Applying cutting-edge technology for reproductive control in bivalves

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Steven Roberts, University of Washington SAFS
Adam Luckenbach, NMFS - Northwest Fisheries Science Center

Managing expectations: this talk has no data

Outline

- Reproductive control in aquaculture
  - Approaches to sterility
    - Ploidy manipulation
    - Germ cell elimination
- Identification genes involved in germ cell development in bivalves
  - Single-cell RNA Sequencing

Why is sterility desirable in aquaculture?

- Improved growth
- Year-round marketability
- Blackboxing of selectively bred lines
- Minimize genetic impact of selectively bred stocks on natural populations
- Escapement is less of a threat when culturing non-native species

Current approach to reproductive control: Ploidy manipulation

- Triploid bivalves have 3 chromosome sets (instead of 2)
- Effectively sterile because they can’t complete meiosis
- Challenges:
  - Takes a long time to develop selected lines (10 years!)
  - Triploids can exhibit compromised performance in the field

Alternative approach: Elimination of germ cells

What are germ cells?

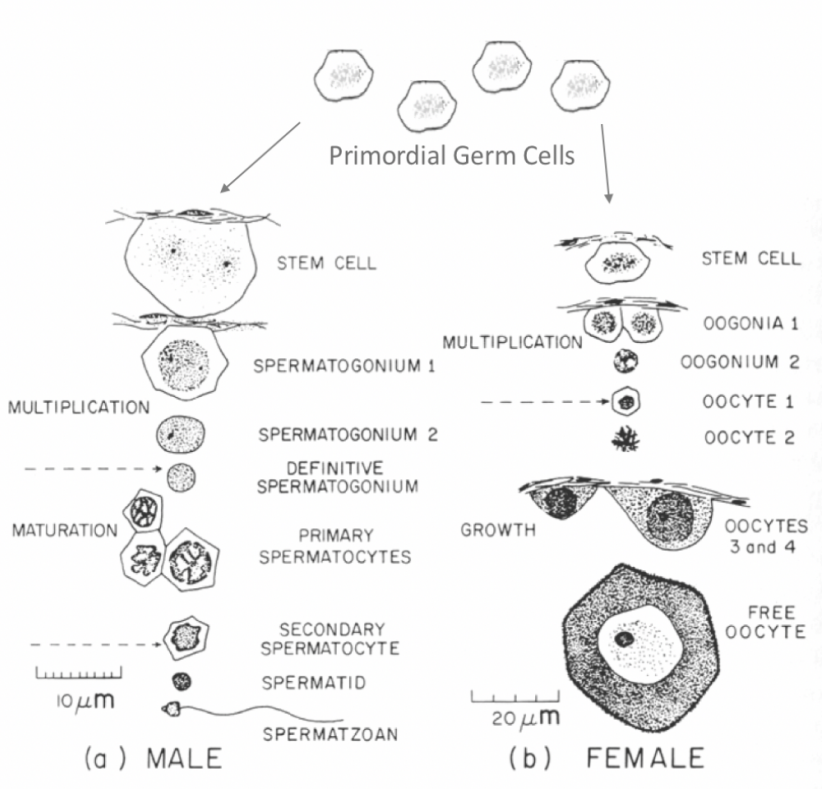
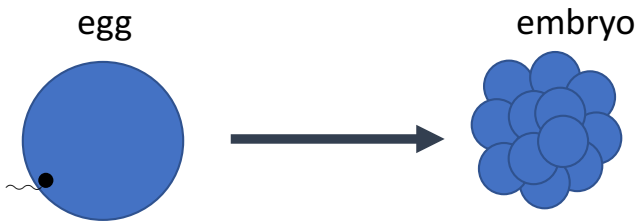


Image modified from (Sastry, 1979)

How does a cell become a germ cell?



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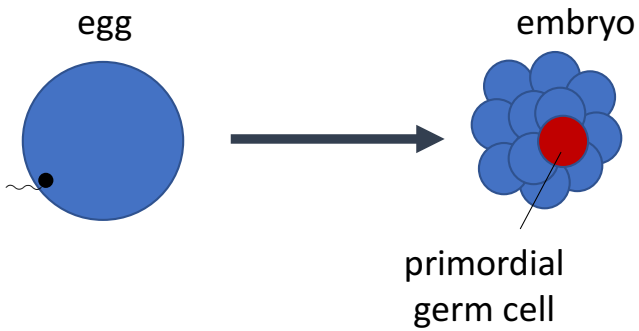

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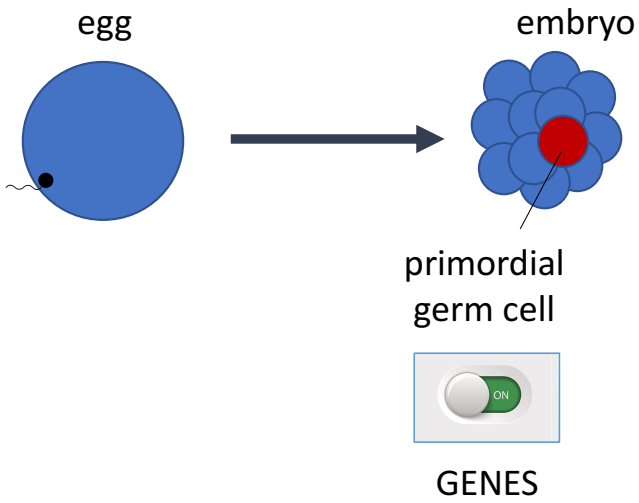

