

***nanog*^{ihb97/+} (CZRC catalog ID: CZ220)**

Nature of the mutation

Between 56 bp to 57 bp of the wildtype *nanog* coding sequence, AT is deleted in exon 1.

Sense Strand Sequence

CAGGTGATGTAAATGGGTCGGTAATTGTTACTATGAGGAGGAGCGCTTCAATCAGCA
TCCGTTTTTCGAGGGTAGTCGAGCCCTTTGGGTTTTTTTTTTTAGTAGTCCGGTTTTTTTT
AATTAGGTGTTTAGTACGTTACTTTCTGCTTGAGTTTATCTAACGGCGAAATGGCGGA
CTGGAAGATGCCAGTGAGTTACAATTTAACCCATCTTATCATGCATATGCATACGGG
CTCATGTACCCGCAAGTGTCAGAGCACGGCGTCCCGAATCTGAGCTGGCCCCGATGCC
GCGTACACACACTCCGGCGGGGTCACAGCGGGCTACTTTACCGCTCAAACACTGCACAG
TCGCCACCCTGGAGCCCGGAGAACGGCGGGCGCCAGCAGCACCTACAGCCAGTACCCG
GGACTCGCAGAACGGACGGCTCTTTCTGTCTACAACAAGACTGAGCCCGACCAA
AAGGCCAAAGATGCAGAGCAGACCAGCAGTGATACACCCAGTGATTCCGAGGCCCA
CACGCCAGgtaagccttcactgaccgaaagttaacacgggtcgggtg**atcgctacactcgactcgata**

Uppercase: Exon/coding sequence

Lowercase: intron/noncoding sequence

atcg : Forward/Reverse primer

atcg : cas9 target site

Genotyping assay

Primers:

nanogTALEN_F2: 5' caggtgatgtaaatgggtcgta 3'

nanogTALEN_F2: 5' tatcgctcgagtgtacgcatg 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: 586 bp

The sequencing results of the CZ220:

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WT          TAGGTGTTTAGTACGTTACTTTCTGCTTGAGTTTATCTAACGGCGAAATGGCGGACTGGA
CZ220       TAGGTGTTTAGTACGTTACTTTCTGCTTGAGTTTATCTAACGGCGAAATGGCGGACTGGA
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WT          AGATGCCAGTGAGTTACAATTTTAACCCATCTTATCATGCATATGCATACGGGCTCATGT
CZ220       AGATGCCAGTGAGTTACAATTTTAACCCATCTTATCATGC--ATGCATACGGGCTCATGT
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WT          ACCCGCAAGTGTGAGAGCACGGCGTCCC GAATCTGAGCTGGCCCGATGCCGCGTACACAC
CZ220       ACCCGCAAGTGTGAGAGCACGGCGTCCC GAATCTGAGCTGGCCCGATGCCGCGTACACAC
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Reference:

He MD, Zhang FH, Wang HL, Wang HP, Zhu ZY, Sun YH. Mutat Res. 2015 Oct; 780:86-96. Efficient ligase 3-dependent microhomology-mediated end joining repair of DNA double-strand breaks in zebrafish embryos.