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Guide RNA Design and Delivery for CRISPR/Cas9 Editing in Annual Killifish

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Background

- *Austrofundulus limnaeus* is a species of annual killifish native to ephemeral ponds in Venezuela
- *A. limnaeus* embryos can enter a stage of dormancy (diapause)
- Diapause embryos are tolerant to environmental extremes
- Their unique development makes *A. limnaeus* a great model organism
- CRISPR-Cas9 has shown to be successful in knocking out gene function in other fish species
- This technology has not been used in the *A. limnaeus* (Fig 1)

Fig. 1: *A. limnaeus* male and female size comparison, (Podrabsky, J. E. et.al, 2017).

Hypothesis:

We hypothesize that targeted knockouts of the tyrosinase gene, a key enzyme in the synthesis of melanin, will lead to fish without the ability to produce black/brown pigment.

Materials and Methods

Design guide RNAs using ChopChop

Set up for microinjections (Microscope, needle with cocktail, embedded embryos, ice – Fig 2)

Green fluorescent protein (GFP) study to test for mRNA translation in embryos

Test injection location, Cell vs. Yolk (Fig 3)

Microinject Embryos

Monitor embryos at 25°C for four days then transfer to 30°C

Hatch embryos – score phenotypes (Fig 4)

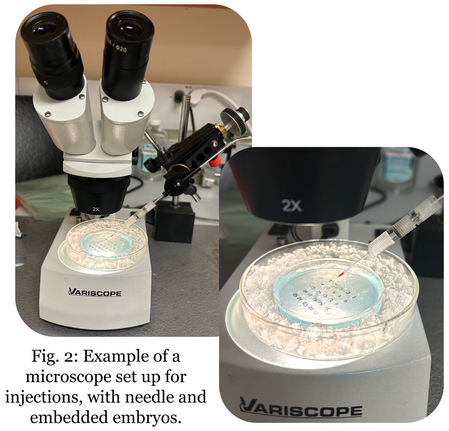
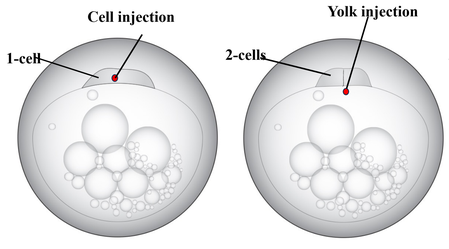


Fig. 2: Example of a microscope set up for injections, with needle and embedded embryos.



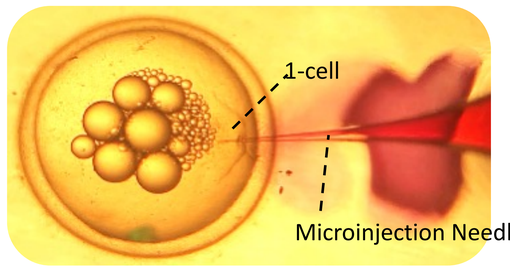


Fig. 3:Example of cell injection vs. a yolk injection locations and an image of a microinjection in progress (photo by Y. Chmykh).

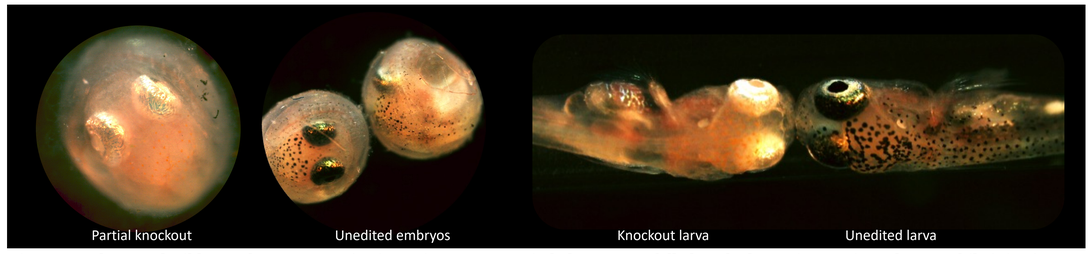
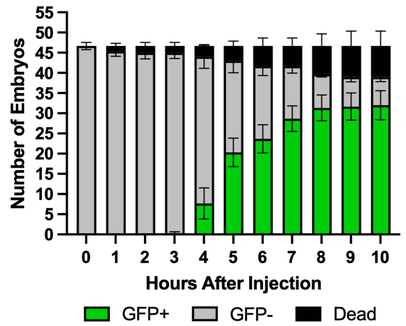


Fig. 4: Knockout and wildtype phenotypes. Injection using cap9 protein led to successfully knocked out pigment in embryos, while injections with cas9 mRNA failed to produce knockouts.

Take Home Messages/ Future Directions

- We discovered for the first time in this species that CRISPR/Cas9 can be used to successfully knockout the tyrosinase gene
- Tyrosinase knockout do not produce black pigment in *A. limnaeus*
- Cas9 mRNA is not an effective way to deliver cas9 to early embryos of *A. limnaeus* based on the delayed translation of the protein
- Cas9 protein is highly effective at inducing genome edits in *A. limnaeus*
- In the future we plan to establish a laboratory strain of tyrosinase knockout fish to aid in viewing embryonic structures during development

Results



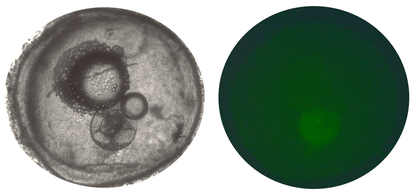


Fig. 5: GFP is translated four hours after injections at Wourms' stage 6-7, the 8-16 cell stages of development.

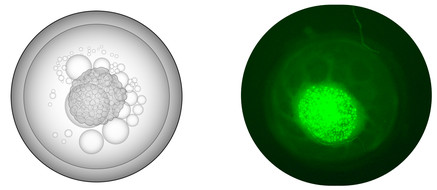


Fig. 6: Top view of GFP+ blastomeres glowing in a Wourms' stage 11 embryo (flat solid blastula, photo by Y. Chmykh).

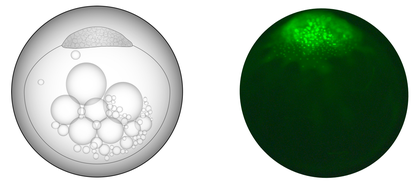


Fig. 7: Side view of Wourms' stage 11 embryo (flat solid blastula) with GFP+ blastomeres glowing (Photo K. M.-V.).

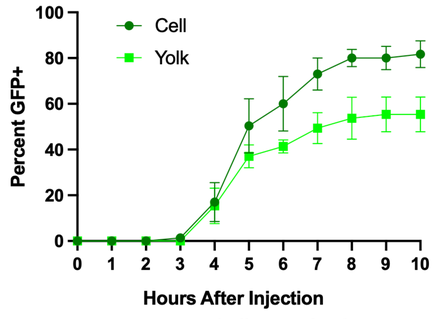


Fig. 8: A greater proportion of cell-injected embryos are GFP+ compared to yolk injected embryos.

Acknowledgement's

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References