kita^{b5}

Nature of the mutation

The *b5* allele contains a single nucleotide deletion (a deletion of T) at codon 846 of the *kita* gene, resulting in a frameshift and a premature stop codon (Parichy et al., Development 126: 3425-3436, 1999).

Genotyping assay

Genotyping of the *b5* 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 *b5* mutation abolishes a site recognized by the Tsp509I restriction enzyme.

Primers:
KIT_03: 5' AGA CAA CCA CAA GCA CTT TAC CC 3'
KIT_04: 5' CCA TAG ATG TTG ACC CAG ACT GG 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: 269 bp

Digestion of the PCR product with the Tsp509I restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	202 bp and 67 bp
PCR product containing the mutation	unaffected	269 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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