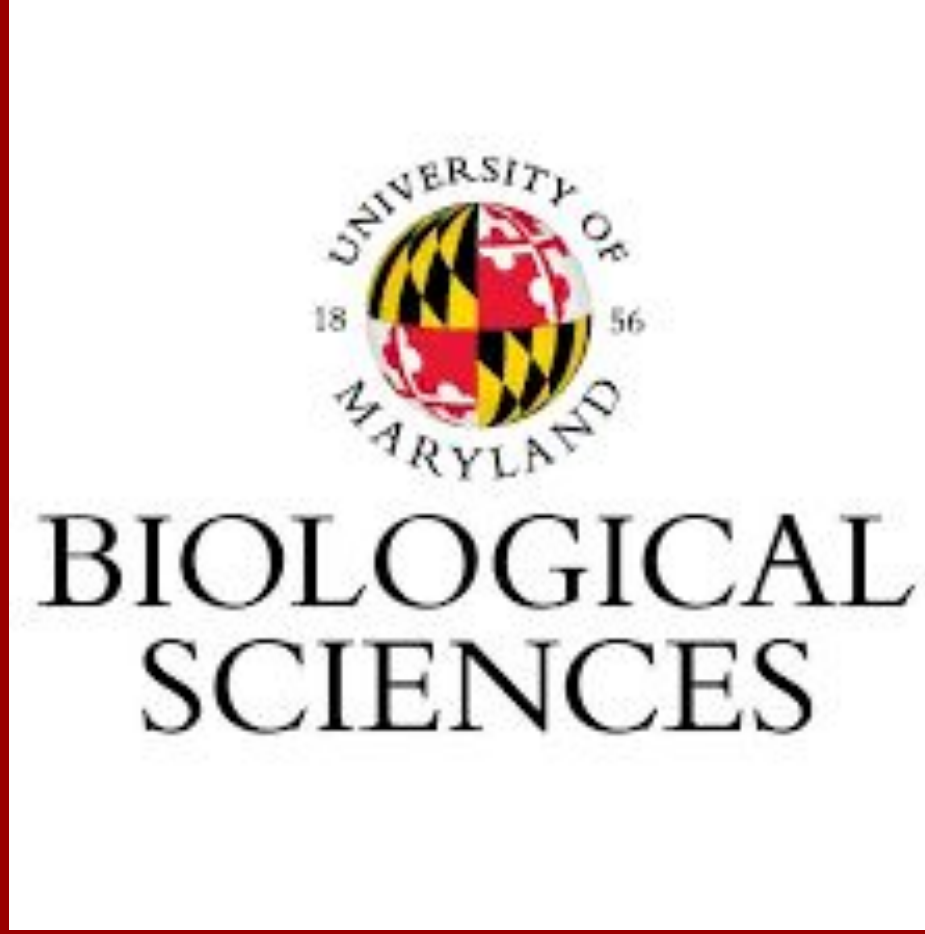


# Exploring the Effect of Putative PGF Synthase Genes in *A. Burtoni* Cichlid



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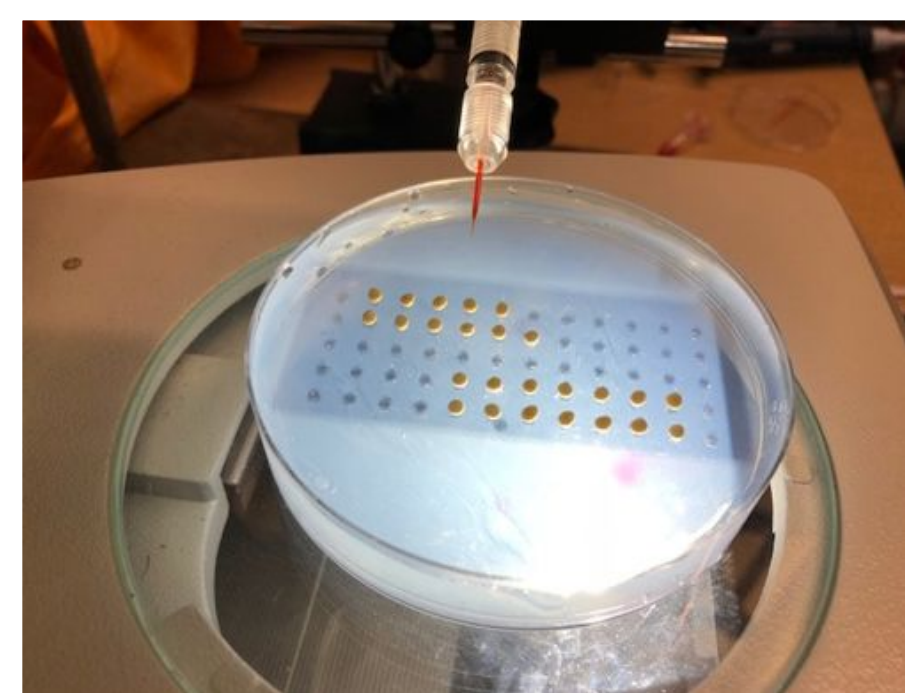
## Introduction

- Investigate the neuroendocrine mechanism behind reproductive behavior
- **Study species:** *Astatotilapia burtoni*
  - Display unique reproductive behaviors (Quivering, circling, mutual pecking & mouthbrooding of embryos)
- **Dual roles of prostaglandin F<sub>2a</sub> (PGF<sub>2a</sub>)**
  - **In females:** A reproductive hormone that initiates mating behavior
    - Knockout of its receptor (Ptgfr) yielded a complete loss of all reproductive displays
  - **In males:** Females release PGF<sub>2a</sub> metabolite(s) that is sensed by male and can elicit preference behaviors.
- **What enzyme is responsible for synthesizing PGF<sub>2a</sub>?**
  - We hypothesize that aldo-keto reductase 1b (AKR1b) is a PGF<sub>2a</sub> synthase
  - Two paralogs in *A. burtoni* cichlid: LOC102291490 (AKR1b-603) & LOC102291786 (AKR1b-604)

