

***flt4*^{ihb438/+} (CZRC catalog ID: CZ1388)**

Nature of the mutation

In 2040 bp of the wild-type *flt4* coding sequence, GTGAAAACATTCGA are inserted in exon 13.

Sense Strand Sequence

tacatcaataaaatgggggttcttgctgaagagatgtgctgttacttcttaattaacattcatttagCTATCCCTGAAGGAT
TCGATATAGAGATGGAGCCCTCAGAGGATCCGCTTGAGCAGGACCTGGTACAGCT
GAAGTGTAATGCAGATAATTCACCTATGAGAACCTGCGGTGGTACCGCCTGGACC
CGCAGACTGTTCCAGAGCTGGACTGCAAGAGTCTGCACCAGTATGCCACATTT
TTGGAGGGACAACACTATCTTTTCAGACCACCAGCAACAACACTGGGTCCTGCAGCTTA
ACATTACCAACATTCAGCTACAGGATGAGGGGAACTATGTGTGTGAGGTGCAGAA
CCGACGAACCGGGGTGAAACATTGTCACCGCAAATACATCCAGTCAAAGgtgaaggt
caacaataactcaacttttattctgtaatttcatttgcagtgcgataaacactaactagctaagctaacttttaaagtagcatcataacctcca
cataaccttcattccgctgtgga

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : Crispr/cas9 target site

Genotyping assay

Primers:

flt4_forward: 5' CACACAGACACACGTACAGT 3'

flt4_reverse: 5' TCCACAGCGGAATGAAGGTT 3'

PCR program:

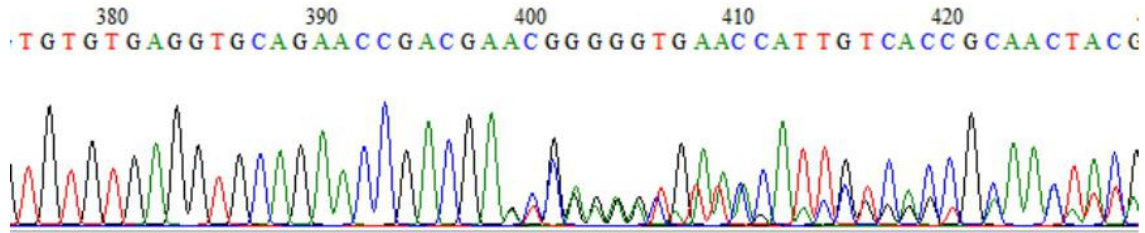
95°C	5min	} 30 Cycles
95°C	30 sec	
55°C	30 sec	
72°C	30 sec	
72°C	8min	
4°C	hold	

Product size: 600 bp





The sequencing results of the CZ1388(+/-):



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WT          GCAGCTTAACATTACCAACATTCAGCTACAGGATGAGGGGAACTATGTGTGTGAGGTGCA
CZ1388      GCAGCTTAACATTACCAACATTCAGCTACAGGATGAGGGGAACTATGTGTGTGAGGTGCA
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WT          GAACCGA-----CGAACCGGGTGAAACATTGTCACCGCAAATACATCCCA
CZ1388      GAACCGACGAGTGAAAACATTCGAACCGGGTGAAACATTGTCACCGCAAATACATCCCA
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WT          GTCAAAGgtgaaggtcaacaatactcaacttttattctggttaatttcatttgcagtgcg
CZ1388      GTCAAAGgtgaaggtcaacaatactcaacttttattctggttaatttcatttgcagtgcg
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