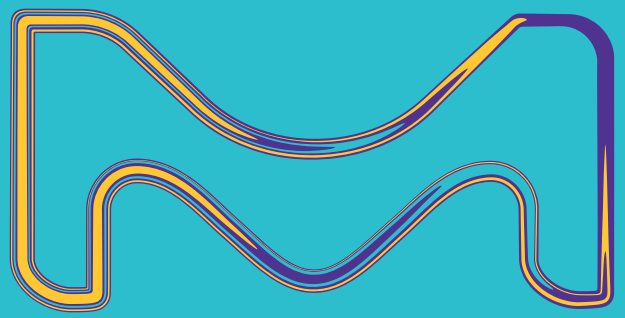
Progress toward transgenesis in Biomphalaria glabrata and implications for snail control

Nicolas J Wheeler, Nathalie Dinguirard, Theresa Maier; Erica KO Namigai, Josh Tycko, Jutta Reinhard-Rupp, Timothy P Yoshino, Mostafa Zamanian



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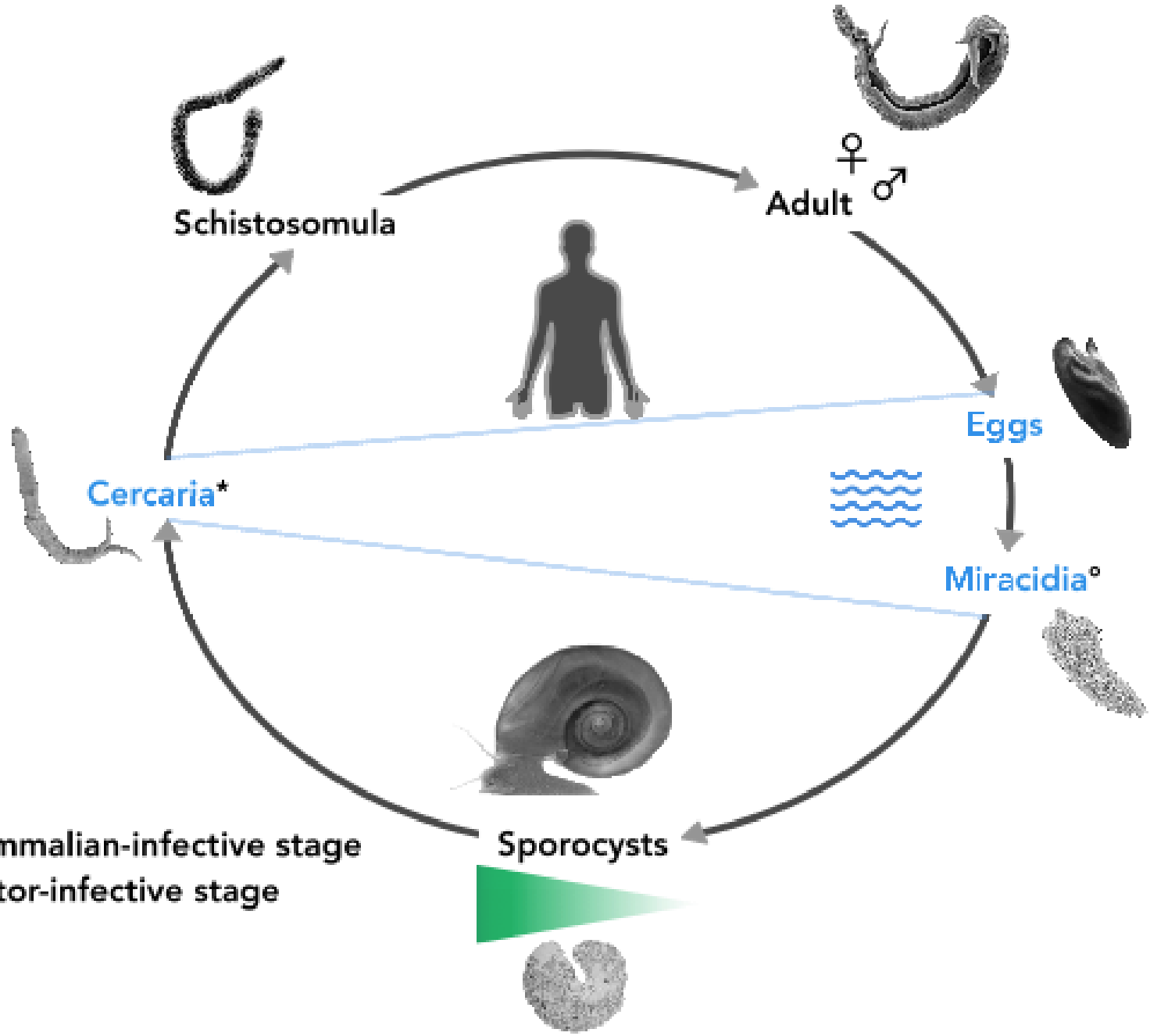
B. glabrata - host of *Schistosoma mansoni*



