

ccna2^{*hi3594aTg*}

Nature of the mutation

hi3594aTg 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 (**Hi3594a_5E04**) anneals to the 5'-portion of the retrovirus that causes the mutation, and the reverse primer (**Hi3594a_5E03**) anneals to the adjacent genomic region flanking the virus.

Primers:

Hi3594a_5E03: 5' CAT CGT AAA CAA CAT CAC GAC TCG 3'

Hi3594a_5E04: 5' TGC CAA ACC TAC AGG TGG GG 3'

PCR program (60_40_30):

- 1_94°C for 3 min
- 2_94°C for 30 s
- 3_60°C for 40 s
- 4_72°C for 30 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: 234 bp

The 234 bp product is specific for the *hi3594aTg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.