dlat ^{m631}

Nature of the mutation

The *m631* allele contains a single T-to-C point mutation that substitutes Leu by Pro at residue 644 of the Dlat protein (Taylor et al., Proc. Natl. Acad. Sci. USA 101(13): 4584-4589, 2004).

Genotyping assay

Genotyping of the *m631* 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 *m631* mutation abolishes a site recognized by the BfaI restriction enzyme.

Primers:
NOA_11: 5' CAA TCA ACT GCC ATC ACT AAT AAC G 3'
NOA_12: 5' AGA GAG AGA GAG AGA GAA CTG CCT G 3'

PCR program (56_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **56**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: 337 bp

Digestion of the PCR product with the BfaI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	182 bp and 155 bp
PCR product containing the mutation	unaffected	337 bp

IMPORTANT NOTE: It is highly recommended to use WT positive controls to monitor whether enzyme digestion has been carried out to completion. Without this control, partially digested WT samples can be mistakenly regarded as heterozygous samples.

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