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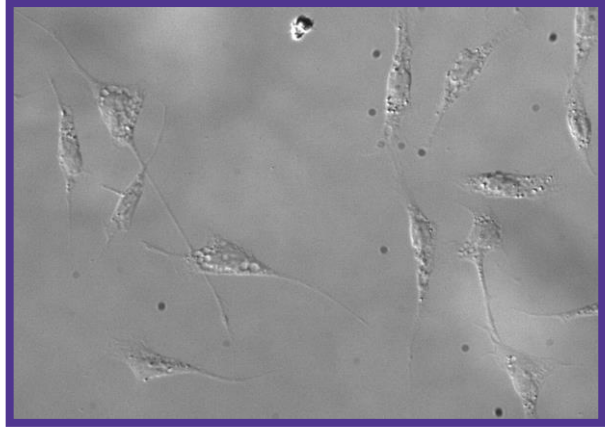
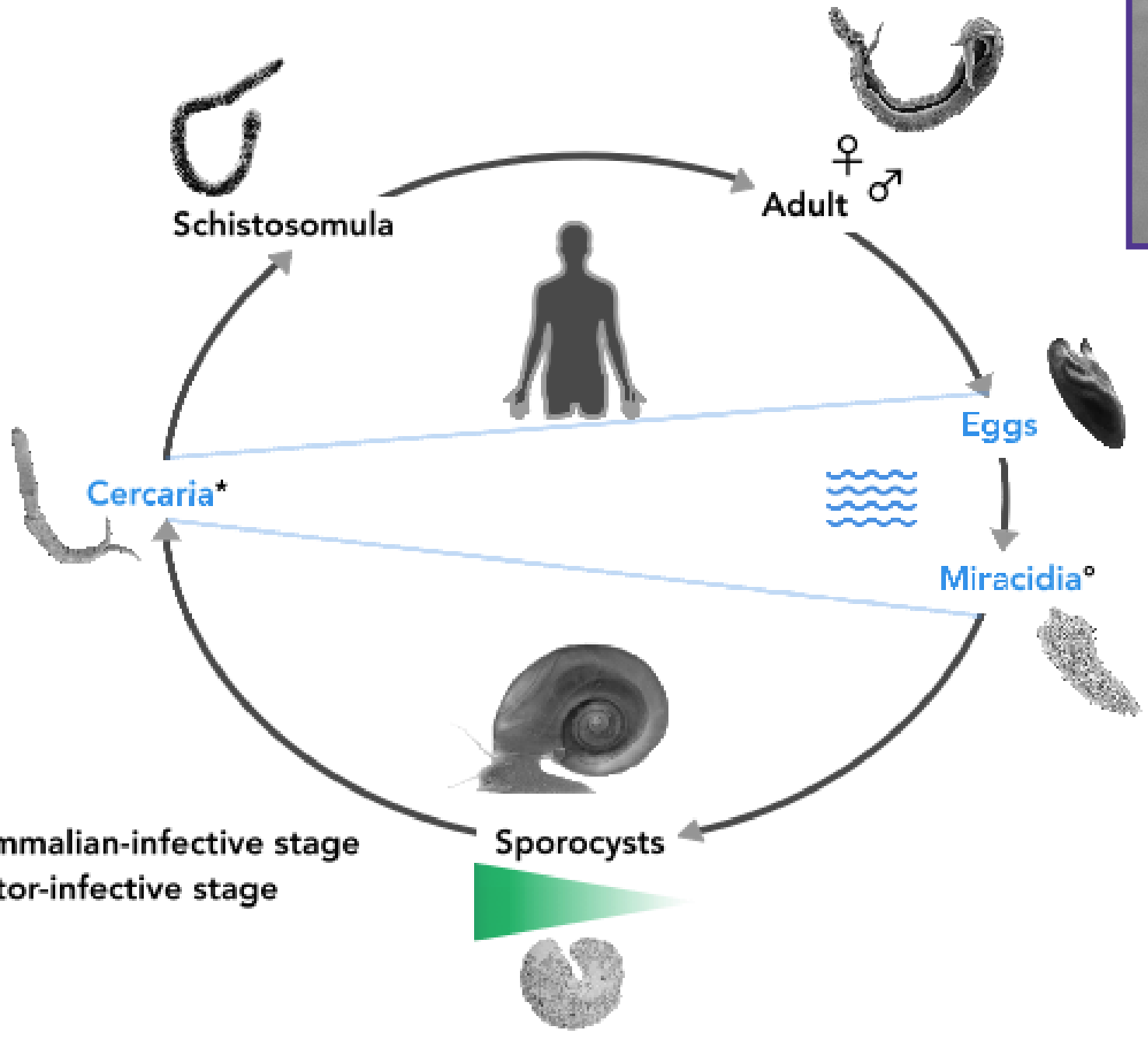


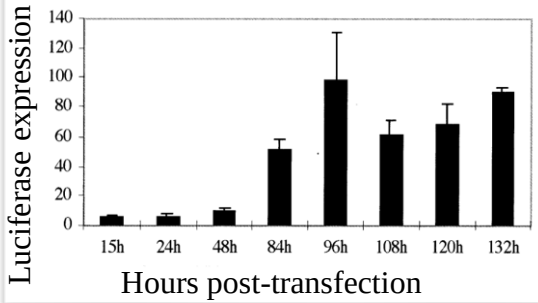
Biomphalaria glabrata embryonic cell line



History of genetic manipulation of *B. glabrata*

Bge

Lardens et al. 1996



Yoshino et al. 1998

	$HSP_{0.5k}$	$HSP_{1.0k}$
26°C	0.04 ± 0.02	0.95 ± 0.76
40°C	14.9 ± 11.9	10.6 ± 1.4

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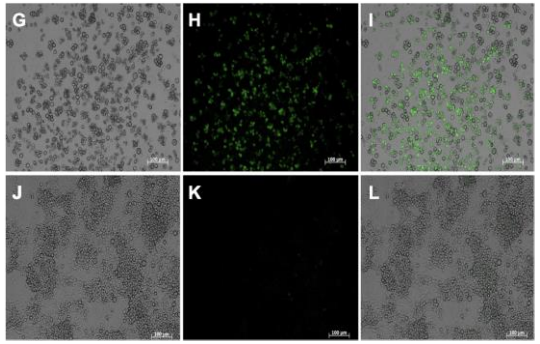

