



***ihb487/+* (AB) (CZRC Catalog ID: CZ 1441)**

Nature of the mutation

The *ihb487* allele contains 6bp deletion and 10bp insertion of the *cdk2* sequence.

Genotyping assay

Genotyping of the *ihb487* allele is based on the sequencing assay.

Primers:

F: 5' CAATCACCCCTAACATAGTCAA 3'

R: 5' AAAGAACTGAAAGCTATCTGG3'

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: 402bp.

Sequence:

Target Position: exon 2

Sense Strand Sequence

CAATCACCCCTAACATAGTCAAGTATGTGCACTGTTTATGAATTTAGTCTCAAAC
CAGTGAATATTTCAATGCACAAATGTCCTCCTGGTTTGTGAGTTTTACAGTTTTCT
TGCCTCCAGGTTGCGTGATGTGATACACACTGAAAATAAACTTTACTTGGTGTTTG
AGTTTCTTCACCAAGACCTGAAGAGGTTTATGGACTCGACGCTGTGTCAGTGGCAT
ATCCTTGCCACTCGTGAAGgtgagttaaggtttgaccattagaagaagtgttaacatcagctaaagctgctgaactgtt
cctttaaaaatatattactcaatattacataaatattttccacatcatcacttactaactttattatctcataccagatagctttcagttcttt

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : Cas9 target site





>Wild type

CCTGAAGAGGTTTATGGACTCGACGTCTGTCAGTGGCATATCCTTGCCACTCGTG
AAG

>ihb487(-6bp,+10bp)

CCTGAAGAGGTTTATGGACTCGACGTCTGTCCTGTCTGTCCATATCCTTGCCACTC
GTGAAG

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

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Wild          CCTGAAGAGGTTTATGGACTCGACGTCTGTC----AGTGGCATATCCTTGCCACTCGTGA
ihb487(-6bp, +10 CCTGAAGAGGTTTATGGACTCGACGTCTGTCCTGTCTGTCCATATCCTTGCCACTCGTGA
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Wild          AC
ihb487(-6bp, +10 AC
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Reference:

None

