erbb2^{st61}

Nature of the mutation

The *st61* allele contains a single T-to-A point mutation that substitutes cysteine for a stop codon at residue 580 of the Erbb2 protein (Lyons et al., Current Biology 15(6): 513-524, 2005).

Genotyping assay

Genotyping of the *st61* 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 *st61* mutation abolishes a site recognized by the BsrGI restriction enzyme.

Primers:
ERB_07: 5' CAT AAA GGT CTA AAA CCA CCG TCA G 3'
ERB_08: 5' TGG ACT CAG CAA AGG ACT TAC G 3'

PCR program (55_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **55**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: 273 bp

Digestion of the PCR product with the BsrGI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	163 bp and 110 bp
PCR product containing the mutation	unaffected	273 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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