



## ***Df(Chr23:mir200a,mir200b,mir429)hza53* (CZRC catalog ID: CZ1431)**

### **Nature of the mutation**

The *hza53* allele was a mutant line, induced by the CRISPR-cas9 in the AB genetic background. deleted an 8.4-kb fragment containing the premiR-200b/-200a/-429a on chromosome 23 (chr23-miR-200-KO). Ectopic expression of miR-429a, miR-200a, and miR-200b, which are located in the miR-200 cluster on chromosome 23, significantly reduced motility traits of sperm.

### **Genotyping assay**

#### **Primers:**

Primer1 (5'-3') F1: CCAAGTGATGCTTACTATCC

R1: CACCGTAGCTTCATCATGTC

Length of expected PCR product (bp):424

PCR protocol: 94° 3 min; 94° 30 Sec, 55° 30 Sec, 72° 40 Sec; 34 cycles;72°10 min

Primer2 (5'-3') F2: GGGAGACCTTTTCTCACAGATG

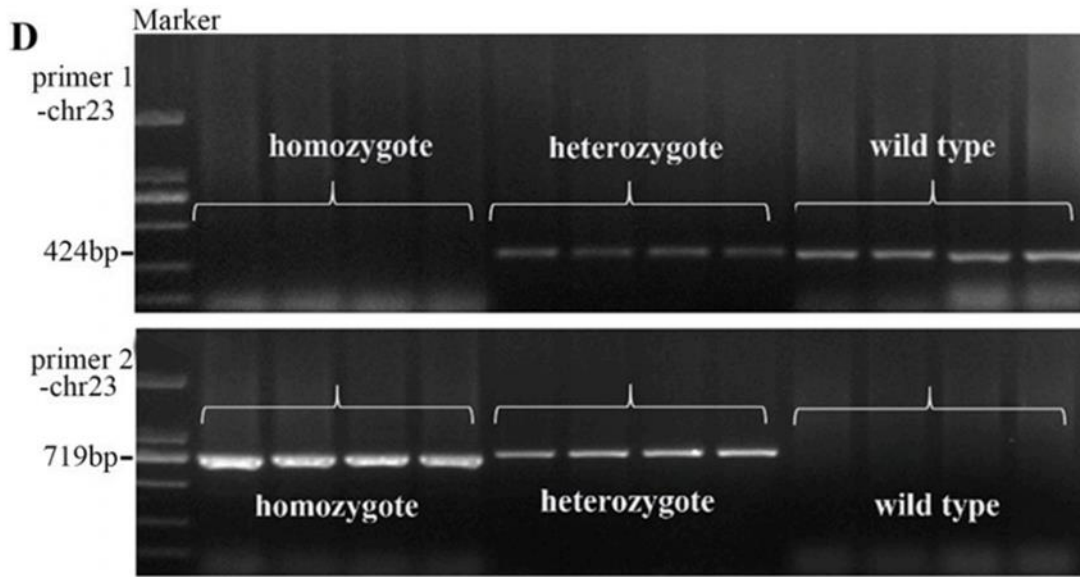
R2: GAGACTACTTAGCAAAGTA

Length of expected PCR product (bp):719

PCR protocol: 94° 3 min; 94° 30 Sec, 58° 40 Sec, 72° 40 Sec; 34 cycles;72°10 min

**Identification of homozygote, heterozygote of chr-23-KO and WT zebrafish. A 424-bp band was detected in the WT and heterozygote zebrafish and no band was observed in the homozygote zebrafish when using primers 1F and 1R. A 719-bp band was detected in the heterozygote and homozygote zebrafish and no band was observed in WT zebrafish when using primers 2F and 2R for genome of chromosome 23.**





### Reference

Xiong, S., Ma, W., Jing, J., Zhang, J., Dan, C., Gui, J.F., Mei, J. (2018) An miR-200 cluster on chromosome 23 regulates sperm motility in zebrafish. *Endocrinology*. 159(5):1982-1991

