spna2^{st60}

Nature of the mutation

The *st60* allele contains a single G-to-T point mutation that introduces a premature stop codon and results in a truncated Spna2 protein (Voas et al., Current Biology 17: 562-568, 2007).

Genotyping assay

Genotyping of the *st60* allele is based on the RFLP assay (**R**estriction **F**ragment **L**ength **P**olymorphism; Botstein *et al.*, Am. J. Hum. Genet. 32: 314-331, 1980). This method is used to detect a mutation that either creates or abolishes a site recognized by a specific restriction enzyme. In the RFLP assay, a sequence of interest is first PCR-amplified and then the PCR product is subjected to restriction enzyme digestion. The presence or absence of the mutation is determined by the resulting restriction pattern. The *st60* mutation creates a site recognized by the BfaI restriction enzyme.

Primers:
SPN_07: 5' CCT TCT TAG CCA GCG ATG ACA AG 3'
SPN_08: 5' GAG AAC ACT GAC CTG TCC AAG ACC 3'

PCR program (55_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **55**ºC for **30** sec
4. 72ºC for **30** sec
5. Go to step 2 (above) for 39 cycles
6. 72ºC for 5 min
7. 8.0ºC hold
8. END

Product size: 302 bp

Digestion of the PCR product with the BfaI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	302 bp
PCR product containing the mutation	cleaved	237 bp and 65 bp

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