hspd1^{zp7}

Nature of the mutation

The *zp7* allele contains a single T-to-A point mutation that changes valine to glutamic acid at amino acid residue 324 of the Hspd1 protein (Makino et al., Proc. Natl. Acad. Sci. USA 102: 14599-14604, 2005).

Genotyping assay

Genotyping of the *zp7* 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 *zp7* mutation creates a site recognized by the Hpy188III restriction enzyme.

Primers:
HPD_01: 5' CAG TTT AGT TCC AAC CCT GCT TAC 3'
HPD_02: 5' GCA AGA CCC ATA GCC TCG TC 3'

PCR program (53_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **53**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: 255 bp

Digestion of the PCR product with the Hpy188III restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	155 bp and 100 bp
PCR product containing the mutation	cleaved	124 bp, 100 bp and 31 bp

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