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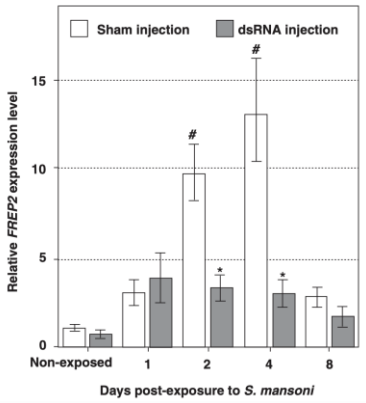

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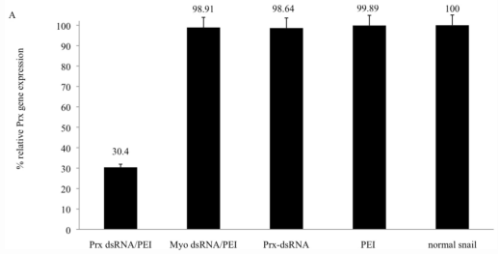

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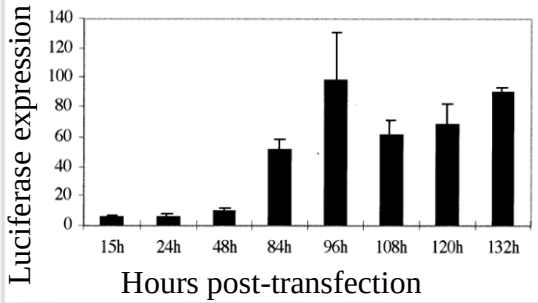

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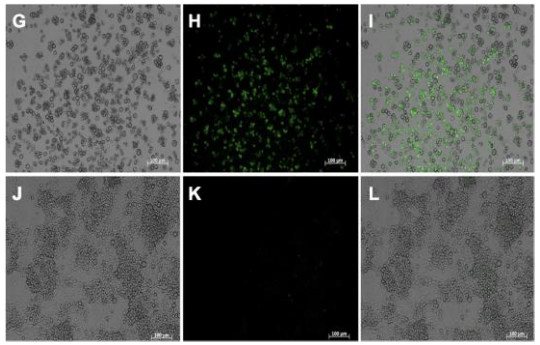
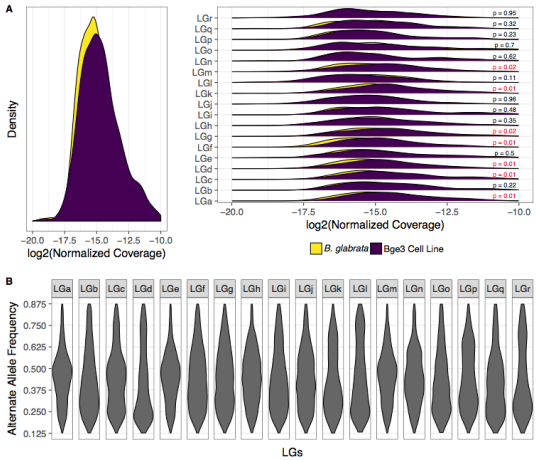


Genome Paper
Wheeler et al. 2018



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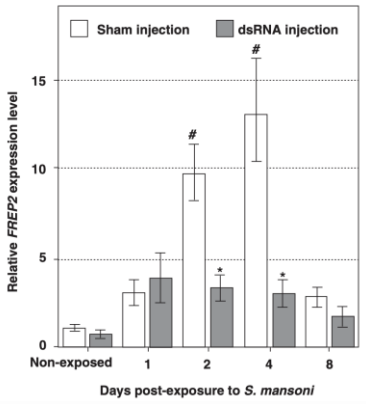

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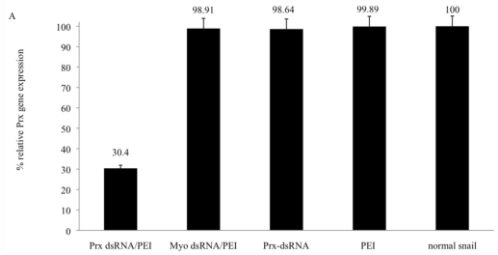

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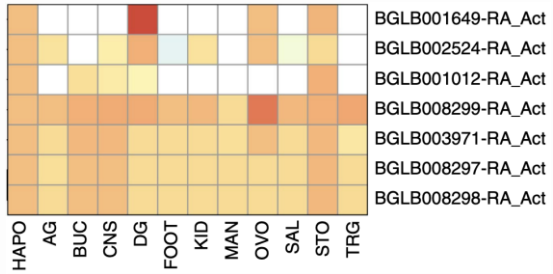

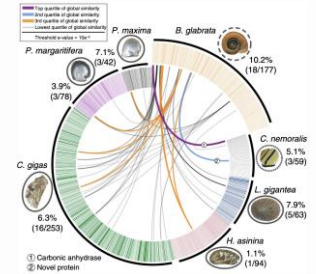

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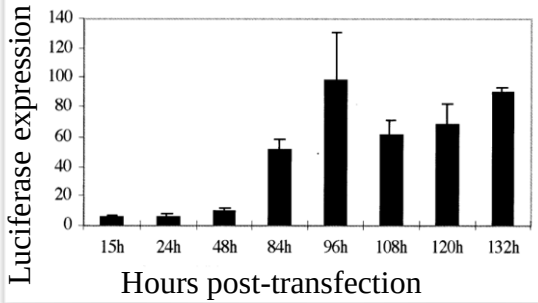

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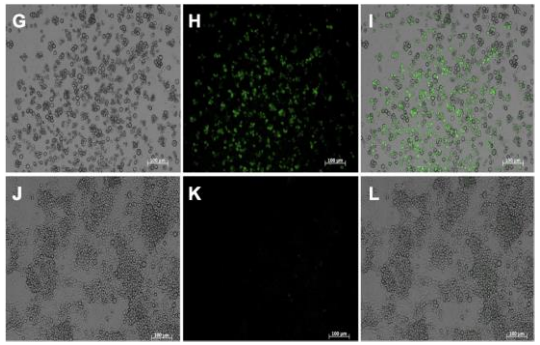

