



***ihb489/+* (AB) (CZRC Catalog ID: CZ 1443)**

Nature of the mutation

The *ihb489* allele contains 4bp deletion of the *mvp* sequence.

Genotyping assay

Genotyping of the *ihb489* 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.

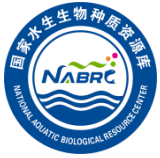
Sequence:

Target Position: exon 6

Sense Strand Sequence

AGTTACTGgtgcgTTAGACaacaacagacaagtgcacacacatatagagataaaacattccagaataaaaactgtttacgttc
cgtcattgtcatcacaacacctgaatgacattctgtggaacacagacatatataataaacatatattttataaaaaagtcattttggaccacaac
aagggttcattgtgtgggtaaacggctgacagttcataattgcatgcatattcaaattcacgatggctgaaatggtctgctgtctcagGT
GAGGAATGGCAGGTCAGTAAGGTTGGAGCGTATCTGCCTGGAGCTCATGAAGAG
GTCGTGGATATTGTCAGCGCTTTCATCCTTACAGACAAGgtacaaccctgatgctttctgccattt
tctcccttatttctgtcc





Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : Cas9 target site

>Wild type

ctgcttgtctcagGTGAGGAATGGCAGGTCAGTAAGGTTGGAGCGTATCTGCCTGGAGCT
CATGAAGAGGTCGTGGATATTGTCAGCGC

>ihb489(-4bp)

CGATGGCTGAAATGGTCTGCTTGTCTCAGGTGAGGAATGGCAGGT----
AAGGTTGGAGCGTATCTGCCTGGAGCTCATGAAGAGGTCGTGGATATTGTCAGCG

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

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Wild          -----ctgcttgtctcagGTGAGGAATGGCAGGTCAGTAAGGTTGGAGC
ihb489(-4bp)  CGATGGCTGAAATGGTCTGCTTGTCTCAGGTGAGGAATGGCAG----GTAAGGTTGGAGC
                *****
                *****

Wild          GTATCTGCCTGGAGCTCATGAAGAGGTCGTGGATATTGTCAGCGC
ihb489(-4bp)  GTATCTGCCTGGAGCTCATGAAGAGGTCGTGGATATTGTCAGCG-
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                *****

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Reference:

None