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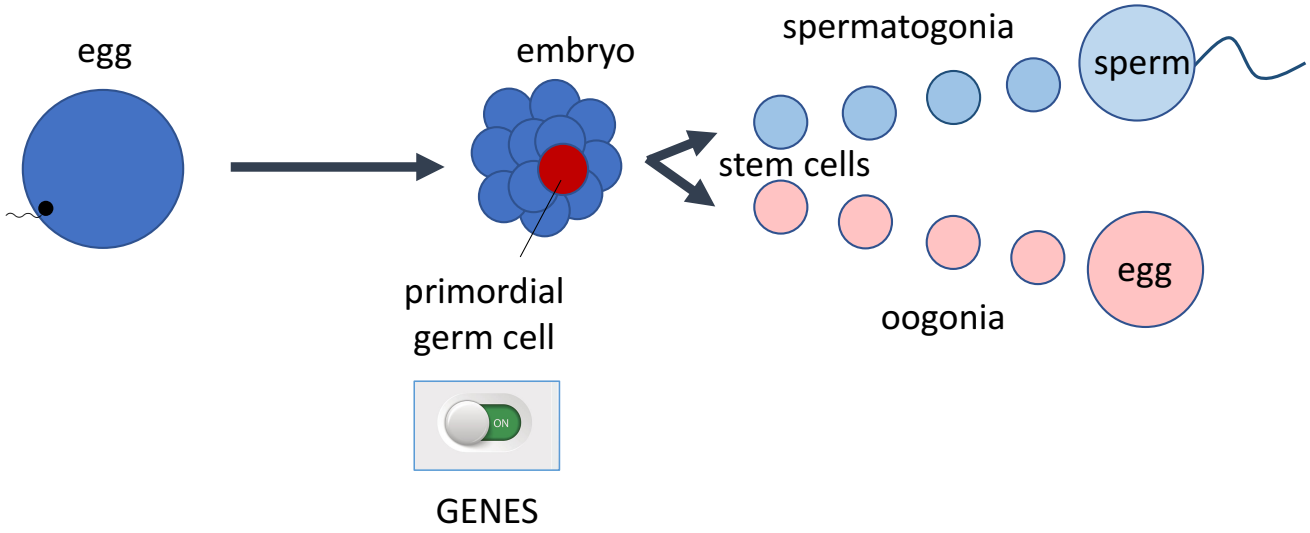

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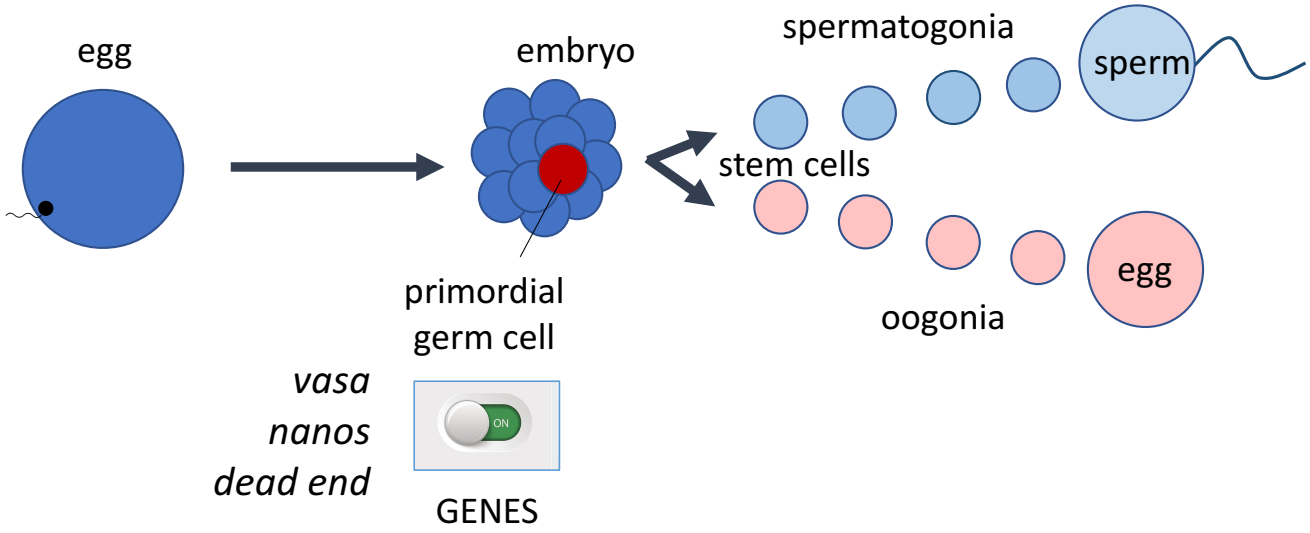

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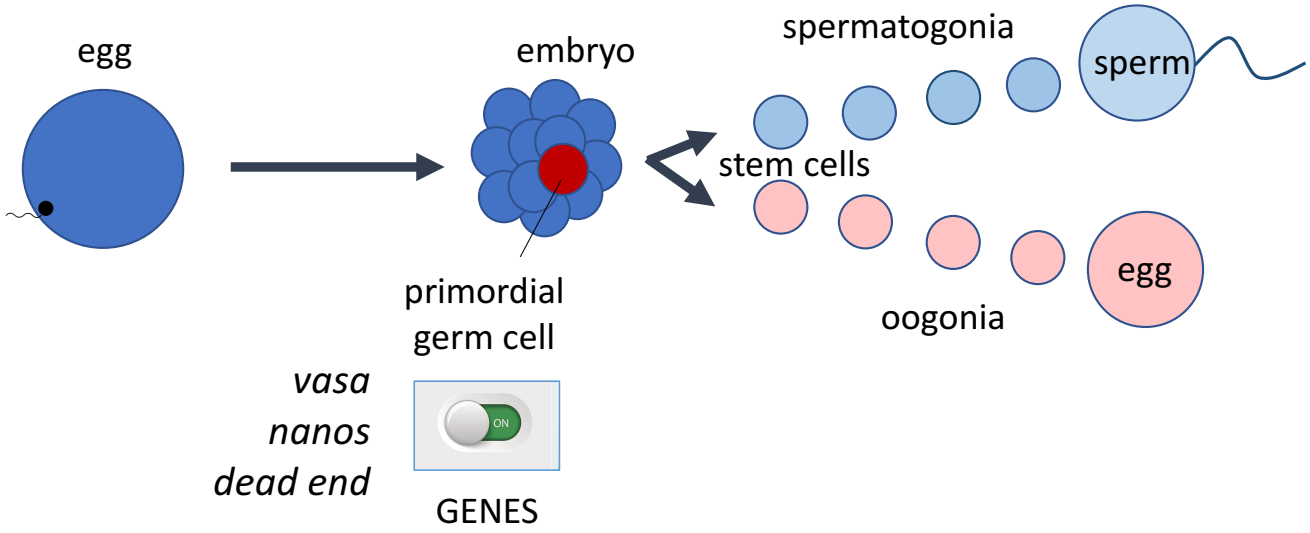

