

## *lepr*<sup>ihb251/+</sup> (CZRC catalog ID: CZ458)

### Nature of the mutation

Between 918 bp to 922 bp of the wild-type *lepr* coding sequence, CCGCA is mutated to GCA in exon 8.

### Sense Strand Sequence

ttgcctcaacacaccttcttgaagttactagtatacaaaagtaagagcttgattagcttggtcagatgtgttgattcaggtgaaacttagctctccaggacactggccctccaggacagagtggacaccctggctaaatggccctattctgttttctcctcaaagTCAGTTACATCCCTGCTGAAGTCTTCACAACGCAGGAGTCAGAGGTGACAGTTTACTCTGTGTTCCA**CAA****CCGCAGCTGGAGCGCC**AGTAAAGCCGTGTGGTTTCTGAACGGCAAGATGAAGATACCAGAAAGCCAGTACAGAGTCATCAATGAGCAGGTGAGCACAGTCACTCTAAAGATGGA  
CAAAGCAGGATTTGACACTCTGATGTGCTGCCTCACATTGGGAGAGAAATCTATGTGCTCCAT**CGCCTATGCCAAAATATACA**

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

**atcg** : Forward/Reverse primer

**atcg** : Crispr/cas9 target site

### Genotyping assay

#### Primers:

**lepr\_forward**: 5' TTGCCTCAACACACCTTCCT 3'

**lepr\_reverse**: 5' TGTATATTTTGGCATAGGCG 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: 441 bp**

### The sequencing results of the CZ458:

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WT      ACATCCCTGCTGAAGTCTTCACAACGCAGGAGTCAGAGGTGACAGTTTACTCTGTGTTCC
CZ458   ACATCCCTGCTGAAGTCTTCACAACGCAGGAGTCAGAGGTGACAGTTTACTCTGTGTTCC
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WT      ACAACCGCAGCTGGAGCGCCAGTAAAGCCGTGTGGTTTCTGAACGGCAAGATGAAGATAC
CZ458   ACAACCGCAGCTGGAGCGCCAGTAAAGCCGTGTGGTTTCTGAACGGCAAGATGAAGATAC
***   ** *****

WT      CAGAAAGCCAGTACAGAGTCATCAATGAGCAGGTCAGCACAGTCACTCTAAAGATGGACA
CZ458   CAGAAAGCCAGTACAGAGTCATCAATGAGCAGGTCAGCACAGTCACTCTAAAGATGGACA
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### Reference:

None