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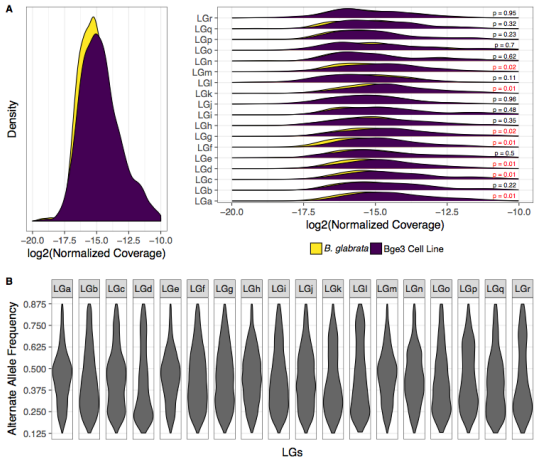

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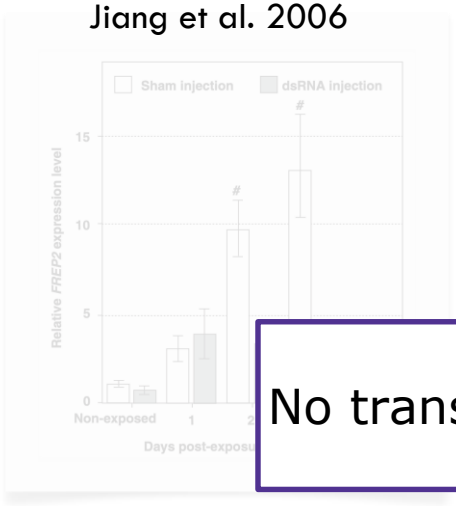

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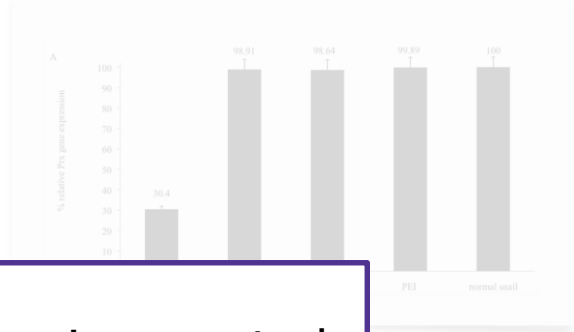


No transgenesis reported

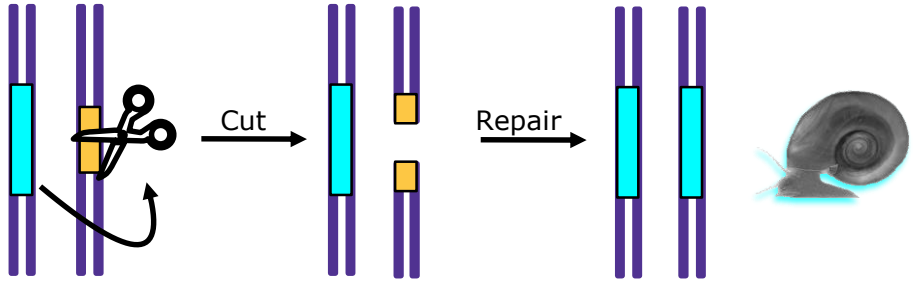
Genome Paper
Adema et al. 2017

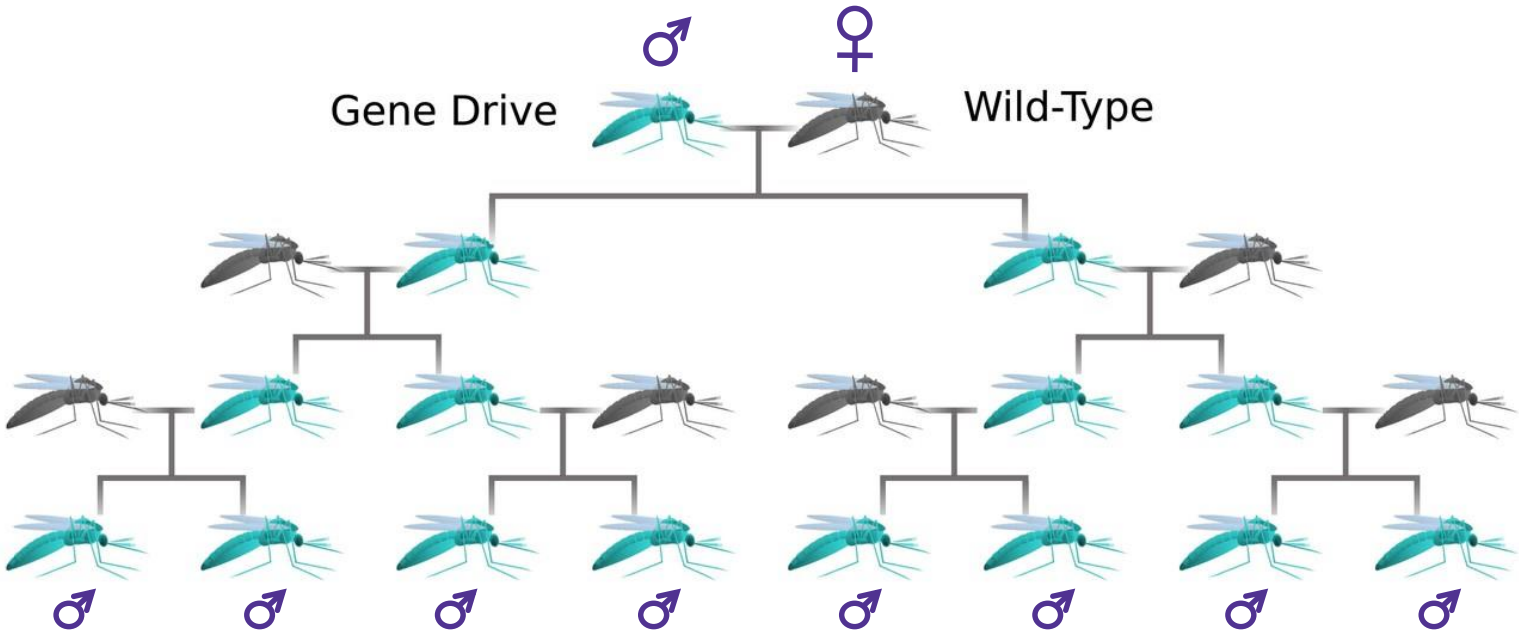
Why transgenesis now?

