCCGAAAGGACAGAGAT
TAACGGATTTGATCCGTTTTTAAACGCCATCACTGGACTGAACGGCAGCGGAAAGTCAAATA
TACTCGACTCCATCTGCTTTTTACTGGGGATTTCGAACCTTTCCCAGgtgagggttaaacatgcttat
ttattatcagaacttagcaatgtgtataaatccgttttaagctgtgactgcgttattttgattgtgttagcttaacattgttta
ttttgttgcagaaaaacgaaatacaatcatttcattgttatatgtgacacctgtaccacaaaactagagatacgtgcattt
tttgatcttaaacctaaataaacaatcttccactgatgaataacgttactttattacgatagggccacatttagc cgagat
gcaaacaactggaag

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

ZKO372a: 31bp deletion

>WT

cgaaaggacagagattaacggatttgatccgtttttaacgccatcactggactgaacggcagcggaaagtcaaataact
cgactccatc

>ZKO372a

cgaaaggacagagattaacggatttgatccagcggaaagtcaaataactcgactccatc

ZKO376

Target Position: exon 3

Sense Strand Sequence

CGAGGAGCTGGACTTACTGTCTTCAAACACTCAAATGCATCAAATCCCAGAAAGAAAAAC
AGACGAATAGTGgtaaaacacttatacctactgaaactgcgtttgtttataactttaaaaagtctaaattgaagcaat
tgtgttgtttgtgctgtaggaactaactttatctttctatgcag**GTTGCAGATT****CAGATAGGCTTCCTATGTA**
GGGAGCAATTTGGGTCTGGATCGCTGCAGTCTAATAATATGTGCAAgtgaggtcatcacgttgttat
tgatttctgagcaacacttaataacctctcaataatggtgtaaattgtgtcaacaggtatgtgggagttcttgacaag
cagactatgcaaatgaaagttcatagtgctcagctgttcaatatgctgccggtataaccaggtaaacagtttacatctgaa
atattatggacataaatgttaggactcttagagttcaaaggaactttactcgtttt**gtgggtgaaattgttgcag**

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

ZKO376a: 10bp deletion

>WT

tttctatgcaggttgcagattcagataggcttcctatgtagggagcaatgtgggtctggatcgctgca

>ZKO376a

tttctatgcaggttgcagattcagataggcagggagcaatgtgggtctggatcgctgca

ZKO388

Target Position: exon 4

Sense Strand Sequence

cagcacgggtatgtgatgctgctacttgaagttagtagtattaatcagaacgatgctgaggtcagttaccatttggtttgttact
gtagGGCATCATTGACATATCAGTTCCTGGTGCTAAAAGGAAGTGTCTGAATGAGGGTGAA
GATCCAGAGGAAGATGTGGAAGAGCAAAGgtgagagagcgctgatcaggaatcggctgtaattcaga
aaagcccttacatttgatcaggtcagagctatgtgtaactgatttgaatctgctgtatggtgtagGAAGATGTTGAG
CCTCAACATCAGGAAGAGAGTGGACCATATGAAGAGGTGTACAAGGACTCCAGCACATTC
TAAAGGtatctttaaacaattgtatatgattaattgagcaatcgggtatgagaagc

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

ZKO388a: 2bp deletion

>WT

gaagatggtgagcctcaacatcaggaagagagtgaccatgaagaggtgtacaaggactc

>ZKO388a

gaagatggtgagcctcaacatcaggaagagtgaccatgaagaggtgtacaaggactc

ZKO388b: 4bp deletion

>WT

aggaagatggtgagcctcaacatcaggaagagagtgaccatgaagaggtgtacaaggactc

>ZKO388b

aggaagatggtgagcctcaacatcaggaagagtgaccatgaagaggtgtacaaggactc

ZKO392

Target Position: exon 2

Sense Strand Sequence

TGACCTGTTGCTCGTCGATCCCAATGAGATGTGTGGATTTGTCATAGCTCACATTTTGGGTGT
CCAGTATGCAGTATTTAGCACAGGACTGTGGTACCCTGCTGAAGTTGGGGCACCAG**GCTCCCC**
TATCTTATGTCCCGGAATTTAACTCTCTACTAACGGACCATATGTCGTTGTTTCAACGGGTAGC
TAACACAGCGGTGTACCTAGTTTCCAGGTTTGGTGTCCAGTTCCTGGTTCTACCTAAATATGA
TCGATTATGAGGAAGTACAACATCCAGCCATCTGTCTCCATGCACGATCTGGTTCAGAACA
GTCGACTCTGGATGCTGTGTACTIONGACTGGACTGGAGTTCCCACGACCAACTTTGCCACAT
GTTGTCTATGTAGGGGGCATCCTCACTAAACCTCCCAGCCCATTACCACAGgtaagaaaccactgc
tattatatttgaaaatTTTTAAAGGGTcctttccttttcatTTtaggatggtaaaatgt**ggtaaagctcaatgcaaaggg**

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

ZKO392a: 13bp deletion, 8bp insertion

>WT

acaggactgtggtaccctgctgaagttggggcaccagctcccctatcttatgtcccggaatttaactctctac

>ZKO392a

acaggactgtggtaccctgctgaagttgggtatatccctatcttatgtcccggaatttaactctctac

