**Supplemental Figure data**

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**Supplemental Figure 1. No mutations in the seed region of miRNA200b/200a/429a were induced by CRISPR/Cas9. A,** gRNA1, gRNA2 and gRNA3 were designed in the seed regions of miRNA200b, miRNA200a and miRNA429a. *Cas9* mRNA and gRNA against each of the miRNA were injected into tilapia embryos at one-cell stage. B, The genomic DNA fragments spanning the target site were amplified using gene specific primers. T7 endonuclease I (T7EI) assay was performed. T7EI assays showed no mutagenesis induced. The control embryos (WT) were wild type.

**Supplemental Table 1 Primers used in the present study**

|  |  |  |
| --- | --- | --- |
| **Primer** | **Sequence (5'-3')** | **Purpose** |
| gRNA-miRNA125a | TAATACGACTCACTATAACCACGCAACCGGCCCTTTGGTTTTAGAGCTAGAAATAGC | miRNA125a targeting |
| miRNA125-S-F1 | GTTCTCTCATTTAACTCGAATG | miRNA125a mutant screening |
| miRNA125-S-R1 | AGAAACCACTTTAACTGCAAG |
| gRNA1-miRNA200b | TAATACGACTCACTATAGGCTATCATCATCATTACCGTTTTAGAGCTAGAAATAGC | miRNA200a/200b/429a targeting |
| gRNA2-miRNA200a | AATACGACTCACTATACTGTTGTTCTAACACTGTCGTTTTAGAGCTAGAAATAGC |
| gRNA3-miRNA429a | TAATACGACTCACTATAGAAGGTAATGCCATTTAATGGAGTTTTAGAGCTAGAAATAGC |
| gRNA4 | TAATACGACTCACTATAGGTTAAGTTCAGGAGTTTAGGTTTTAGAGCTAGAAATAGC |
| gRNA5 | TAATACGACTCACTATAGACAGAATTCGGTACAGCCTGGTTTTAGAGCTAGAAATAGC |
| gRNA6 | TAATACGACTCACTATAGAAACTTTGACTCAAGGCACAGTTTTAGAGCTAGAAATAGC |
| miR200-F1 | GCCCAGTCTTCTGTGACAGCAAG | miRNA200a/200b/429a mutant screening |
| miR200-R1 | GGTTGGCAGATAAGTTAAAGAG |
| miR429-F2 | GAACTACTATAGTTCTTAGG |
| miR429-R2 | GCGGTAAGAGTAAGAACGCCG |
| *vasa*-3'-UTR-gRNA1 | TAATACGACTCACTATAGGCTGCTGACGATGAAGAATGTTTTAGAGCTAGAAATAGC | *vasa* 3'-UTR targeting |
| *vasa*-3'-UTR-gRNA2 | TAATACGACTCACTATAGGTGTGTTTGAATCATTCCGTTTTAGAGCTAGAAATAGC |
| *vasa*-3'-UTR-S-F1 | GGTTAAAGTTGTGTTACACTG | *vasa* 3'-UTR mutant screening |
| *vasa*-3'-UTR-S-R1 | CCTTGTGAGGTGCTGGGTCC |
| *vasa*-qPCR-F1 | AAATCAACAGGATCGGAGCG | Real-time PCR |
| *vasa*-qPCR-R1 | TGTGGCAATAAAATCAGCCTGTC |
| *β-actin*-F | GGCATCACACCTTCTACAACGA |  |
| *β-actin*-R | ACGCTCTGTCAGGATCTTCA |
| M13+ | CGCCAGGGTTTTCCCAGTCACGAC | Clone screening and sequencing |
| M13- | AGCGGATAACAATTTCACACAGGA |

**Supplemental Table 2 Mutation rates of miRNA125 in each mutant induced by CRISPR/Cas9.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Target** | **Number of F0 tested** | **Mutant** | **Frequency (%)** | **Indel frequency (%)** | | | | | | | | **Average indel frequency (%)** |
| **#1** | **#2** | **#3** | **#4** | **#5** | **#6** | **#7** | **#8** |
| **miRNA125** | 25 | 12 | 48 | 18 | 23 | 56 | 34 | 68 | 33 | 74 | 28 | 41.7 |

Note: Twenty five F0 fish were screened by restriction enzyme digestion. Among the twelve mutants, eight fish, which have uncleaved DNA band with stronger intensity compared with other four fish, was selected to calculate the mutation frequency. The indel mutation frequency within each individual was estimated by quantifying the uncleaved DNA band intensity to total DNA band intensity of the restriction enzyme digestion.

**Supplemental Table 3 Mutation rates of *vasa*-3’UTR in each mutant induced by CRISPR/Cas9.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Target** | **Number of F0 tested** | **Mutant** | **Frequency (%)** | **Deletion frequency (%)** | | | | | | | | **Average deletion frequency (%)** |
| **#1** | **#2** | **#3** | **#4** | **#5** | **#6** | **#7** | **#8** |
| ***Vasa* 3’-UTR** | 22 | 8 | 31.8 | 23 | 34 | 11 | 43 | 16 | 12 | 9 | - | 21.1 |

**Note:** F0 fish were screened by PCR amplification. The PCR products with expected size, including wild type and mutated bands, were purified and cloned into pGEM-T easy vector. Forty single colonies were randomly selected and screened by PCR. The deletion frequency was determined by calculating number of mutated clones versus total clones sequenced.