

***flt4*^{ihb439/+} (CZRC catalog ID: CZ1389)**

Nature of the mutation

Between 2040 bp and 2044bp of the wild-type *flt4* coding sequence, AACCG are deleted in exon 13.

Sense Strand Sequence

tacatcaataaaatggggttcttgctgaagagatgtgctgttacttcttataattaacattcatttagCTATCCCTGAAGGAT
TCGATATAGAGATGGAGCCCTCAGAGGATCCGCTTGAGCAGGACCTGGTACAGCT
GAAGTGTAATGCAGATAATTCACCTATGAGAACCTGCGGTGGTACCGCCTGGACC
CGCAGACTGTTCCAGAGCTGGACTGCAAGAGTCTGCACCAGTATGCCACATTT
TTGGAGGGACAACACTATCTTTTCAGACCACCAGCAACAACACTGGGTCCTGCAGCTTA
ACATTACCAACATTCAGCTACAGGATGAGGGGAACTATGTGTGTGA**GGTGCAGAA**
CCGACGAACCGGGGTGAAACATTGTCACCGCAAATACATCCAGTCAAAGgtgaaggt
caaacaataactcaacttttattctgtaatttcatttgcagtgcgataaacactaactagctaagctaacttttaaagtagcatcataacctcca
cata**aaccttcattccgctgtgga**

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:

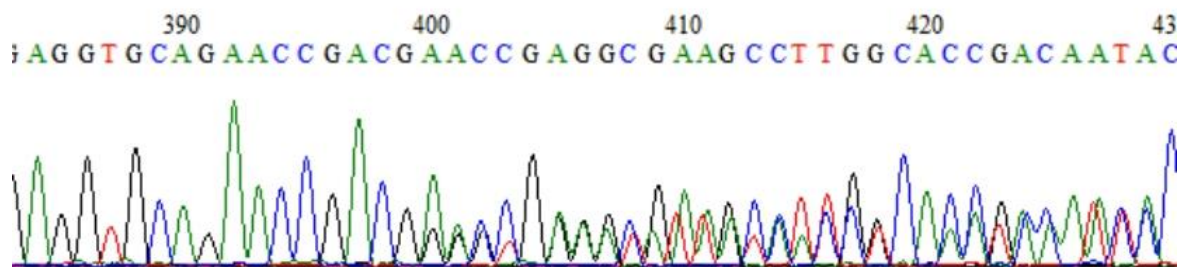
95°C	5min	} 30 Cycles
95°C	30 sec	
55°C	30 sec	
72°C	30 sec	
72°C	8min	
4°C	hold	

Product size: 600 bp





The sequencing results of the CZ1389(+/-):



WT	GTATGCCACATTTTTGGAGGGACAACATCTTTTCAGACCACCAGCAACAACACTGGGTCTT
CZ1389	GTATGCCACATTTTTGGAGGGACAACATCTTTTCAGACCACCAGCAACAACACTGGGTCTT

WT	GCAGCTTAACATTACCAACATTCAGCTACAGGATGAGGGGAACTATGTGTGTGAGGTGCA
CZ1389	GCAGCTTAACATTACCAACATTCAGCTACAGGATGAGGGGAACTATGTGTGTGAGGTGCA

WT	GAACCGACGAACCGGGGTGAAACATTGTCACCGCAAATACATCCCAGTCAAAGgtgaagg
CZ1389	GAACCGA----CGGGGTGAAACATTGTCACCGCAAATACATCCCAGTCAAAGgtgaagg

