lhx2^{tv42z}

Nature of the mutation

The *tv42z* allele contains a single C-to-A point mutation that substitutes Cys by a stop codon in the Lhx2 protein (Seth et al., Development 133: 725-735, 2006).

Genotyping assay

Genotyping of the *tv42z* allele is based on the RFLP assay (**R**estriction **F**ragment **L**ength **P**olymorphism; Botstein *et al.*, Am. J. Hum. Genet. 32: 314-331, 1980). 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 *tv42z* mutation creates a site recognized by the DdeI restriction enzyme.

Primers:
BEL_07: 5' CAC TCA TCC CTA AAG CCA TTA C 3'
BEL_06: 5' GAC TCC AGG TTC AGT TTA CAC TC 3'

PCR program (55_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **55**ºC for **30** sec
4. 72ºC for **30** sec
5. Go to step 2 (above) for 39 cycles
6. 72ºC for 5 min
7. 8.0ºC hold
8. END

Product size: 301 bp

Digestion of the PCR product with the DdeI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	301 bp
PCR product containing the mutation	cleaved	275 bp and 26 bp

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