

## *alkbh4*<sup>tsu20/+</sup> (CZRC catalog ID: CZ410)

### Nature of the mutation

Between 90 bp to 94 bp of the wild-type *alkbh4* coding sequence, TGTGG is mutated to A in exon 3. The mutated *alkbh4* codes for a truncated protein containing 65 aa, of which 188 aa are identical to wildtype *alkbh4*.

### Sense Strand Sequence

catgagacatctgtctatccaaagGAC TTTGGGCCAAAGGTGAACTTCAAGAAGCGCCGTGTCCATG  
TGGGAAGCTTCAGCGGCCTGCCTGCTATCAGTCGTCGGCTGCTGGTCCGGATGTCAGA  
TTTACCCCAGTTATCTTCGTTCAAACCAGTGGAGCAGTGCAACCTGGACTATGATTCT  
CTCAGAGGCTCCGCCATCGACCCACACTTAGACGACAGCTGGCTGTGGGGAGAAAAC  
CTGGTCACCGTTAACCTGCTCTCGGACACCGTTCTGACCTTGAGCTTGATCAGGGCT  
GGGAGACATGGAGCAGGGCGAGGTGAGGGTGGCTGTGCGTCTTCCCCGAGGTCTC  
TGTTGTGCTCTACGGAGATGCACGGCACCGGTGGAAACATGCCATCCACAGAAAAG  
ACATTCACGGCCGAGAGTGTGCAGCACCTTTCGGGAGCTCTCGGCTGAGTTTCTCCC  
CGGAGGACAGCAGGAGAAACTGGGCTCAGAGTTGTTGGATATAGCGCTGAGCTTCCA  
GGGTGCTCCTCTA

**Uppercase:** Exon/coding sequence

**Lowercase:** intron/noncoding sequence

**atcg:** Forward/Reverse primer

### Genotyping assay

#### Primers:

**CZ410\_forward:** 5' CATGAGACATCTGTCTATCCAAAGGAC 3'

**CZ410\_reverse:** 5' TAGAGGAGCACCTGGAAGC 3'

#### PCR program:

95°C 5min

95°C 30 sec

58°C 30 sec

72°C 30 sec

72°C 8min

4°C hold

} 30 Cycles

**Product size: 539 bp**

### The sequencing results of the CZ410:

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WT          GACTTTGGGCC AAAGGTGA ACTTCAAGAAGCGCCGTGTC CATGTGGGAAGCTTCAGCGGC
CZ410       GACTTTGGGCC AAAGGTGA ACTTCAAGAAGCGCCGTGTC CA----AGAAAGCTTCAGCGGC
*****
WT          CTGCCTGCTATCAGTCGTCGGCTGCTGGTCCGGATGTCAGATTACCCAGTTATCTTCG
CZ410       CTGCCTGCTATCAGTCGTCGGCTGCTGGTCCGGATGTCAGATTACCCAGTTATCTTCG
*****
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### Reference:

Qingrui Sun, Xingfeng Liu, Bo Gong, Di Wu, Anming Meng, Shunji Jia. Alkbh4 and Atrn act maternally to regulate zebrafish epiboly. *Int J. Biol. Sci.* 2017, 13(8):1051-1066