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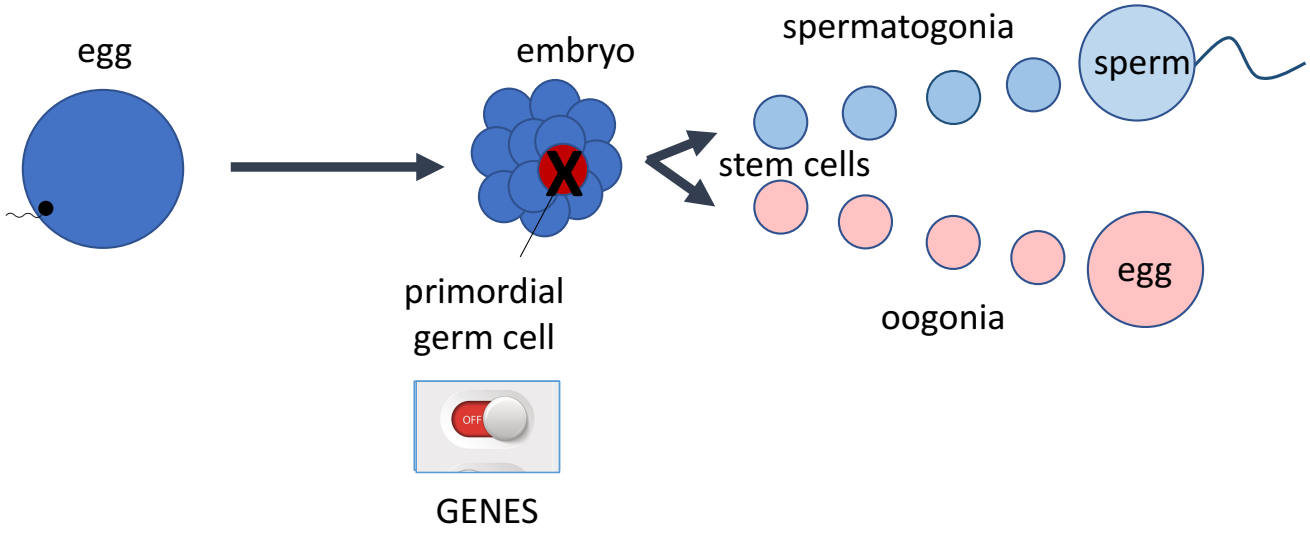

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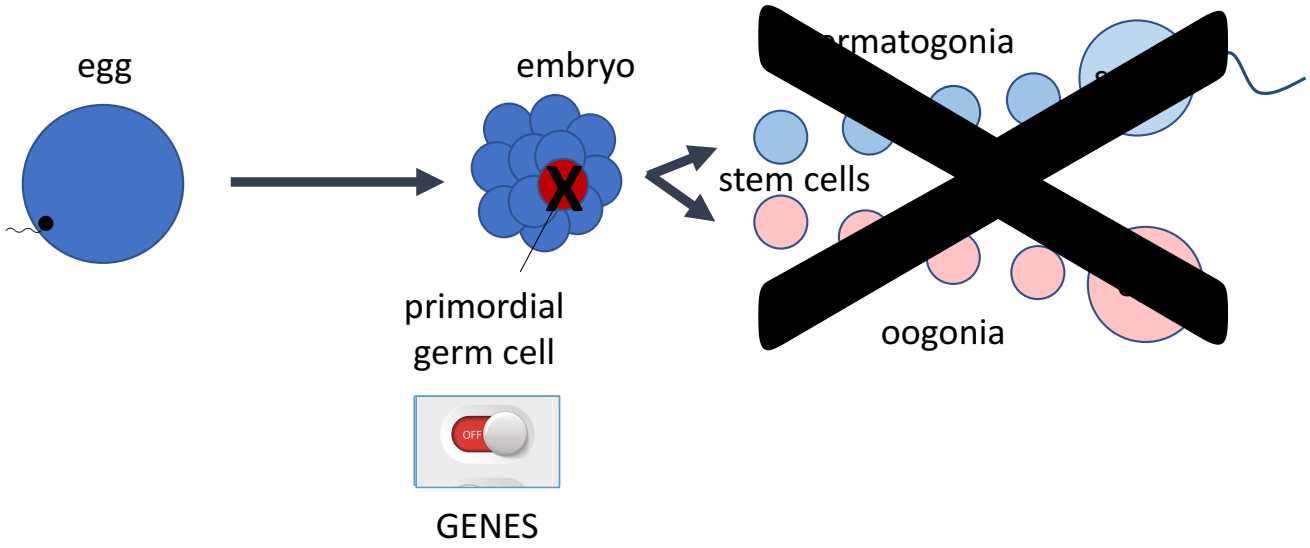

