

***gart*<sup>hi3526bTg</sup>****Nature of the mutation**

*hi3526bTg* 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 (**Hi3526B\_3E01**) anneals to the 3'-portion of the retrovirus that causes the mutation, and the reverse primer (**Hi3526B\_3E02**) anneals to the adjacent genomic region flanking the virus.

**Primers:**

**Hi3526B\_3E01:** 5' GCT CAA TAA AAG AGC CCA CAA CC 3'

**Hi3526B\_3E02:** 5' CGA CTC GAT TCA TTC AGA CAG CTC 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: 318 bp**

The 318 bp product is specific for the *hi3526bTg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.