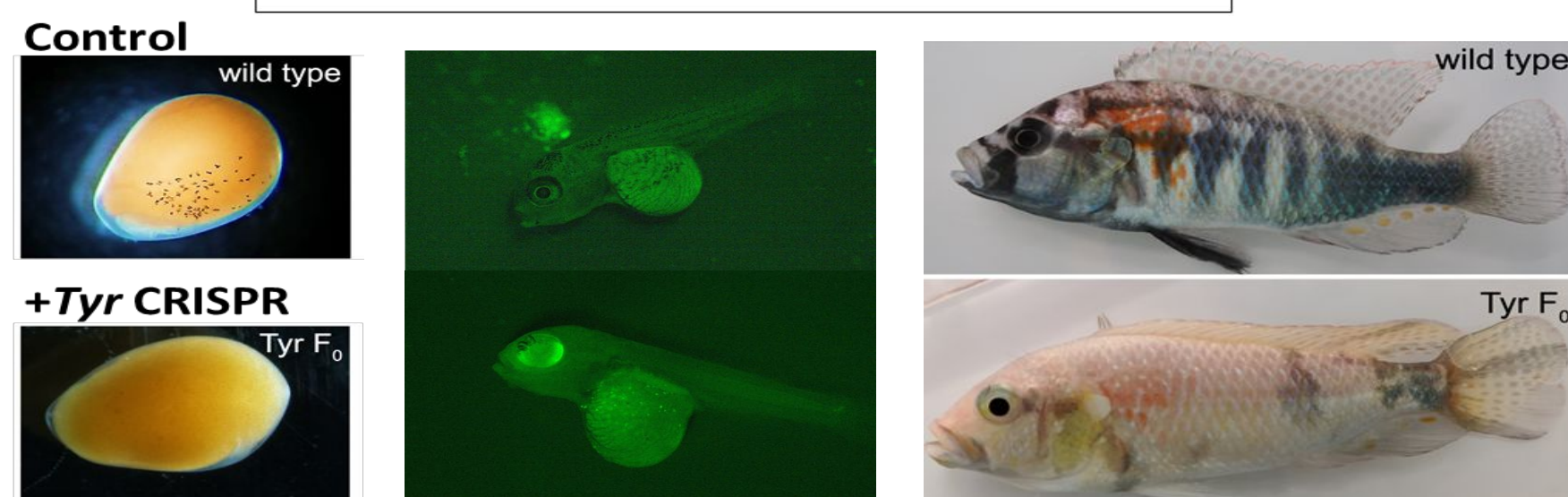


## Optimizing CRISPR/Cas9 Gene Editing Protocol

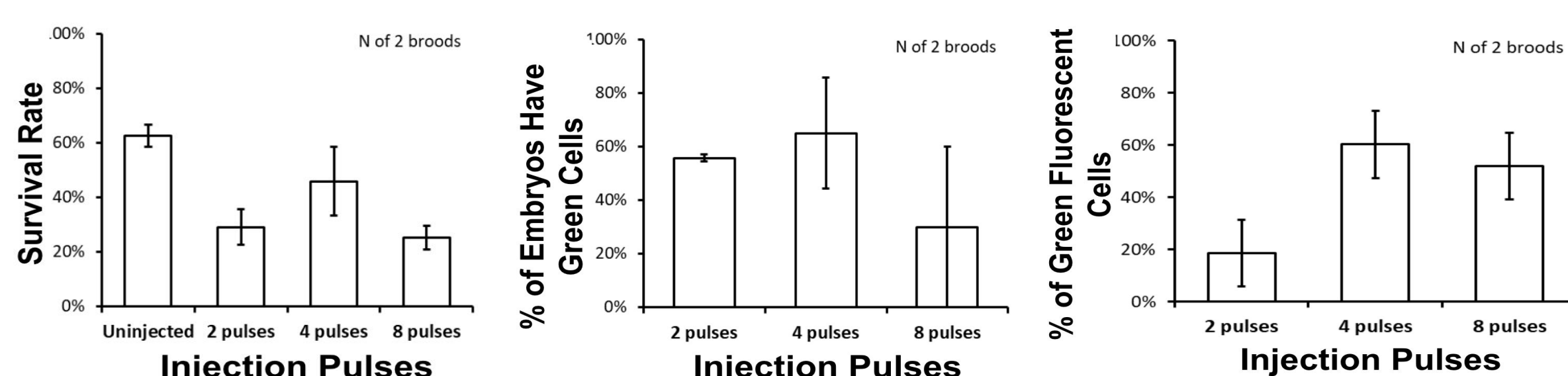
- Cas9 and gRNA mixture that is injected for DNA modification



- Tyrosinase (*Tyr1*) facilitates the creation of melanin
- Knockout yields a pigmentless individual