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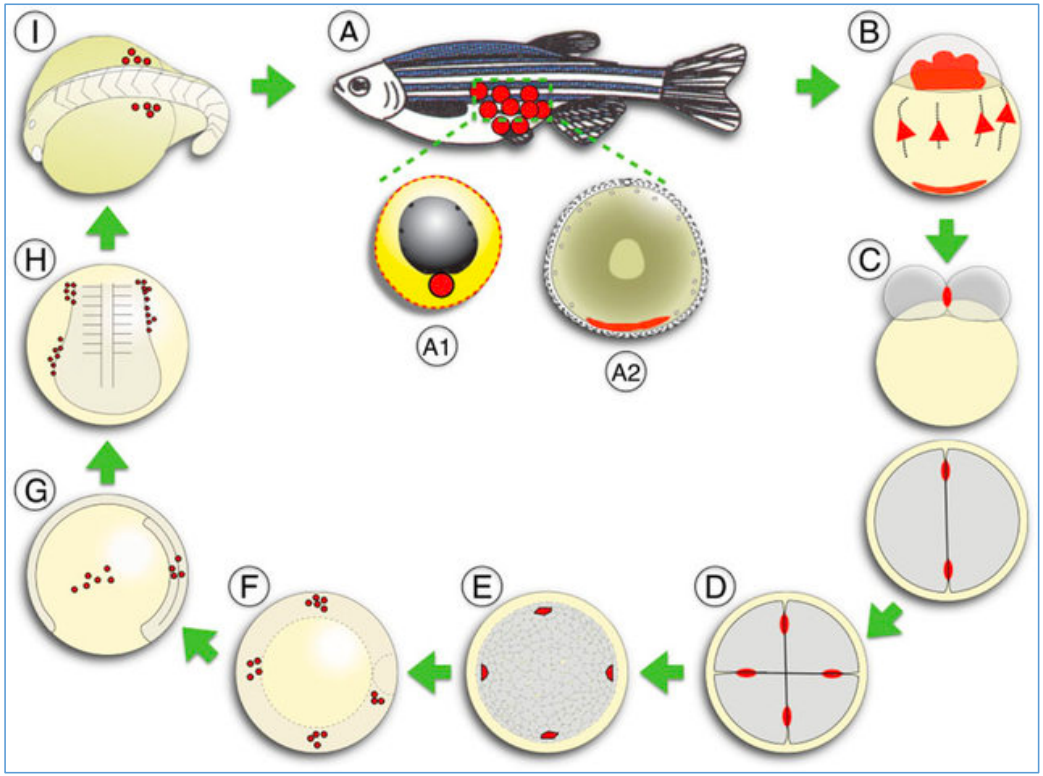
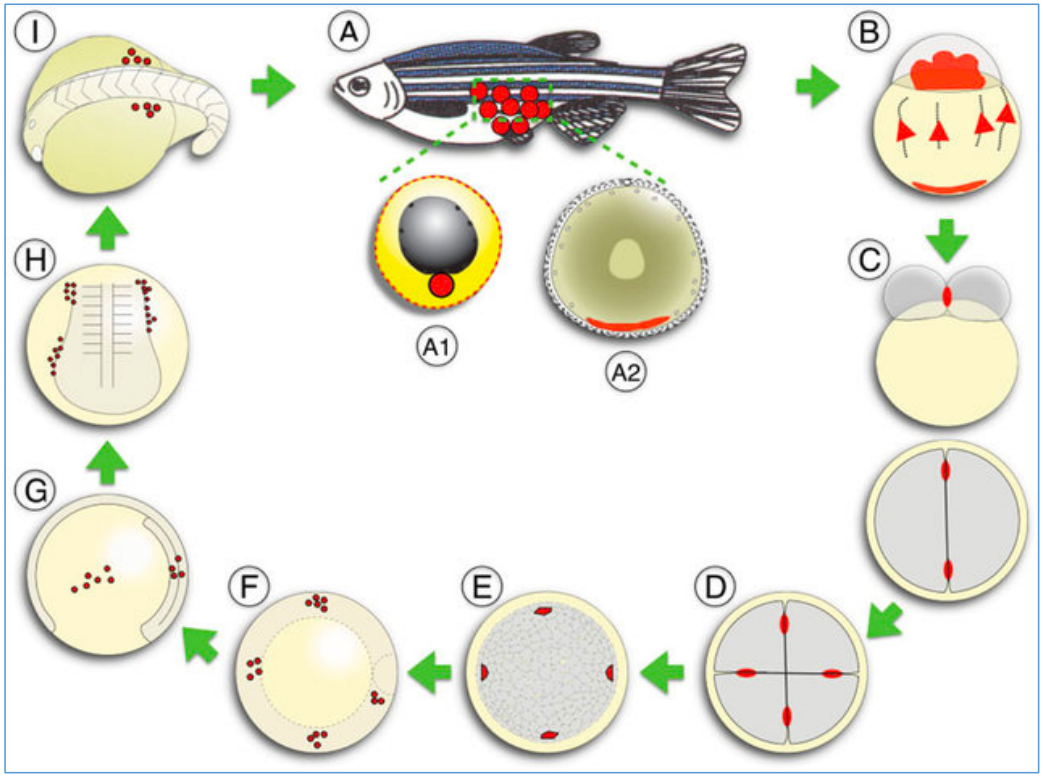


Doesch 2014; *Critical reviews in biochemistry and molecular biology*

Example: germ cell elimination in finfish

Dead end (dnd): important for proper migration of PGCs



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morpholino: short synthetic RNA; turns off a specific gene (temporarily)

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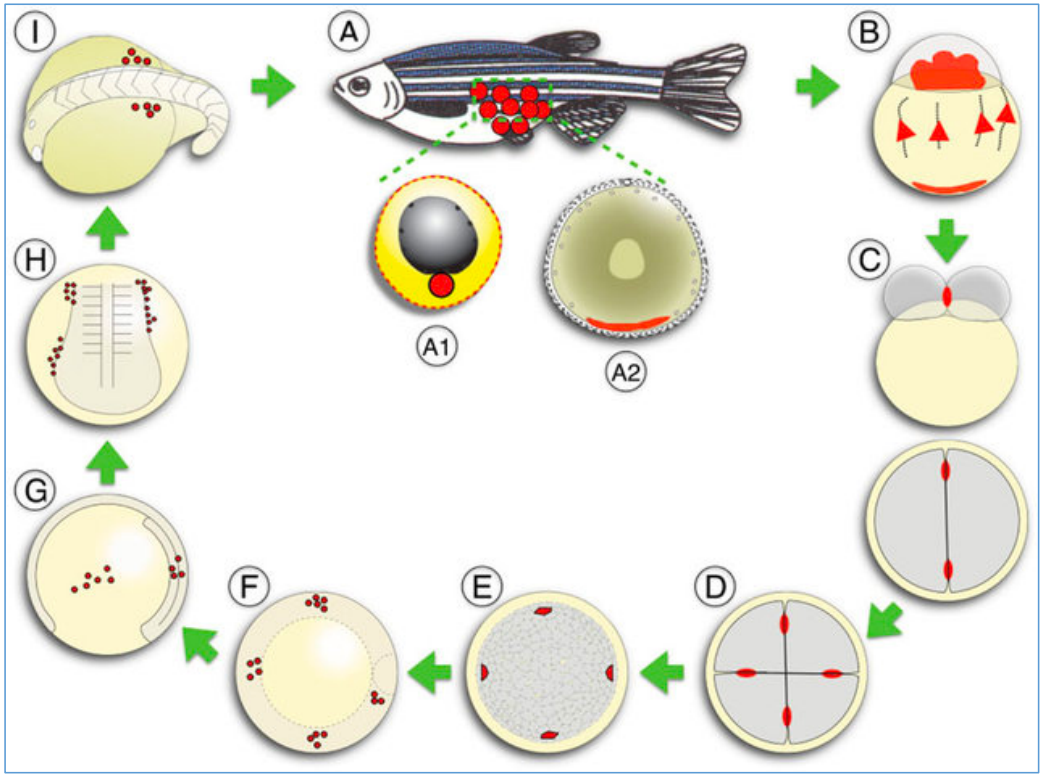

