

***ndella*^{ihb95/+} (AB) (CZRC catalog ID: CZ217)**

Nature of mutation

ihb 95 allele was a mutant line, induced by the CRISPR-cas9 in the TU genetic background. Three nucleotides, CAG, located in the splice acceptor site of intron 4, are deleted. Plus the adjacent twenty-nine-nucleotide coding sequence, AGCCACCATAACATCTCTGGAGGACTTTG, is mutated into GAGGCTGAGCAGAGGCTGA. This mutation may lead to mis-splicing of the *ndella* transcript, resulting in the use of an alternative acceptor site (changing the 5' boundary of the downstream exon 5) or simply the retention of the intron 4.

Genotyping assay

Primers:

Ihb95_forward: 5' TCAATTTATTGTGTCCGTGCT 3'

Ihb95_reverse: 5' CTTGGAATGAGGAACTGTCTG 3'

PCR program:

94°C 4min
94°C 30 sec
55°C 30 sec } 30 Cycles
72°C 30 sec
72°C 5min
4°C hold

Product size: 421 bp

The sequencing results of the parents:

CZ217 (+/-)

ctgtaaccacagAGCCACCATAACATCTCTGGAGGACTTTGAGCAGAGGC (wild-type)