Genome editing →

Driving endonuclease gene

Endonuclease target site





Sex-biasing drive → Population suppression

Adapted from Esvelt et al. 2014, Elife

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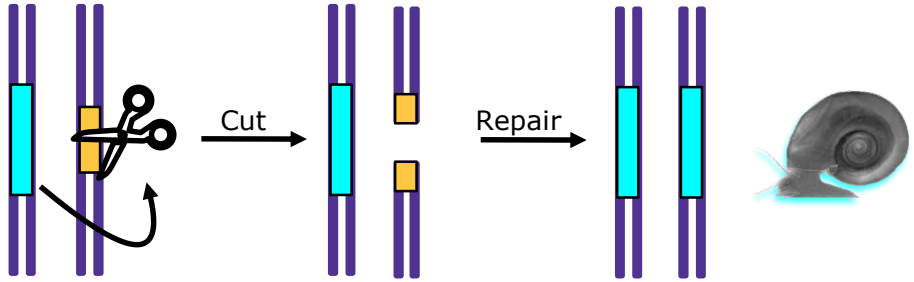
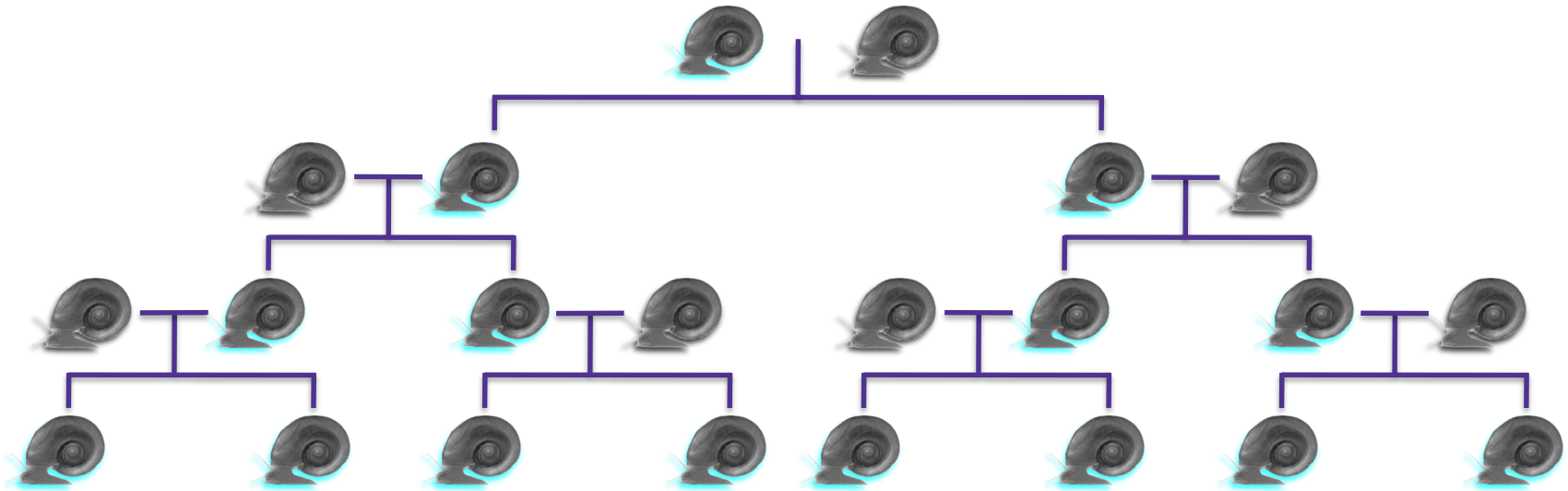
Genome editing →