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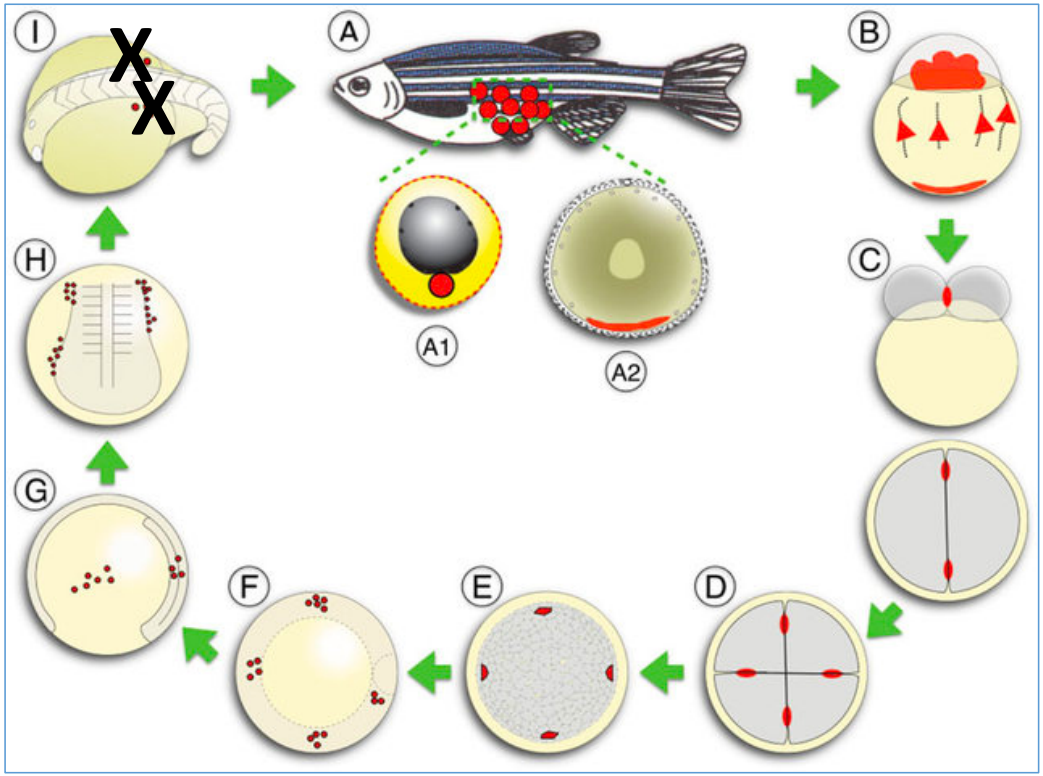

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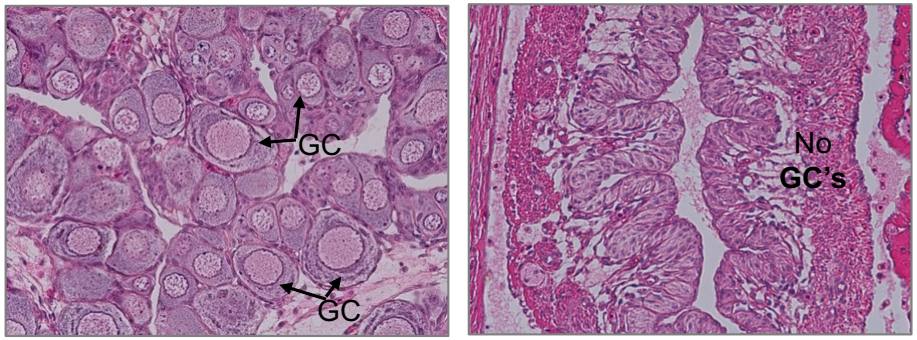
Example: germ cell elimination in finfish

Sablefish (*dead end* knockdown)
morpholino-Vivo using bath immersion

Ovary of control female	**Ovary of *dnd*-silenced female**



Gonad sections photographed at 40X magnification

GC = germ cell; dnd = dead end gene

Adam Luckenbach (NWFSC)
Ten-Tsao Wong, Yoni Zohar (University of Maryland)

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Example: germ cell elimination in ~~finfish~~ bivalves

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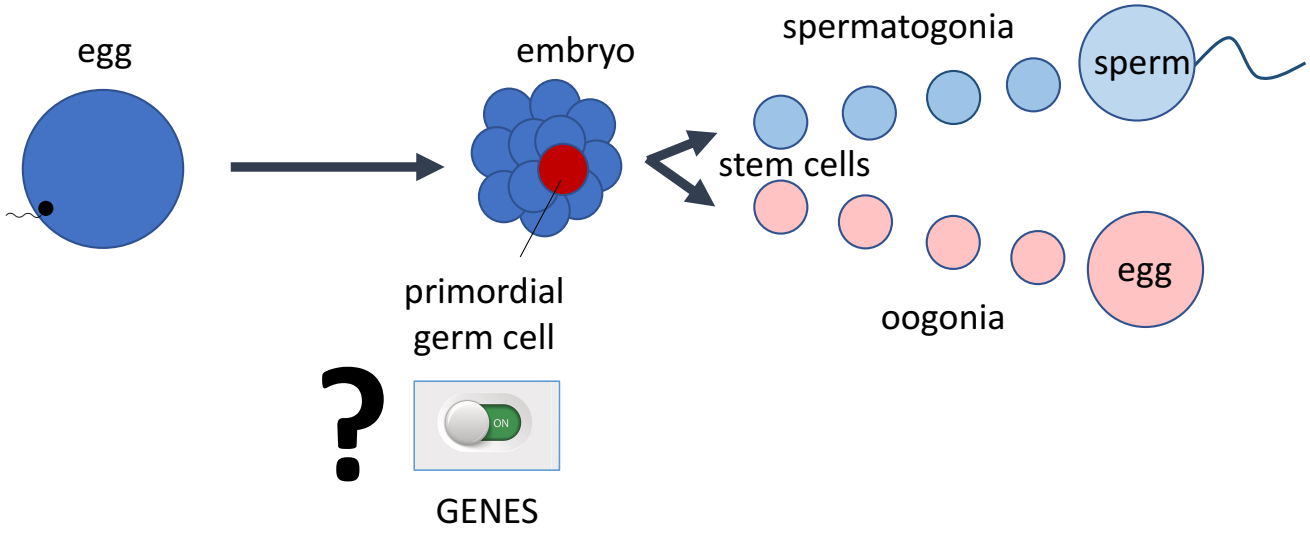

