

## *fndc5b*<sup>ihb253/+</sup> (CZRC catalog ID: CZ460)

### Nature of the mutation

Between 169 bp to 176 bp of the wild-type *fndc5b* coding sequence, GTCATTGG is deleted in exon 2.

### Sense Strand Sequence

atttggtttgctgtatggaattaaatcatattccaaagtgtgtgtgtgtgtcaacatcagccattagttatacattcattttccagacgctc  
atattttcaaagtccagacatattcttcagatgaatattgacctataaagcgacagagatcttactgtatgtcacaccagtagctttatccct  
tcatacttttttcgctcttctgtcatcctcagACAGTTTATCAGCTCCGCTCAATGTGACCATCAAAGCACTGGAGGGGA  
ACTCAGCGATTGTGACATGGGACATTCTGGAGGGAGACCCAGTCATTGGGTTTGCCATCACCCAGCAGgtat  
aaaatcattttatggtcaatttttgtgtaaaaatgggaaaatcgctcataactcaagtgaatgaggcgatagcggaggaatgcttgt  
gtaagcatgtagttatgtgtcgtatgcagactggtttgcttttgata

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : Crispr/cas9 target site

### Genotyping assay

#### Primers:

*fndc5b*\_forward: 5' ATTTGTGTTTGCTGTATGGA 3'

*fndc5b*\_reverse: 5' TATCAAAGCAAACCCAGTCT 3'

#### PCR program:

95°C 5min

95°C 30 sec

58°C 30 sec

72°C 30 sec

72°C 8min

4°C hold

} 30 Cycles

Product size: 488 bp

### The sequencing results of the CZ460:

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*****  
WT          CTCCGCTCAATGTGACCATCAAAGCACTGGAGGGGAAGCTCAGCGATTGTGACATGGGACA  
CZ460       CTCCGCTCAATGTGACCATCAAAGCACTGGAGGGGAAGCTCAGCGATTGTGACATGGGACA  
*****  
  
WT          TTCTGGAGGGAGACCCAGTCATTGGGTTTCCATCACCAGCAGgtataaaatcattta  
CZ460       TTCTGGAGGGAGACCCAG-----TTTCCATCACCAGCAGgtataaaatcattta  
*****  
  
WT          tggccaatttttgtgtaaaacatgggaaaatcgccataactcaagtgaatgaggcg  
CZ460       tggccaatttttgtgtaaaacatgggaaaatcgccataactcaagtgaatgaggcg  
*****
```

### Reference:

None