

eya1^{hi2933Tg}

Nature of the mutation

hi2933Tg constitutes a retrovirus-induced mutation:

- Amsterdam *et al.* Genes Dev. 13(20):2713-2724 (1999)
- Golling *et al.* Nat. Genet. 31(2):135-140 (2002)
- Amsterdam *et al.* Proc. Natl. Acad. Sci. USA 101(35):12792-12797 (2004)

Genotyping assay

The forward primer (**Hi2933_3E04**) anneals to the 3'-portion of the retrovirus that causes the mutation, and the reverse primer (**Hi2933_3E03**) anneals to the adjacent genomic region flanking the virus.

Primers:

Hi2933_3E03: 5' CTC GGC TCC AAT AAA ATG AAC TC 3'

Hi2933_3E04: 5' CAA GGA CCT GAA ATG ACC CTG 3'

PCR program (62_40_40):

- 1_94°C for 3 min
- 2_94°C for 30 s
- 3_62°C for 40 s
- 4_72°C for 40 s
- 5_Go to step 2 (above) for 34 cycles
- 6_72°C for 5 min
- 7_8.0°C hold
- 8_END

Product size: 348 bp

The 348 bp product is specific for the *hi2933Tg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.