Driving endonuclease gene

Endonuclease target site





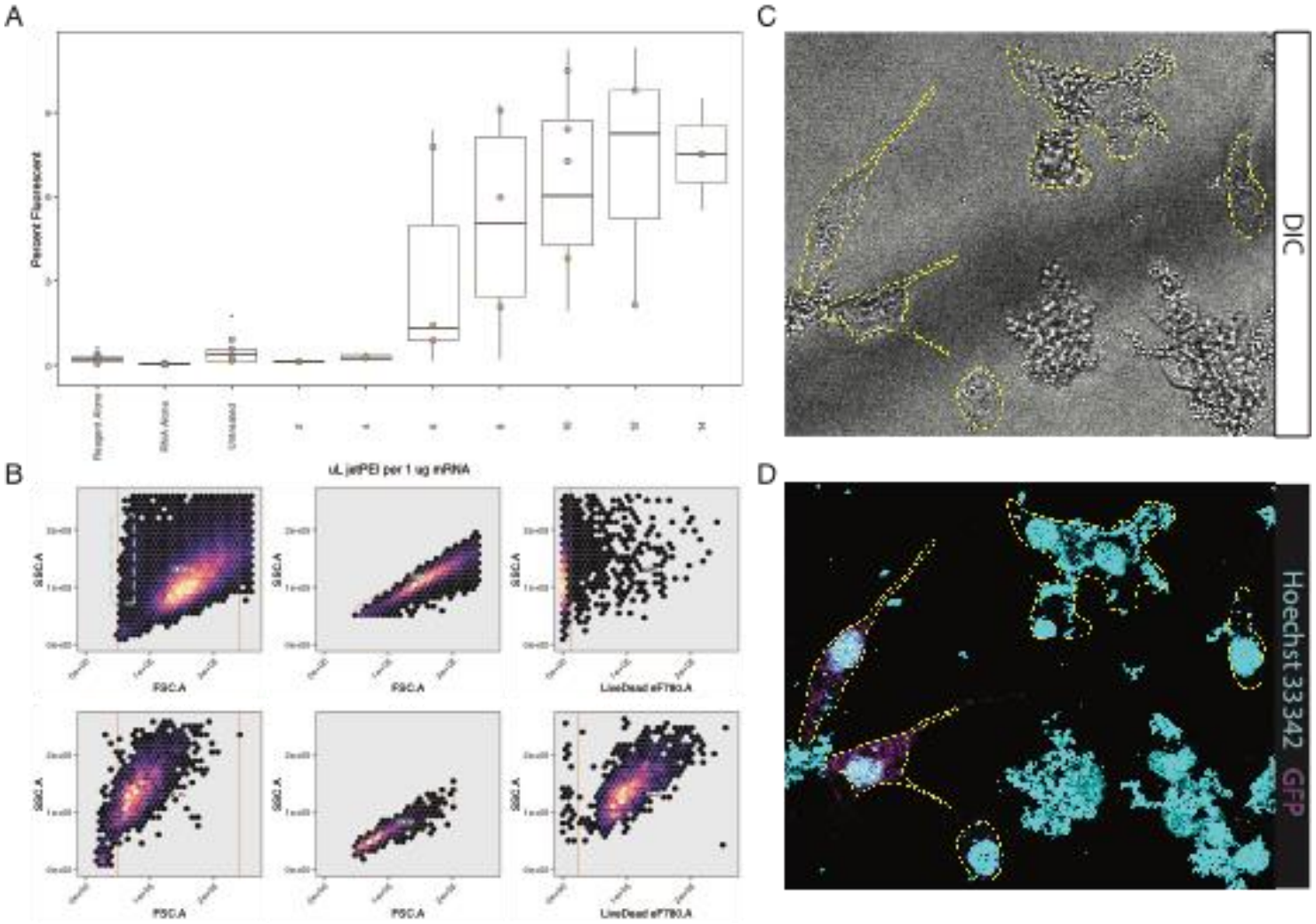
Population modification drive → Helminth-resistant population

Goals

1. Re-establish and update the transgenesis protocol for the Yoshino lab strain of Bge
2. Develop transgenesis at the egg stage
3. Develop transgenesis at the adult stage

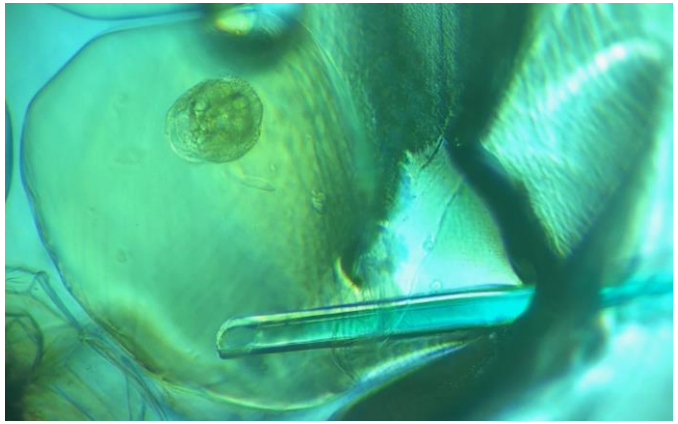
1. Re-establish and update the transgenesis protocol in the Yoshino lab strain of Bge

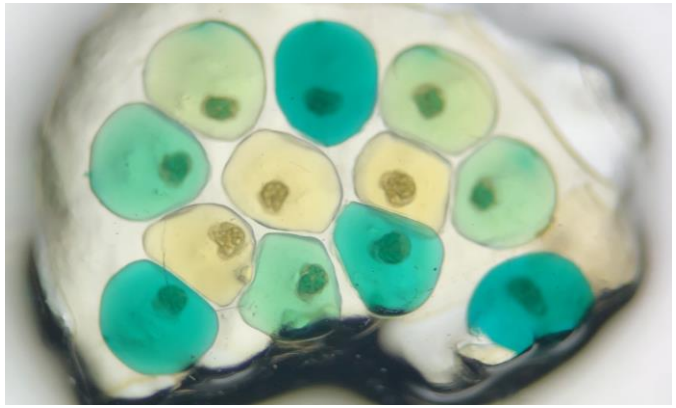
- GFP-encoding mRNA
- jetPEI at higher ratios than previously used and reported
- 6-12% transfection efficiency
- Little cytotoxicity (though evidence of some cell lysis)