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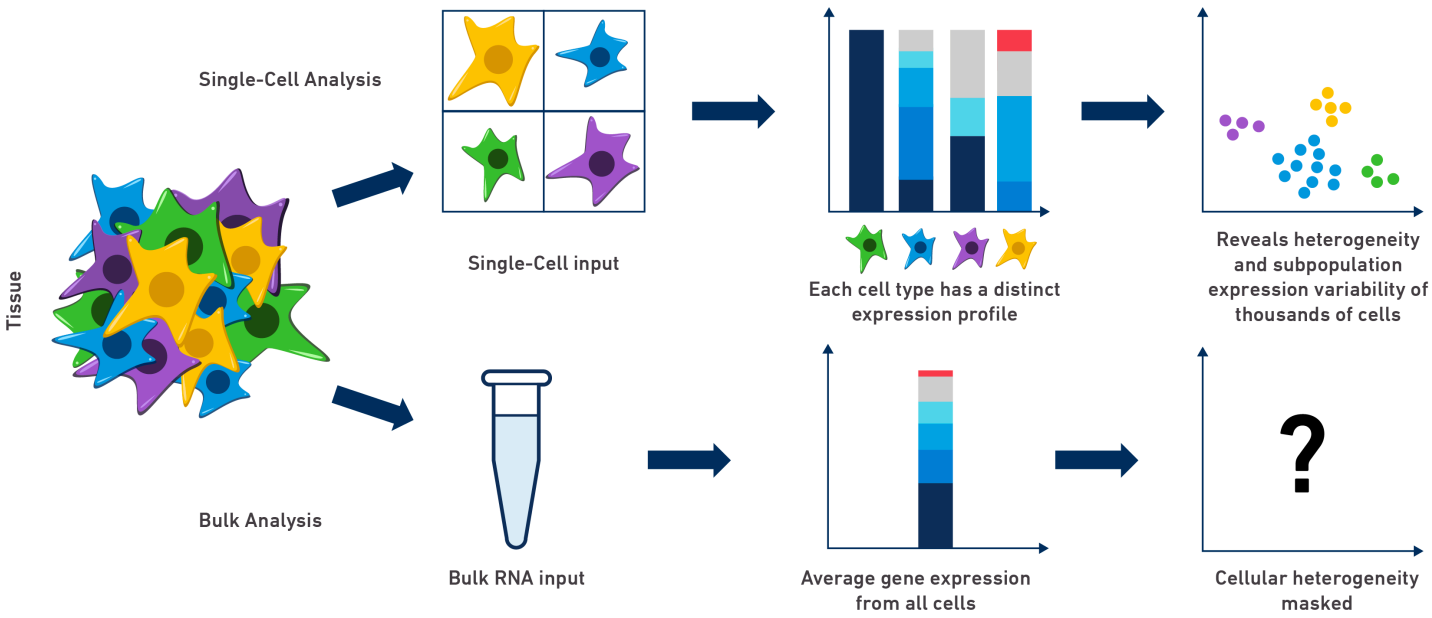
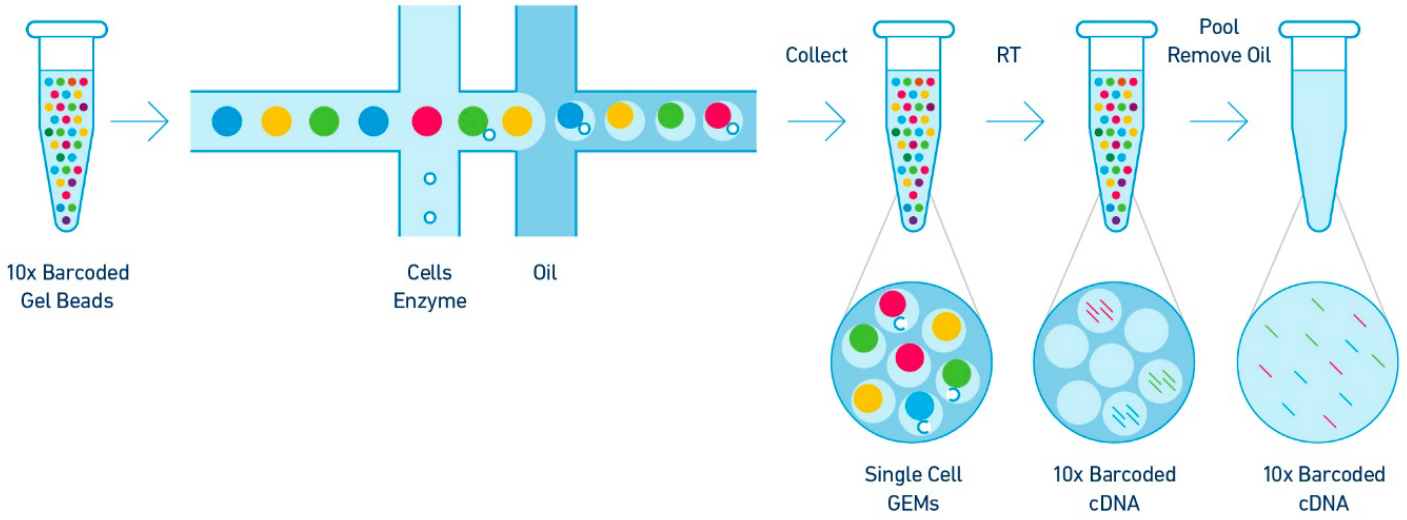


Image: 10x Genomics website

Gene expression: single cell RNA-Seq

1. Dissociate tissue to single cell suspension
2. Prepare cDNA libraries of single cells
3. Sequencing on high-throughput sequencer (Illumina)



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Example: *C. elegans* development scRNA-Seq

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- Sequenced cells from all developmental stages of *C. elegans* to understand key mechanisms of cell fate decisions
- 86,046 cells were sequenced

Science

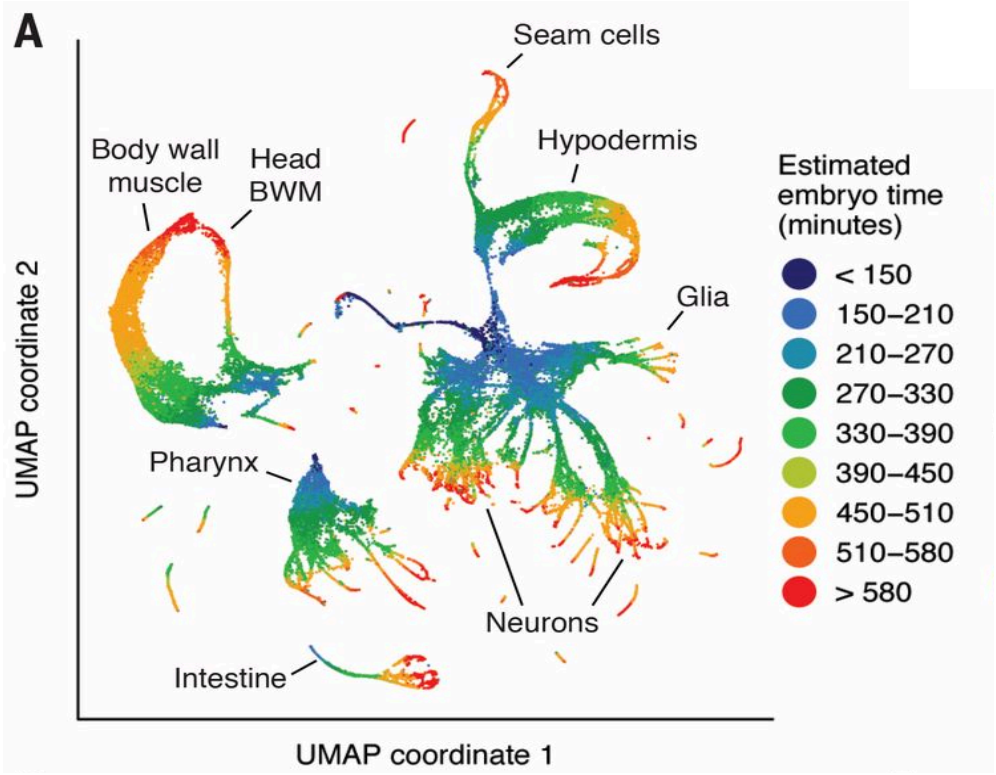
RESEARCH ARTICLES

Cite as: J. S. Packer *et al.*, *Science* 10.1126/science.aax1971 (2019).

