

naca*^{hi5Tg}*Nature of the mutation**

hi5Tg 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 (**Hi5_3E02**) anneals to the 3'-portion of the retrovirus that causes the mutation, and the reverse primer (**Hi5_3E01**) anneals to the adjacent genomic region flanking the virus.

Primers:

Hi5_3E01: 5' AAC GTC CCC AAA CAG TTC CAC 3'

Hi5_3E02: 5' CTT GCA GTT GCA TCC GAC TTG 3'

PCR program (58_40_40):

- 1_94°C for 3 min
- 2_94°C for 30 s
- 3_58°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: 287 bp

The 287 bp product is specific for the *hi5Tg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.