GENERATION OF GOLDEN GOLDFISH *Carassius auratus auratus* VIA TYROSINASE GENE TARGETING BY CRISPR/CAS9

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Introduction

Goldfish (*Carassius auratus*), regarded as one of the world's earliest ornamental fish, has garnered significant attention from researchers due to its diverse range of color patterns and unique morphological variations. Tyrosinase (*tyr*) serves as the rate-limiting enzyme in the enzymatic cascade responsible for melanin biosynthesis. In our study, we have successfully developed a highly efficient and precise genome editing technology for *Carassius auratus* tyrosinase (*tyr*), resulting in the creation of a strikingly golden goldfish. The duplicated *tyr* genes (*tyrA* and *tyrB*) were first identified in *C. auratus*, and the CRISPR/Cas9 was used to disrupt both *tyr* genes. The edited albino mutants displayed a complete absence of melanocytes in both their eyes and body surface, whereas mosaic mutants exhibited varying degrees of melanin reduction. Notably, disrupting only *tyrA* or *tyrB* failed to yield a reduction in melanin content. The whole-genome resequencing was employed to comprehensively screen the off-target sites in the mutant individuals at the genome-wide scale. Our findings underscored the indispensable role of *tyr* genes in melanin synthesis within goldfish, while also demonstrating the remarkable efficiency and accuracy of the CRISPR/Cas9 editing system in generating novel phenotypes in fish.

Results

➢Detection of tyr-sgRNA effectiveness

Six sgRNAs were design to target *tyr* genes, namely tyr-sgRNA1, tyrsgRNA2, tyr-sgRNA3, tyr-sgRNA4, tyrsgRNA5 and tyr-sgRNA6. Both tyr-sgRNA1 and tyr-sgRNA2 could cause target mutations in *tyrA* and *tyrB* simultaneously. Specifically, tyr-sgRNA3 exclusively induced mutations in *tyrA*, leaving *tyrB* unaffected. Conversely, tyr-sgRNA4 specifically edited *tyrB* .

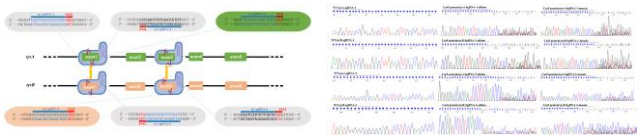

Fig.1 Sanger sequencing of PCR products in the injected embryos.

➢Identification of mutate phenotype

The observed phenotypes of the edited fish can be classified into two categories based on body and eye coloration: albino mutants and mosaic mutants. Complete albino mutants exhibited a complete absence of melanocytes both in their eyes and on their body surfaces. The mosaic mutants, also known as incomplete albino mutants, were characterized by varying degrees of decrease in melanin on the body surface and eye.

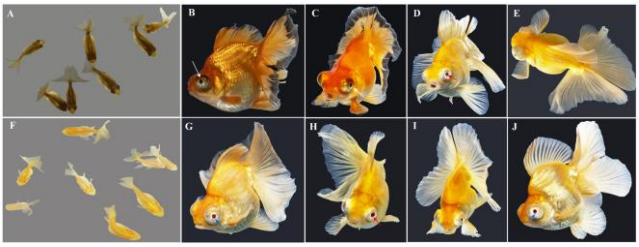

Fig.2 The albino mutants injected with the mixture of tyr-sgRNA1/tyr-sgRNA2/Cas9 protein. A-C displayed the wide type goldfish. D-J represented the albino mutants. White, red and blue arrow respectively indicated black, vivid and wined-red eyes.

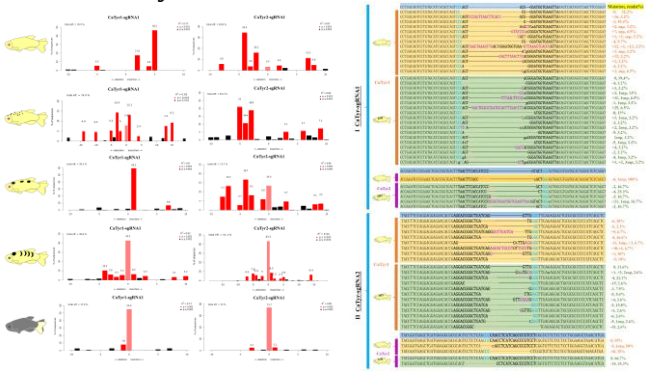

Fig.3 The mutation type on genomic level of tyr-sgRNA1 and tyr-sgRNA2 at albino and mosaic mutants by whole-genome resequencing.