Zebrafish International Resource Center

eomesa^{fh105}

Nature of the mutation

The *fh105* allele contains a single C-to-A point mutation that changes Tyr into a premature stop codon at amino acid 100, resulting in truncation of the Eomesa protein (Moens C., personal communication).

Genotyping assay

Genotyping of the *fh105* allele is based on the dCAPS assay (**d**erived **C**leaved **A**mplified **P**olymorphic **S**equence; Neff *et al.*, The Plant Journal 14(3): 387-392, 1998). In this assay, a restriction enzyme recognition site that includes the single nucleotide polymorphism (SNP) is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The PCR product modified in this manner is then subjected to restriction enzyme digestion and the presence or absence of the SNP is determined by the resulting restriction pattern.

To genotype the *fh105* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *fh105* mutation create a MseI restriction enzyme site in the amplified product. The MseI site is not present in the PCR product derived from the WT DNA template.

Primers:
fh105_03d: 5' ATG AGC TCT CCA GCA CCC GCA GTT A 3'
fh105_02: 5' AAC CGT AAT GCA GAG ACG ACG AAT A 3'

PCR program (60_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **60**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: 186 bp

Digestion of the PCR product with the MseI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	186 bp
PCR product containing the mutation	cleaved	163 bp and 23 bp

Zebrafish International Resource Center (ZIRC)
5274 University of Oregon
Eugene, OR 97403-5274, USA
Phone: 541-346-6028
Email: genotyping@zebrafish.org
Web: http://zebrafish.org