pou5f1^{hi1940Tg}

Nature of the mutation

hi1940Tg 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 (**Hi1940_5E03**) anneals to the 5'-portion of the retrovirus that causes the mutation, and the reverse primer (**Hi1940_5E02**) anneals to the adjacent genomic region flanking the virus.

Primers:
Hi1940_5E02: 5' CCG TAA TTT GGG ACA GTC CAG G 3'
Hi1940_5E03: 5' CCT GAC CTT GAT CTG AAC TTC TCT ATT C 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: 199 bp
The 199 bp product is specific for the *hi1940Tg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.

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