

Harnessing the power of single-cell RNA sequencing to control reproductive development in bivalves

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Lauren Saunders, UW, Genome Sciences

Cole Trapnell, UW, Genome Sciences

Managing expectations: this talk has no data

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I have exciting updates to share!

Outline

- Reproductive control in aquaculture
 - Ploidy manipulation
 - Germ cell elimination approach
- Identify genes involved in germ cell development in bivalves
 - Single-cell RNA Sequencing

Why is sterility desirable in aquaculture?

- Improved growth
- Year-round marketability
- Minimize genetic impact of selectively bred stocks on natural populations

Current approach to reproductive control: Ploidy manipulation

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

Alternative approach: Germ cell elimination

What are germ cells?

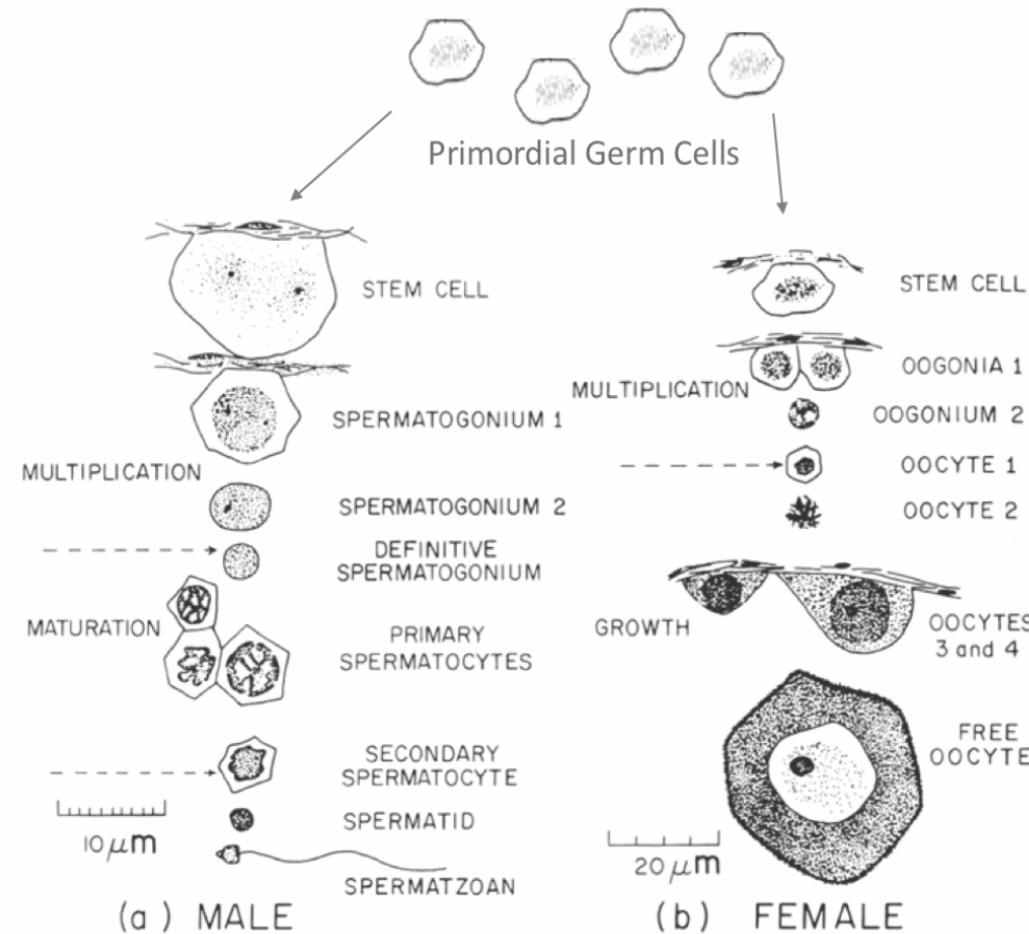
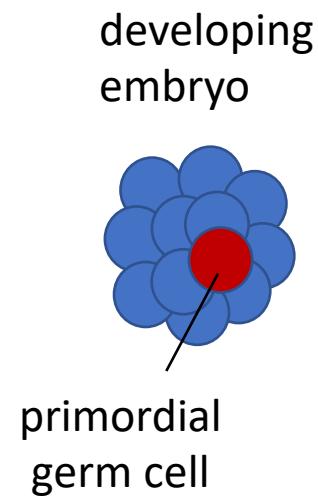
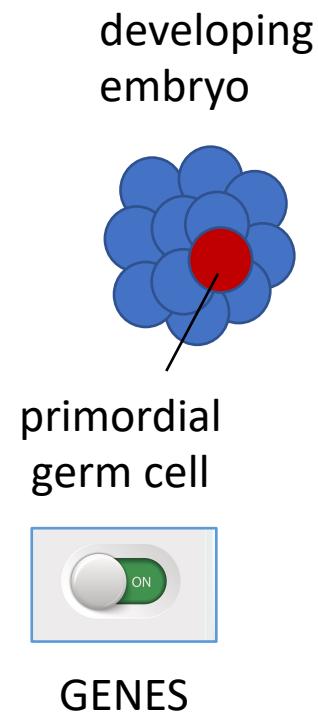


