

CZ227: *cpamd8*^{ihb72/+}

Nature of the mutation

The *ihb72* allele contains 4bp deletion from exon 3 of the *cpamd8* sequence.

Genotyping assay

Genotyping of the *ihb72* allele is based on the sequencing assay.

Primers:

F: 5' CGTGGGAAGAAGCCGTGAG3'

R: 5' GGAAACATTGTGGTTATTAGGC 3'

PCR program:

1. 94°C for 3 min
2. 94°C for 30 sec
3. 58°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: 480bp.

Target Position: exon 3

Sense Strand Sequence

cgtggaagaagccgtgagcgtcaccctctcaatatggtggaggacacacgagtacaactacagctcagcgtcaagggc
gagattgtggcacacagccatggcacagtaagaggtgaatattacaaaactcaagaaaactgattatccccctcagttatcaa
cactaaacctaattctcagaaatacagacatcataaacttttagtattcgtaaaaatttttcagtaaagctctttatctactataa
aatcgcgatgctgatgaagaattccgggtgcttgtgcagttttacttgacatgttttttccatttcagATAAGGGCAC
CATTAAATAAaggtgagccaatcatgtcatcagcccatttgtctcttgccaaaataacccatgcaacatacagact
ggttttggctcagggtcataaaaatcagcttaatgggacttccatgcctaataaccacaatgtttcc

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

突变序列:

***ihb72*: 4bp deletion**

>WT

acatgtttattttccatttcagATAAGGGCACCATTAAAATAAaggtgagccaatcatgcatc

> *ihb72*

acatgtttattttccatttcagATAAGGGCACCATTAAAaggtgagccaatcatgcatc

CLUSTAL format alignment by MAFFT FFT-NS-i (v7.215)

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WT          acatgtttattttccatttcagATAAGGGCACCATTAAAATAAaggtgagccaatcatg
cpamd8-a    acatgtttattttccatttcagATAAGGGCACCATTAAA---AAaggtgagccaatcatg
*****

WT          catc
cpamd8-a    catc
****
```