



## ***ihb490/+* (AB) (CZRC Catalog ID: CZ 1444)**

### **Nature of the mutation**

The *ihb490* allele contains 3 bp deletion and 18bp insertion of the *mvp* sequence.

### **Genotyping assay**

Genotyping of the *ihb490* allele is based on the sequencing assay.

### **Primers:**

#### **Genotyping assay**

F: 5' AGTTACTGGTGCgTTAGACA 3'

R: 5' GGACAAGAAATAAGGGAGAA3'

### **PCR program:**

1. 94 °C for 3 min
2. 94 °C for 30 sec
3. 53 °C for 30 sec
4. 72 °C for 50 sec
5. Go to step 2 (above) for 29 cycles
6. 72 °C for 5 min
7. 12.0 °C hold

**Product size:** 412bp.

### **Sequence:**

Target Position: exon 6

Sense Strand Sequence

AGTTACTGgtgcttagacacaataacagacaagtgcacacacatatagagataaaacattccagaataaaaactgtttacgttc  
cgctcatgttcaccaaacctgaatgacattctgtggaacacagacatatataataaacatatattttataaaaaagtcattttggaccaaac  
aagggttcattgtgtgggtaaacggctgacagttcataattgcatgcatattcaaattcacgatggctgaaatggtctgctgtctcagGT  
GAGGAATGGCAGGTCAGTAAGGTTGGAGCGTATCTGCCTGGAGCTCATGAAGAG  
GTCGTGGATATTGTCAGCGCTTTCATCCTTACAGACAAGgtacaaccctgatgctttctgccattt  
tctcccttatttctgtcc





Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : Cas9 target site

>Wild type

ctgcttgtctcagGTGAGGAATGGCAGGTCAGTAAGGTTGGAGCGTATCTGCCTGGAGCT  
CATGAAGAGGTCGTGGATATTGTCAGCGC

>ihb490(-3bp,+18bp)

AAATGGTCTGCTTGTCTCAGGTGAGGAATGGCAGTGAGGTAGTGAGATGGGTAGT  
AAGGTTGGAGCGTATCTGCCTGGAGCTCATGAAGAGGTCG

CLUSTAL format alignment by MAFFT FFT-NS-i (v7.471)

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Wild          -----ctgcttgtctcagGTGAGGAATGGCA-----GGTCAGTAAGGT
ihb490(-3bp, +18 AAATGGTCTGCTTGTCTCAGGTGAGGAATGGCAGTGAGGTAGTGAGATGGGTAGTAAGGT
                  *****
                  ** .*****
  
```

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Wild          TGGAGCGTATCTGCCTGGAGCTCATGAAGAGGTCGTGGATATTGTCAGCGC
ihb490(-3bp, +18 TGGAGCGTATCTGCCTGGAGCTCATGAAGAGGTCG-----
                  *****
  
```

**Reference:**

None

