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:

<table>
<thead>
<tr>
<th>Product type</th>
<th>Product digestion</th>
<th>DNA fragments after digestion (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR product derived from the WT template</td>
<td>cleaved</td>
<td>571 bp and 193 bp</td>
</tr>
<tr>
<td>PCR product containing the mutation</td>
<td>unaffected</td>
<td>759 bp</td>
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