prkci ^{m567}

Nature of the mutation

The *m567* allele contains a single G-to-A point mutation that substitutes Trp by a stop codon, resulting in a protein that is truncated by 69 amino acids (Horne-Badovinac et al., Current Biology 11(19): 1492-1502, 2001).

Genotyping assay

Genotyping of the *m567* 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 *m567* mutation abolishes a site recognized by the BsrI restriction enzyme.

Primers:
PKC_03: 5' GCA AAC TAT TAG CTG GAC AAT TAG AGC 3'
PKC_04: 5' ACT CAT TGC TTC CTC TGC GTC 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: 254 bp

Digestion of the PCR product with the BsrI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	172 bp and 82 bp
PCR product containing the mutation	unaffected	254 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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