Primer pairs: WT (bp) HEZ(+/KI) (bp) HOZ(KI/KI)(bp)

1. Il6HA5se1 435 435

+Il65HADR1

2. Il63HAUF2 308 308

+ Il6HA3As1

3. mKate2sense2

+ Il6HA3As1 448 448

4. Il6HA5se1

+mKate2As2 456  456

PCR reaction components:

Final concentration

Genomic DNA x ug

10x polymerase buffer 5 ul

5 mM dNTP mix 2 ul

Forward primer (10 pmol/l) 2 ul

Reverse primer (10 pmol/l) 2 ul

DNA polymerase 1 ul

H2O x ul

Total volume 50 ul

PCR reaction condition

1. Initial denaturation 96oC 3 min

2. Denaturation 96oC 30 sec

3. Annealing 56oC 30 sec

4. Extension 72oC 1 min

5. Back to step 2 for 35 cycles

6. Final extension 72 C 10 min

7. Hold 4oC

Primer sequences:

1. Il6HA5se1: GATTCTTTCGATGCTAAACG

2. Il6HA3As1: TCTAACACCTCAAAGCCAAG

3. Il65HADR1: CAACTGGATGGAAGTCTCCTGC

4. Il63HAUF2: ACTGGATATAATCAGGTAGAAACTTGTC