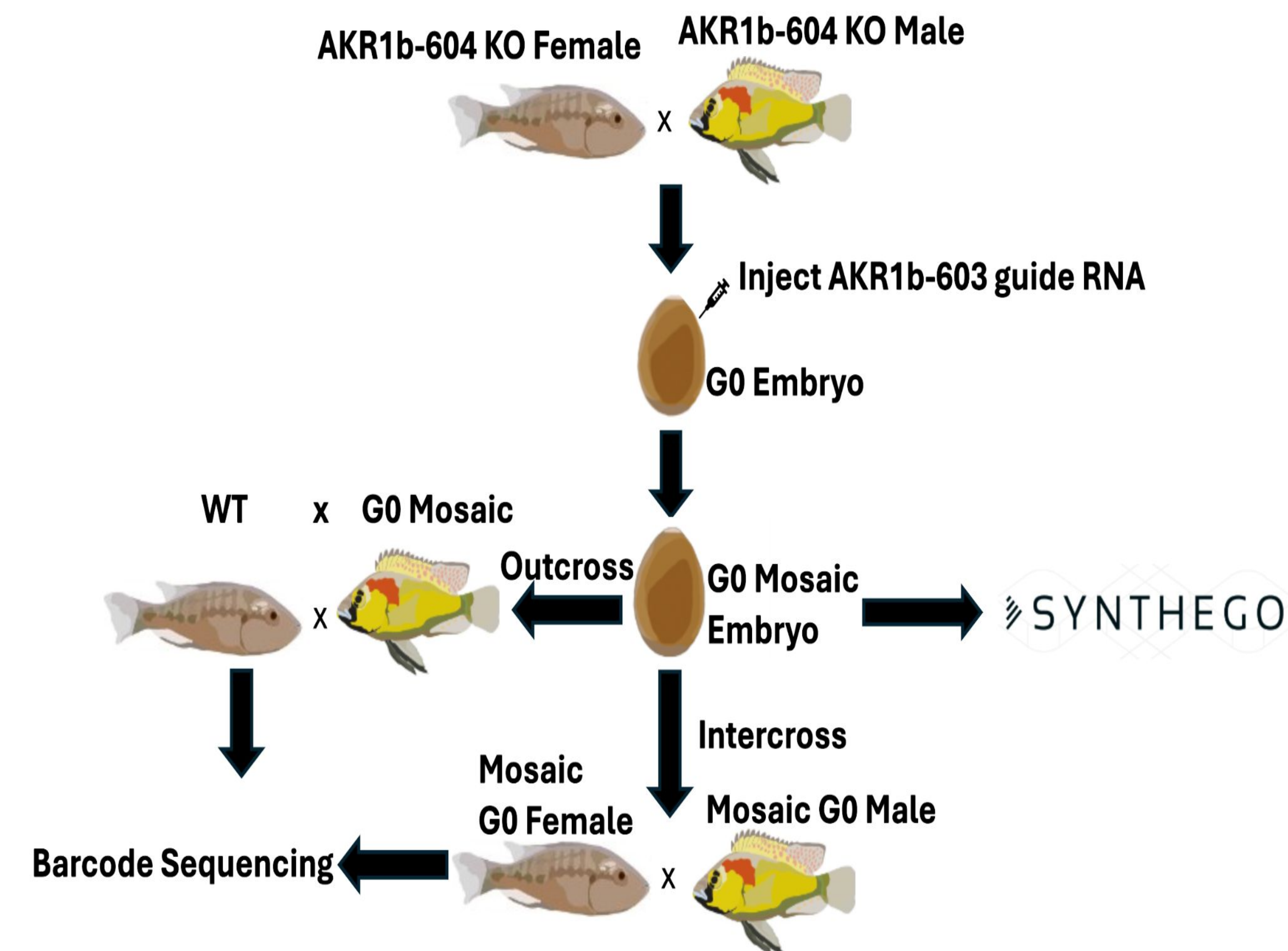


- Determined the quantity of individuals with black vs green pigment cells, along with precise counts, after embryo injection with varying pulses.