Image modified from (Sastry, 1979)

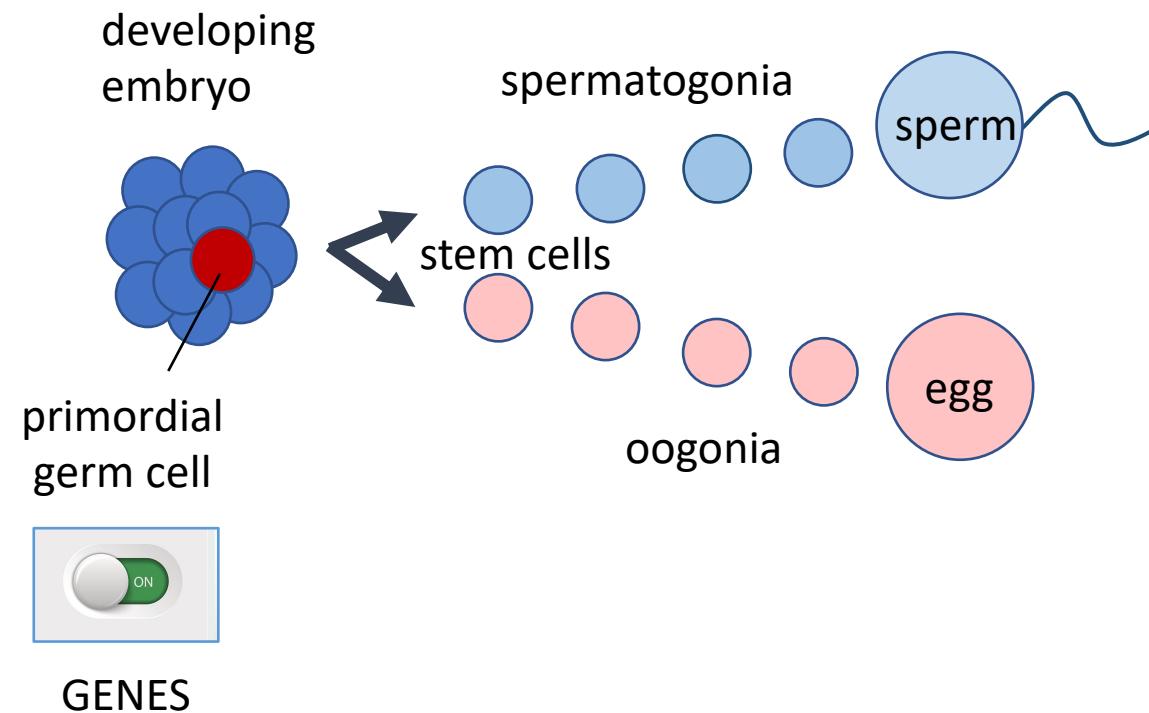
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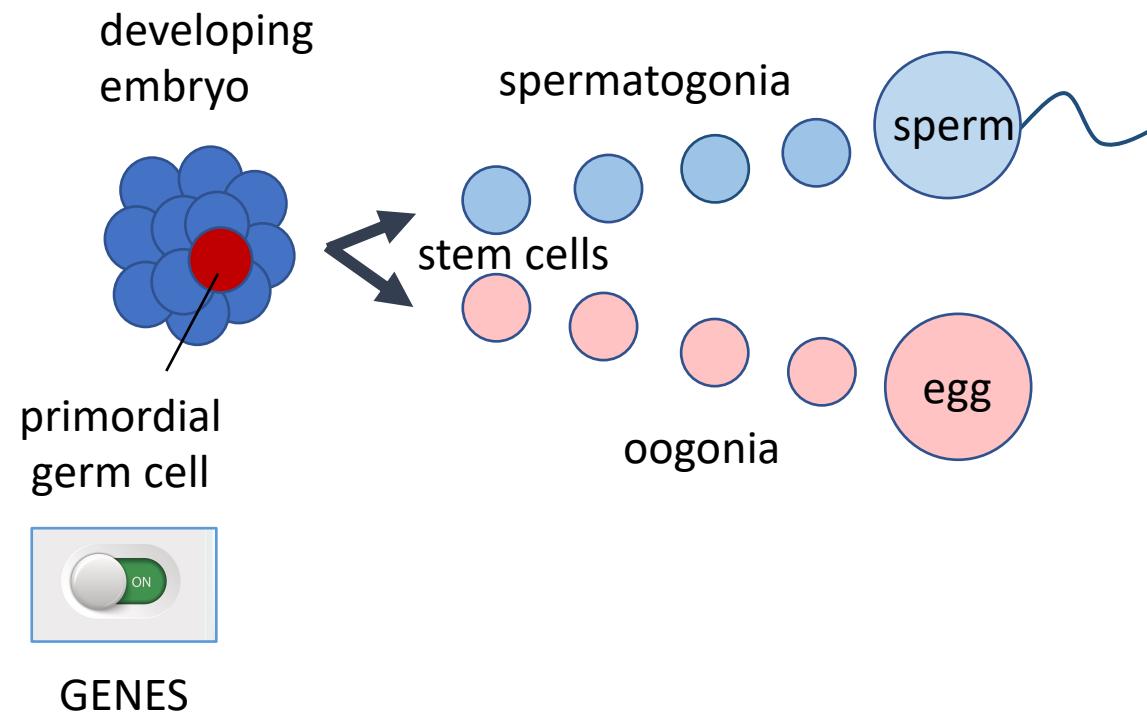