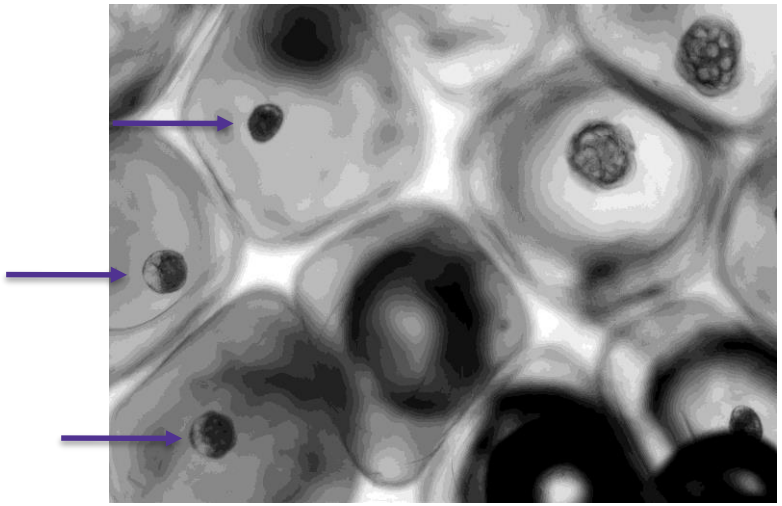


2. Develop transgenesis at the egg stage

- Establish delivery techniques
  - Injection of multiple eggs per clutch
- Dye stains embryo and is retained in the egg
- Injection does not harm development or hatching if performed at post-gastrula stage (trocophore)







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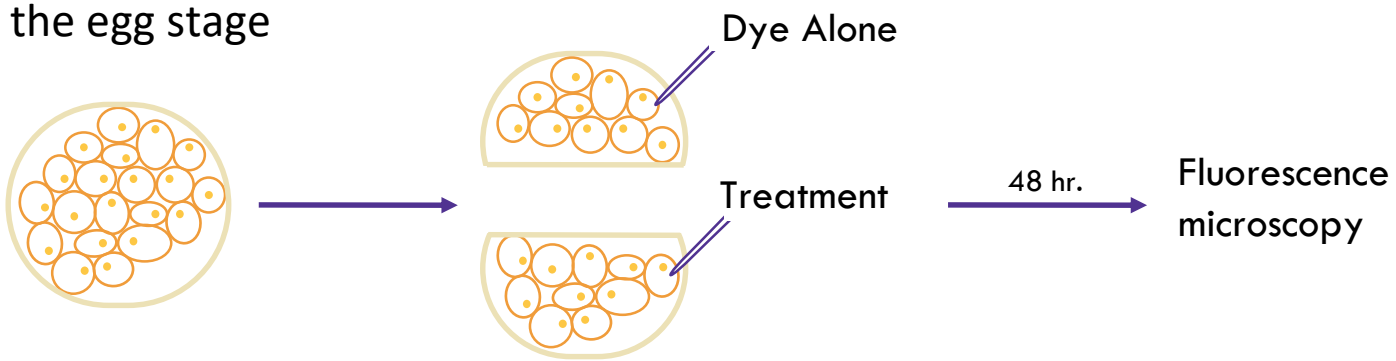

