Zebrafish International Resource Center

erbb3b^{st48}

Nature of the mutation

The *st48* allele contains a single C-to-T point mutation that introduces a premature stop codon at residue 563 of the Erbb3b protein (Lyons et al., Current Biology 15: 513-524, 2005; Pogoda et al., Developmental Biology 298: 118-131, 2006).

Genotyping assay

Genotyping of the *st48* 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 *st48* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch creates an Hpy8I restriction enzyme site in the amplified product derived from the WT DNA template. The Hpy8I site is not present in the PCR product containing the *st48* mutation.

Primers:
ERB_01: 5' GTG TCT GTT GCA GAA TGG TAA A 3'
ERB_02: 5' AGA AAA CCC TGA CTG GAC CA 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: 119 bp

Digestion of the PCR product with the Hpy8I restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	99 bp and 20 bp
PCR product containing the mutation	unaffected	119 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.