

## *pou5f1*<sup>hi349Tg</sup>

### Nature of the mutation

*hi349Tg* 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 (**MSL4**) anneals to the 5'-portion of the retrovirus that causes the mutation, and the reverse primer (**349c**) anneals to the adjacent genomic region flanking the virus.

#### **Primers:**

**349c:** 5' ACG TCT TCT TTT AAG GAG ACT CTG ACT A 3'

**MSL4:** 5' GCT AGC TTG CCA AAC CTA CAG GT 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: 258 bp**

The 258 bp product is specific for the *hi349Tg* mutant genomic DNA. No PCR product is generated for wild-type genomic DNA.