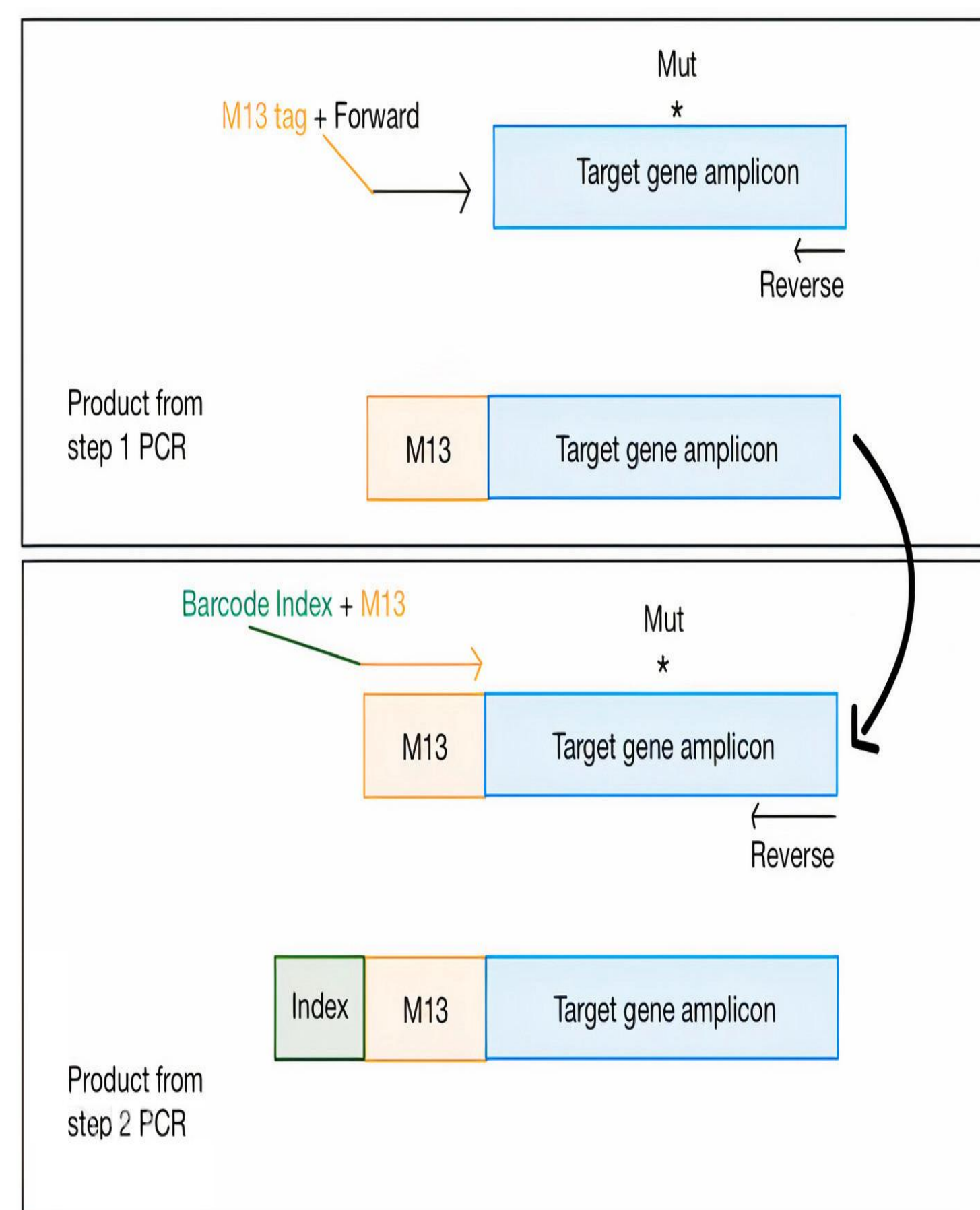


- Optimized protocol are used for producing AKR knockout

## Generation of an AKR Dual-Knockout Line



### Barcode Sequencing

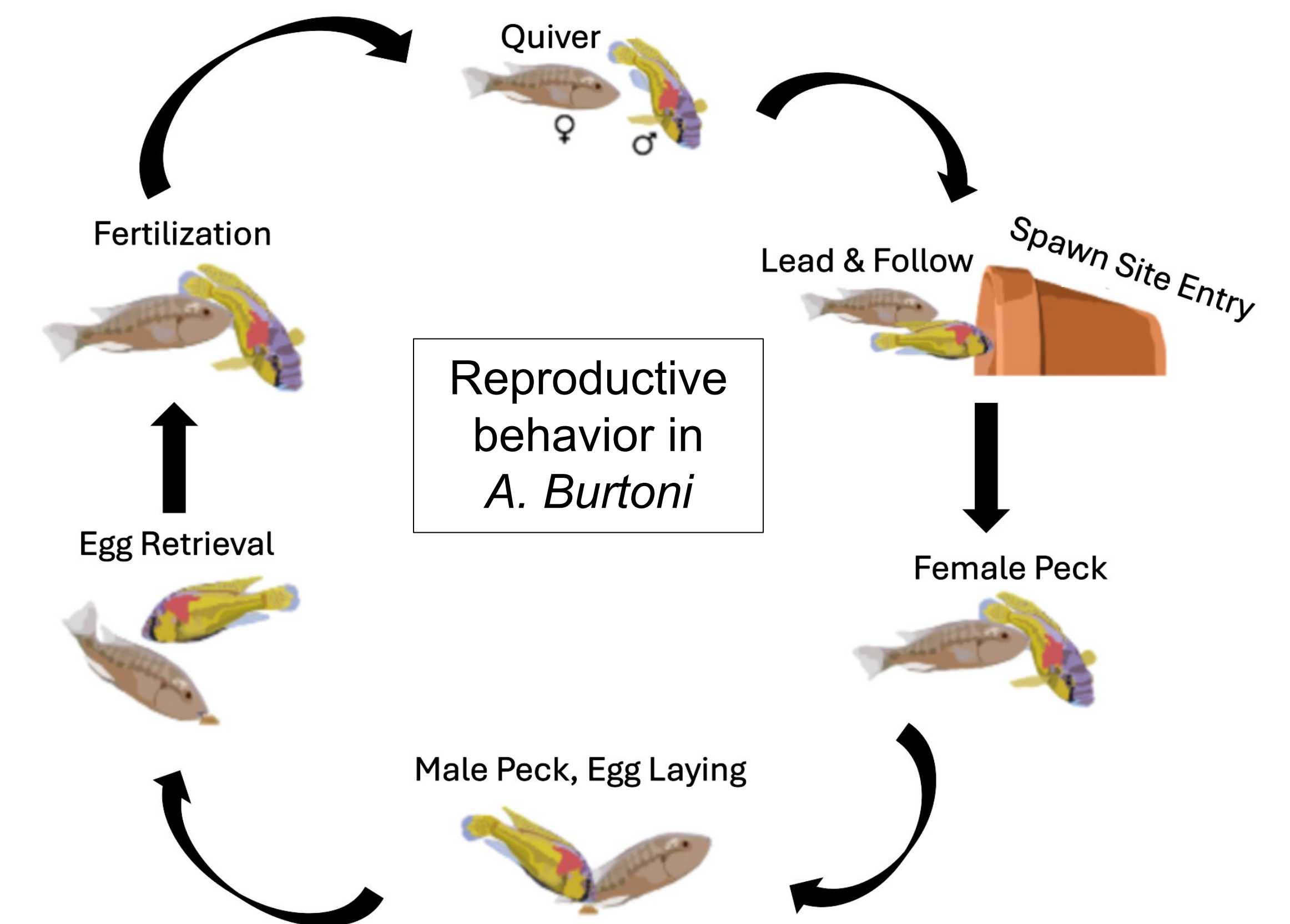


### Synthego ICE Analysis



## Observing for Dysfunctions in Female Reproductive Behaviors

- Mutant females were housed, tagged, and free mating with males was permitted
- Animals were recorded and spawning events were tracked per individual
- Predict that reproductive cycles in mutant females will be longer or completely abolished
  - Reproductive cycle: the duration between two spawning events
- Predict that serum PGF<sub>2a</sub> levels will be lower in mutant females



## Investigation of Male Mouthbrooding Behavior

- In wild-type *A. burtoni* cichlid, females mouthbrood embryos
- However, male mouthbrooder was discovered in AKR1b dual-KO mutant line
- **Do male and female shared a similar neural circuitry in mouth brooding behavior?**
- Previous data suggested that female releases PGF<sub>2a</sub> metabolites to inhibit male retrieving eggs during spawning.
- Test male mouthbrooding behavior in:
  - (1) WT males mate with AKR1b mutant female
  - (2) WT males mate with PGF<sub>2a</sub>-injected AKR1b mutant female

