

vars*^{hi558Tg}*Nature of the mutation**

hi558Tg 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 (**Hi558_5E02**) anneals to the 5'-portion of the retrovirus that causes the mutation, and the reverse primer (**Hi558_5E01**) anneals to the adjacent genomic region flanking the virus.

Primers:

Hi558_5E01: 5' GCA CGT TCT TCT GTT ATT GCG TC 3'

Hi558_5E02: 5' CAA ACC TAC AGG TGG GGT CTT TC 3'

PCR program (60_40_40):

- 1_94°C for 3 min
- 2_94°C for 30 s
- 3_60°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: 302 bp

The 302 bp product is specific for the *hi558Tg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.