dll4 j16e1

Nature of the mutation

The *j16e1* allele contains a single G-to-A point mutation that substitutes glycine (270) by serine at a conserved site in an EGF-like domain of the Dll4 protein. (Leslie et al., Development 134: 839-844, 2007).

Genotyping assay

Genotyping of the *j16e1* 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 *j16e1* mutation creates a site recognized by the BpmI restriction enzyme.

Primers:
DEL_03: 5' GTA TTT CAG ATG CAG AGA AGG ATG G 3'
DEL_04: 5' GGT GTA ACT GCC CTG ACC TGT ATT C 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: 442 bp

Digestion of the PCR product with the BpmI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	442 bp
PCR product containing the mutation	cleaved	306 bp and 136 bp

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