

***lhx1a*^{ihb25/+} (AB) (CZRC catalog ID: CZ24)**

Nature of mutation

ihb 25 allele was a mutant line, induced by the TALEN in the AB genetic background. 133 bp to 136 bp of the wildtype *lhx1a* coding sequence, CGAG, is mutated into GTT. The mutated *lhx1a* codes for a protein containing 85 aa, in which only the sequence of the first 44 aa is identical to wildtype Lhx1a. The homozygous mutants die in the larvae stage.

Genotyping assay

Primers:

ihb25_forward: 5' TCCTTCAGCGATAACAACCTT 3'

ihb25_reverse: 5' TTCAACTACCCTGGCAAA 3'

PCR program:

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

Product size: 987 bp:

Digestion of the PCR product with the XhoI restriction enzyme:

PCR Product	Product digestion	DNA fragments after digestion (bp)
AB	unaffected	242 and 745
<i>Ihb25 allele</i>	affected	987

The sequencing results of the parents:

CZ24 (+/-) cagagaaatgcttctctcgagaaaggaaaactatat (wild-type)

