Cite this as: Yuewen JIANG, Qihua PAN, Zhi WANG, Ke LU, Bilin XIA, Tiansheng CHEN. 2024. Efficient genome editing in medaka (*Oryzias latipes*) using a codon-optimized SaCas9 system. *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*, 25(12):1083-1096. http://doi.org/10.1631/jzus.B2300899

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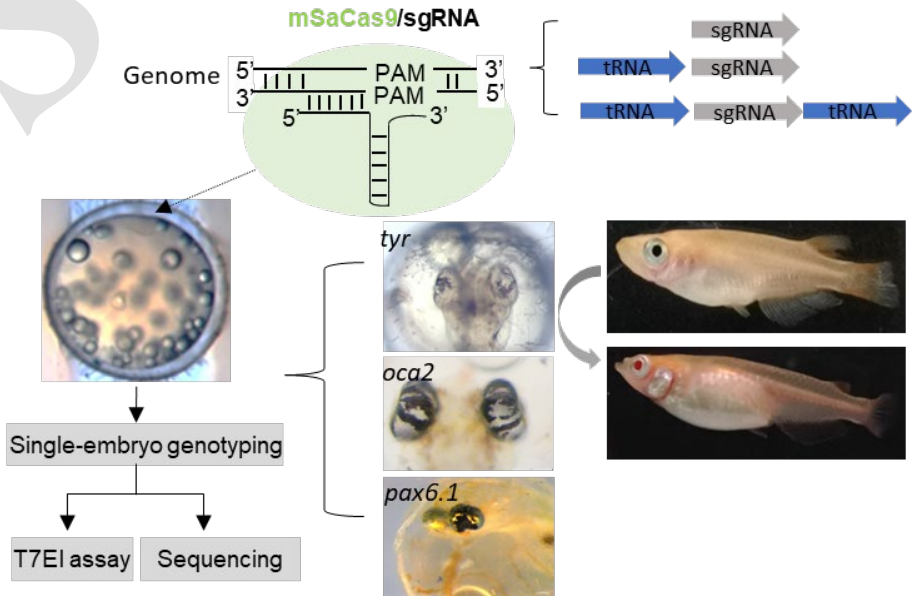
Efficient genome editing in medaka (*Oryzias latipes*) using a codon-optimized SaCas9 system

Key words: *Staphylococcus aureus* Cas9 (SaCas9); Medaka; Transfer RNA (tRNA); Gene editing; Tyrosinase (*tyr*); Oculocutaneous albinism II (*oca2*); Paired box 6.1 (*pax6.1*)

Research Summary

This article mainly established a codon-optimized SaCas9 system for gene editing in medaka, and stable inherit mutants are generated by SaCas9.

- Editing *tyr*, *oca2* and *pax6.1* gene in medaka.
- Editing *tyr* gene using SaCas9/tRNA-sgRNA systems.
- SaCas9 can mediate gene editing in both sgRNAs, and gene editing can occur in different cells during embryonic cleavage.
- The PAM sequence NNGRRT is necessary for gene editing efficiency.



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Innovation points

- **SaCas9 gene editing system is optimized in medaka.**

- **The cassette of tRNA-sgRNA mediates the gene editing both in RNA and DNA contents.**

- **The all-in-one cassette CMV-SaCas9-tRNA-sgRNA-tRNA is also functional in embryos.**

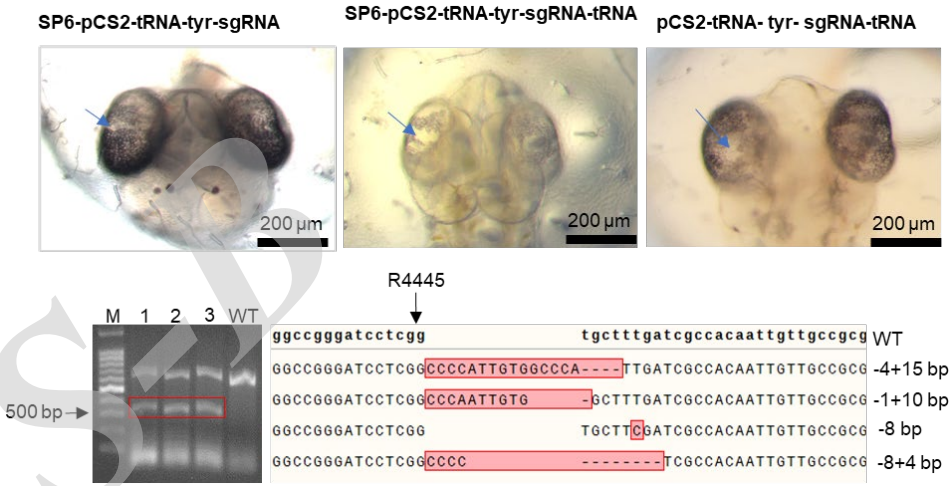

Figure 6

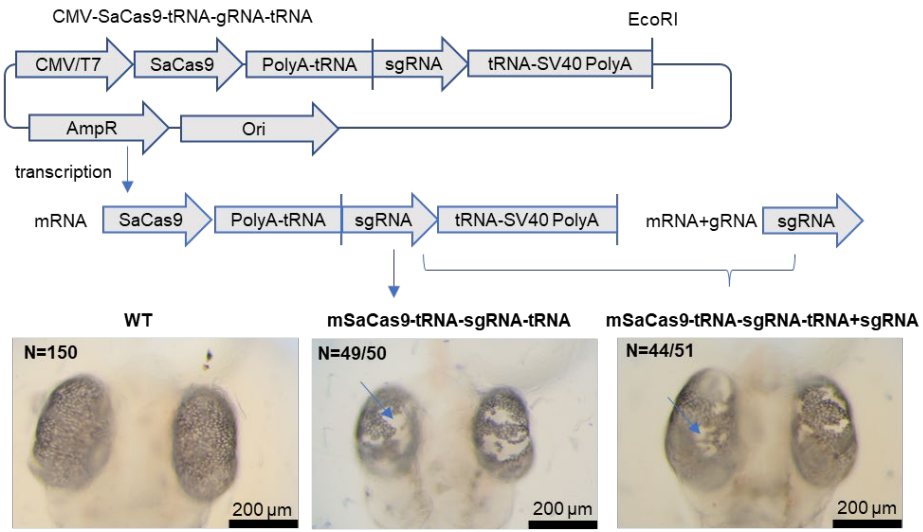

Figure 7

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Innovation points

A series of table and figures were generated to summarize the editing ability of the SaCas9 system in medaka genome.

Table 2 | Injection contents and mutation ratio in the experiment.

Figure 2-4 | Analysis of sgRNAs knockout in three genes.

Figure 5 | Analysis of sgRNA knockout without PAM in *tyr* gene.

Figure 6 | Analysis of tRNA-sgRNA in RNA or DNA content in *tyr* gene knockout results.

Figure 7 | Analysis of all-in-one vector of mSaCas9-tRNA-sgRNA-tRNA in RNA content in *tyr* gene knockout results.

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