Zebrafish International Resource Center



# *mafb*$^{b337}$

## Nature of the mutation

The *b337* allele contains a single C-to-T point mutation that changes a glutamine codon to a stop codon and truncates the protein upstream of its DNA binding domain (Moens et al., Development 125: 381-391, 1998).

## Genotyping assay

Genotyping of the *b337* 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 *b337* mutation abolishes a site recognized by the PvuII restriction enzyme.

**Primers:**
**VLA01:** 5' CAG CCC CAC AGA ACA GAA GAA CC 3'
**VLA02**: 5' ACA AGC TGG TCG TCG GAG AAG C 3'

**PCR program (62_30_30):**

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **62**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: 525 bp**

**Digestion of the PCR product with the PvuII restriction enzyme:**

| Product type | Product digestion | DNA fragments after digestion (bp) |
|---|---|---|
| PCR product derived from the WT template | cleaved | 352 bp and 173 bp |
| PCR product containing the mutation | unaffected | 525 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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