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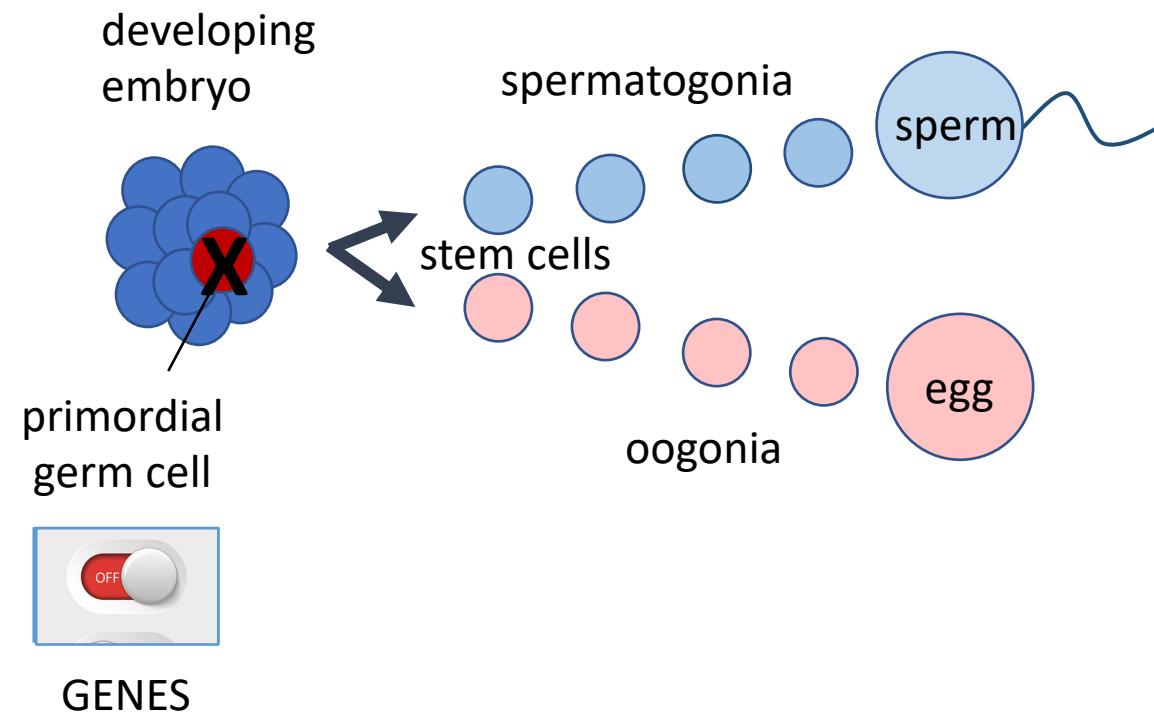