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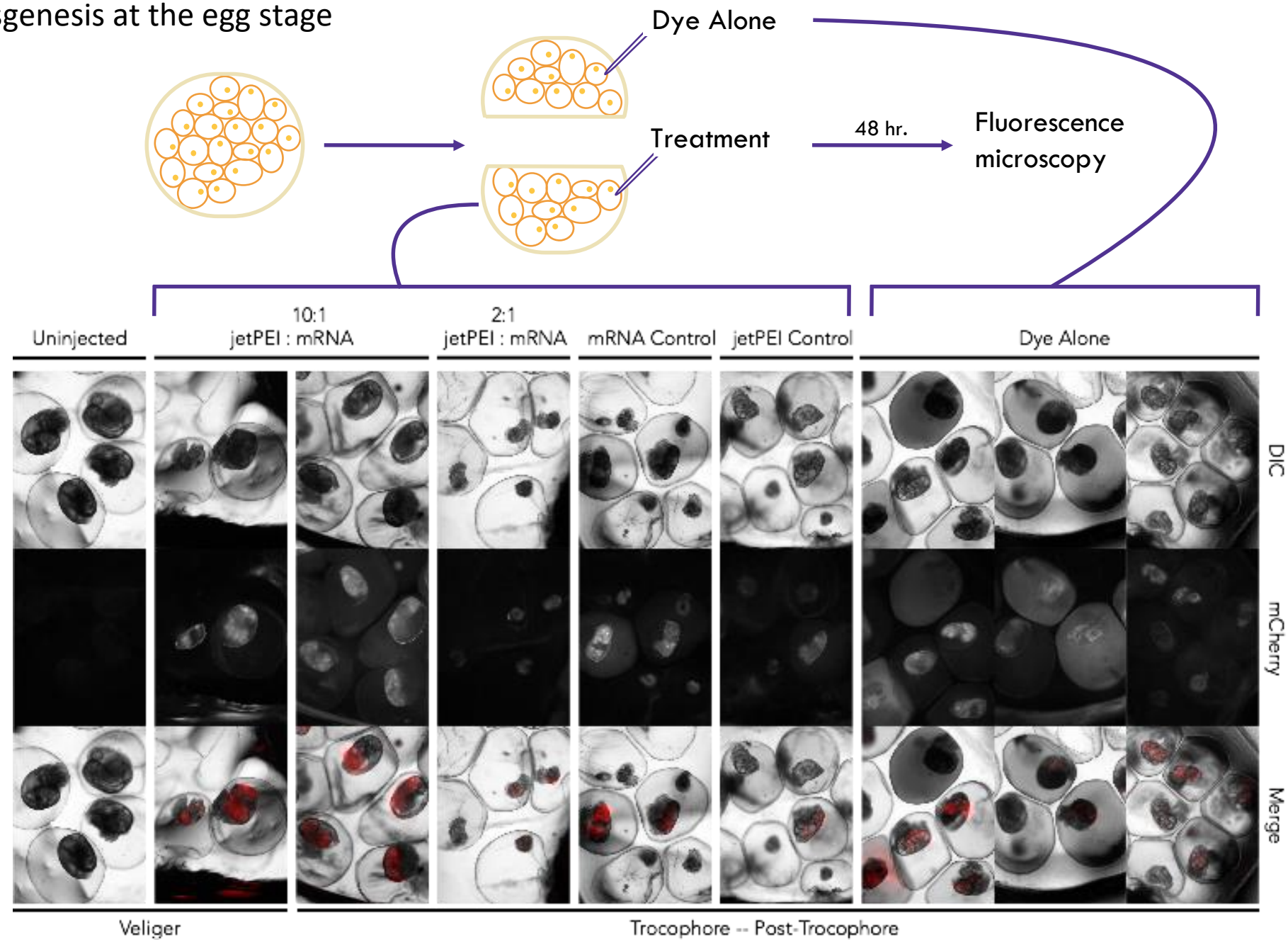
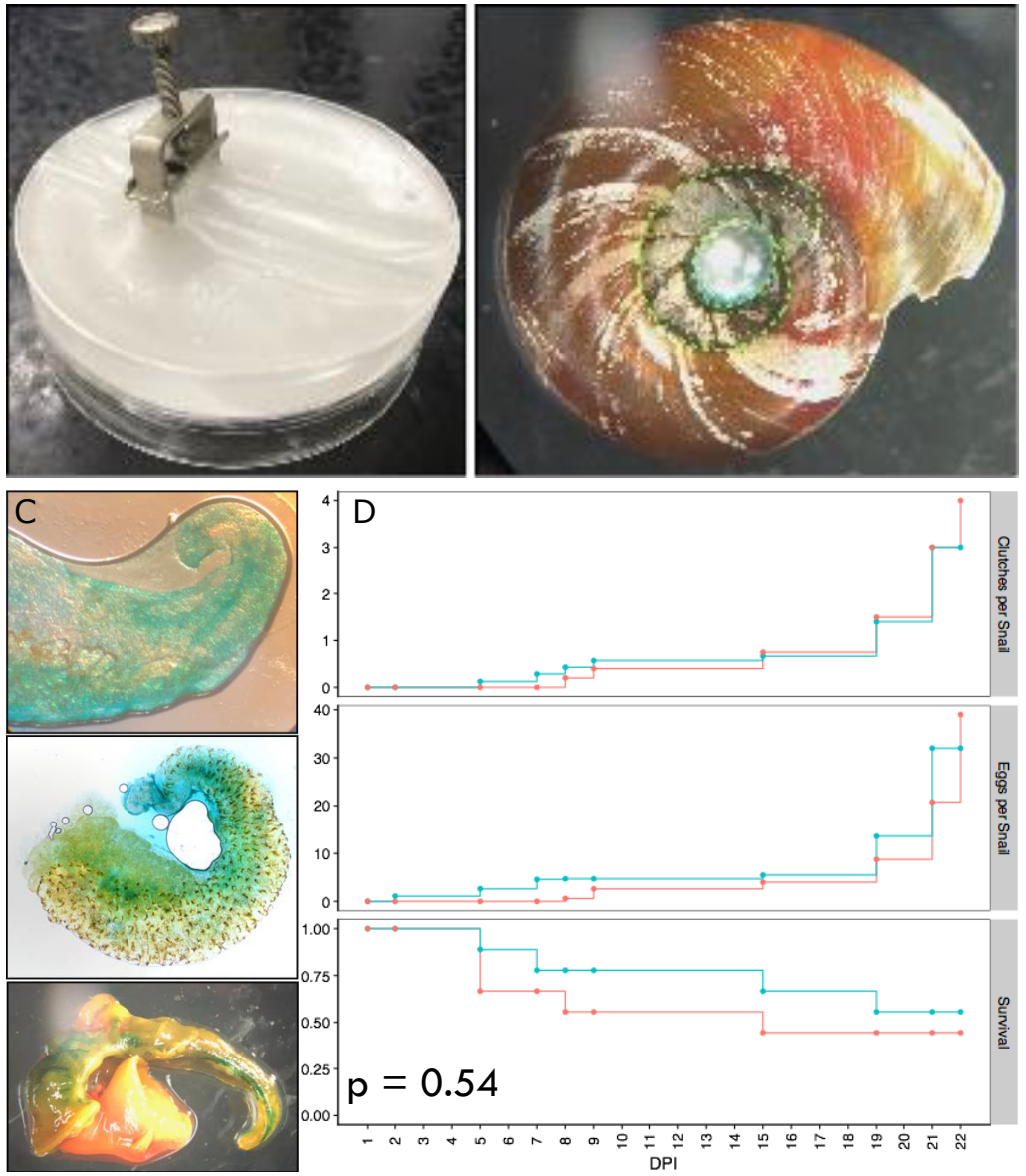


3. Develop transgenesis at the adult stage

- Establish delivery techniques
  - Injection of ovotestis
- Dye stains site of injection
- Rapid injection can cause dye to spread throughout the organism
- Injection does not reduce fecundity or increase mortality

E. H. Michelson, Trans. Am. Microsc. Soc., vol. 77, no. 3, pp. 316–319, 1958.



Future Directions

- Inject eggs and electroporate into embryos
- Inject snails ovotestis with higher ratios of transfection reagent, as optimized with Bge
- Inject snail ovotestis and eggs with lentiviral particles
- Develop sperm-mediated gene transfer using artificial insemination
- Transfect Bge with Cas9 mRNA and selected small guide RNAs to perform genome editing
  - Chemical selection with 6-thioguanine
  - KO lines
  - Parameters for homology-directed repair for B. glabrata

University of Wisconsin-Madison

Merck Global
Health Institute

Thank You

Zamanian Lab
Mostafa Zamanian

Yoshino Lab
Tim Yoshino
Nathalie Dinguirard

Erica Namigai
Theresa Meier
Josh Tycko
Gabrielle Disselhoff
Jutta Reinhard-Rupp