

tp53^{zdf1}**Nature of the mutation**

The *zdf1* allele contains a single T-to-A point mutation that changes Met to Lys at residue 214 of the Tp53 protein (Berghmans et al., Proc. Natl. Acad. Sci. USA 102(2): 407-412, 2005).

Genotyping assay

Genotyping of the *zdf1* 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 *zdf1* mutation creates a site recognized by the MboII restriction enzyme.

Primers:

TPA_03: 5' ACA TGA AAT TGC CAG AGT ATG TGT C 3'

TPA_04: 5' TCG GAT AGC CTA GTG CGA GC 3'

PCR program (53_30_30):

1. 94°C for 3 min
2. 94°C for 30 sec
3. 53°C for 30 sec
4. 72°C for 30 sec
5. Go to step 2 (above) for 39 cycles
6. 72°C for 5 min
7. 8.0°C hold
8. END

Product size: 336 bp**Digestion of the PCR product with the MboII restriction enzyme:**

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	336 bp
PCR product containing the mutation	cleaved	140 bp and 196 bp

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