

CZ97:*socs3b*^{*ihb36*}

Nature of the mutation

The *ihb36* allele contains a 10bp deletion of the *socs3b* coding sequence.

Genotyping assay

Genotyping of the *ihb36* allele is based on the RFLP assay. 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 *ihb36* mutation creates a site recognized by the PstI restriction enzyme.

Primers:

CZ37-F: 5' GCTGATGTCATAGCAGATG 3'

CZ37-R: 5' TGCCTGTGGCCGTTTT 3'

PCR program:

1. 94°C for 3 min
2. 94°C for 30 sec
3. 55°C for 30 sec
4. 72°C for 30 sec
5. Go to step 2 (above) for 29 cycles
6. 72°C for 5 min
7. 12.0°C hold

Product size: 454bp

Digestion of the PCR product with the PstI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	311bp and 143bp
PCR product containing the mutation	unaffected	454 bp