

ihb24

CZ37:*tbr1b*

Nature of the mutation

The *ihb24* allele contains a deletion from 299bp to 303bp (CTGCA) of the *tbr1b* coding sequence. The mutated *tbr1b* codes for a protein containing 220 aa, in which only the sequence of the first 100 aa is identical to wildtype *tbr1b*.

Genotyping assay

Genotyping of the *ihb24* allele is based on the RFLP assay. This method is used to detect a mutation that either creates or abolishes a site recognized by a specific restriction enzyme. In the RFLP assay, a sequence of interest is first PCR-amplified and then the PCR product is subjected to restriction enzyme digestion. The presence or absence of the mutation is determined by the resulting restriction pattern. The *ihb24* mutation creates a site recognized by the PstI restriction enzyme.

Primers:

CZ37-F: 5' TGGATGACCAGCAGAAGTGA 3'

CZ37-R: 5' CCTTGCGGAGAAGAGTTGC 3'

PCR program:

1. 94°C for 3 min
2. 94°C for 30 sec
3. 59°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: 764 bp or 759bp

Digestion of the PCR product with the PstI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	571 bp and 193 bp
PCR product containing the mutation	unaffected	759 bp