A lineage-resolved molecular atlas of *C. elegans* embryogenesis at single-cell resolution

Jonathan S. Packer[1]*, Qin Zhu[2]*, Chau Huynh[1], Priya Sivaramakrishnan[3], Elicia Preston[3], Hannah Dueck[3]†, Derek Stefanik[4], Kai Tan[3,5,6,7], Cole Trapnell[1], Junhyong Kim[4]‡, Robert H. Waterston[1]‡, John I. Murray[3]‡

Example: *C. elegans* development scRNA-Seq



Packer et al. 2019, *Science*

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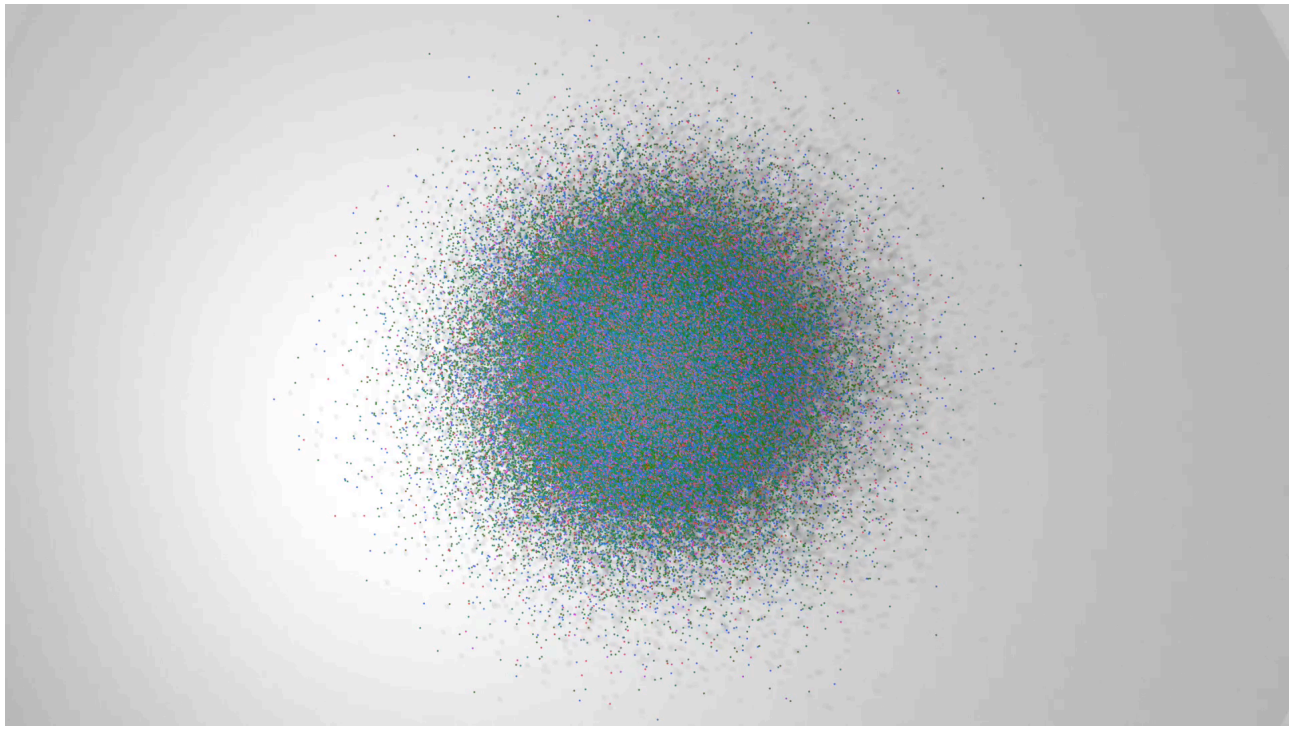

Movie: Cole Trapnell

Approach: single cell RNA-Seq in bivalve embryos

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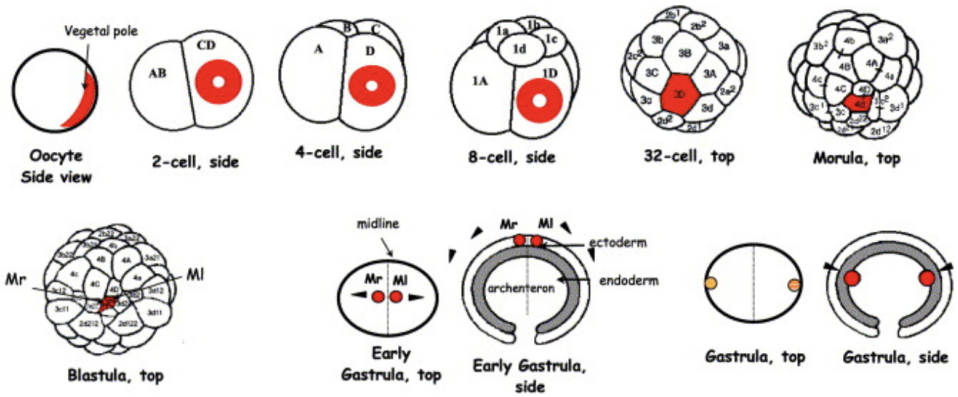

red = *vasa* expressed

Fabioux et al. 2004, *Biochem Biophys Res Commun.*

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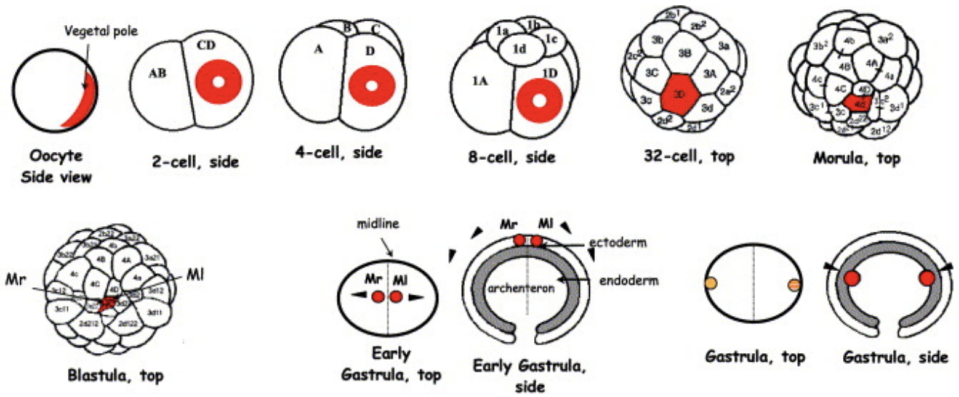

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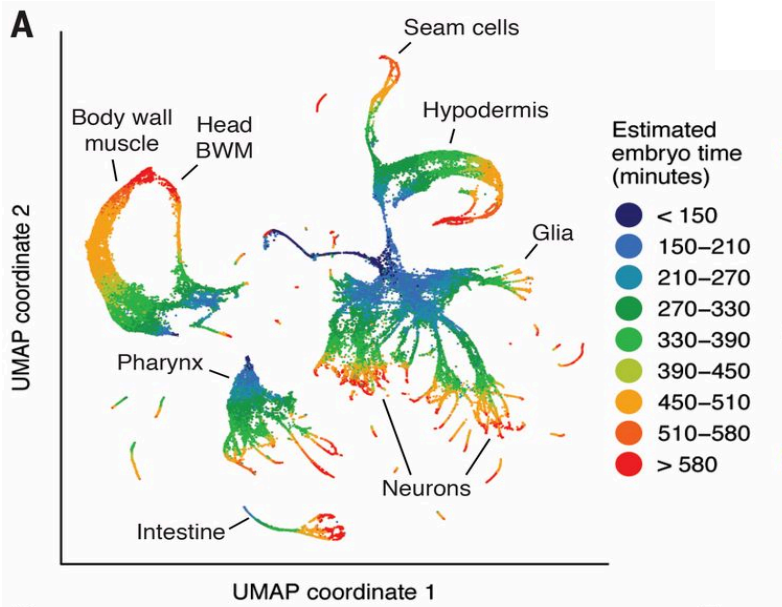
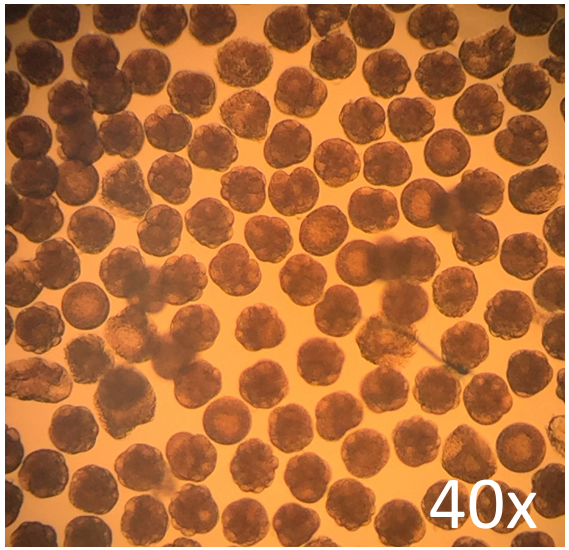
Example trajectory analysis:



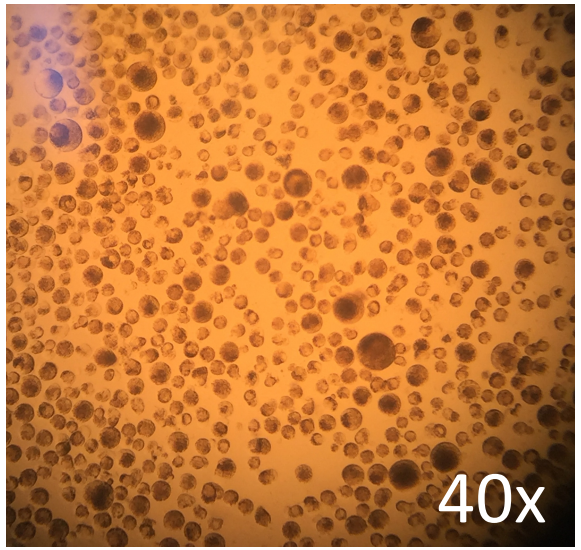
Approach: single cell RNA-Seq in bivalve embryos

- Testing cell dissociation protocols
- Optimizing method for collection of multiple developmental stages
- Generate scRNA-Seq libraries and perform differential expression and trajectory analysis

Early cleavage embryos

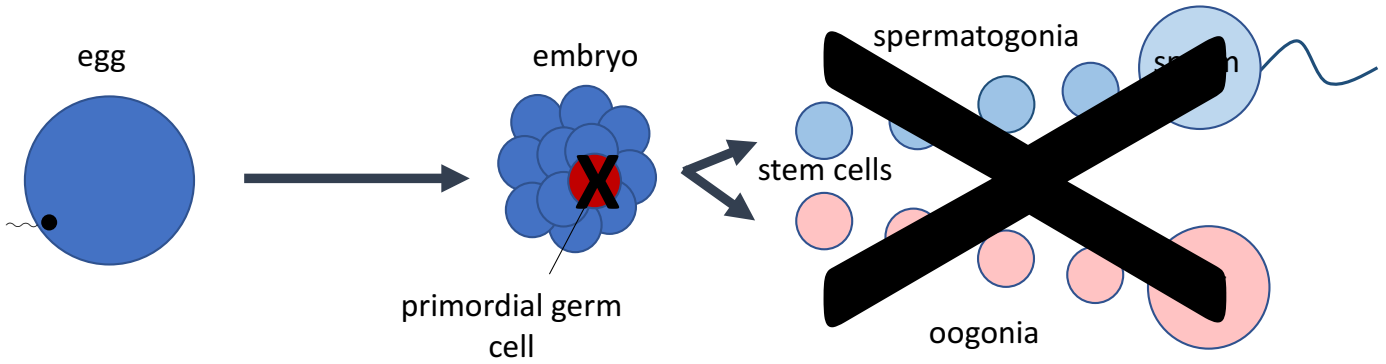


Post-dissociation



Expected outcomes

- Identify candidate genes involved in primordial germ cell formation in bivalves
- Future work will evaluate how silencing these genes impacts germ cell development
- Ultimately, developing a germ cell elimination protocol for bivalves will confer all the benefits of sterility while avoiding the challenges of ploidy manipulation



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Molly Jackson

Beniot Eudeline

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