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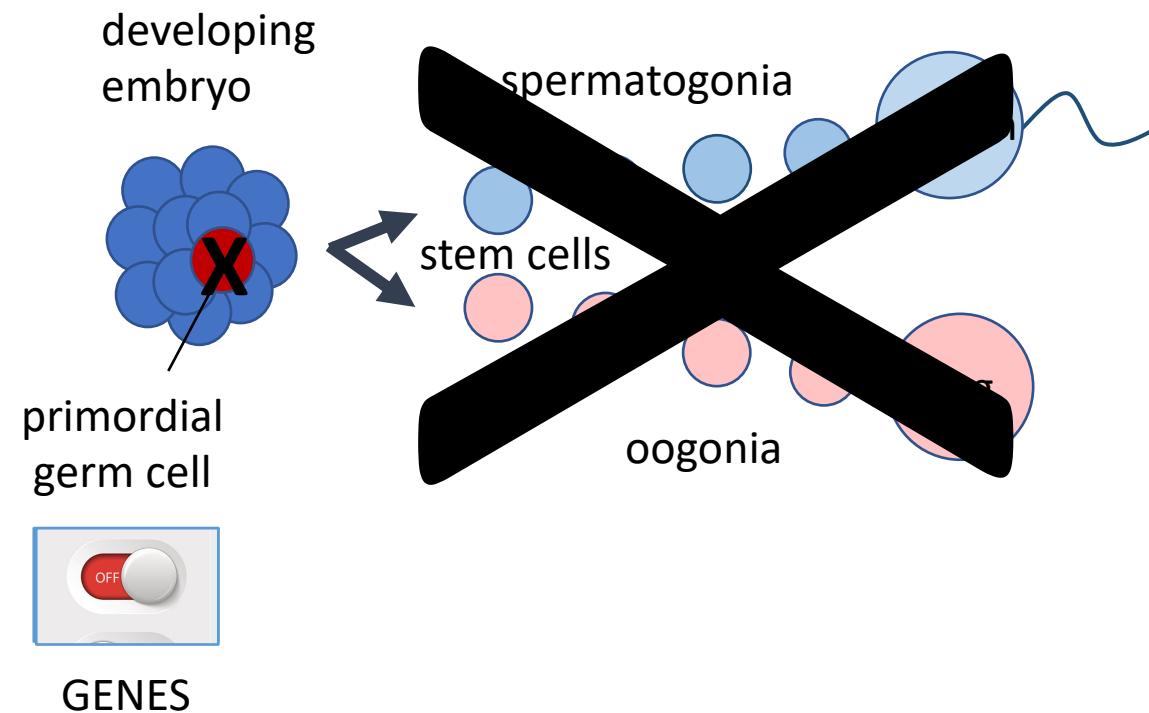
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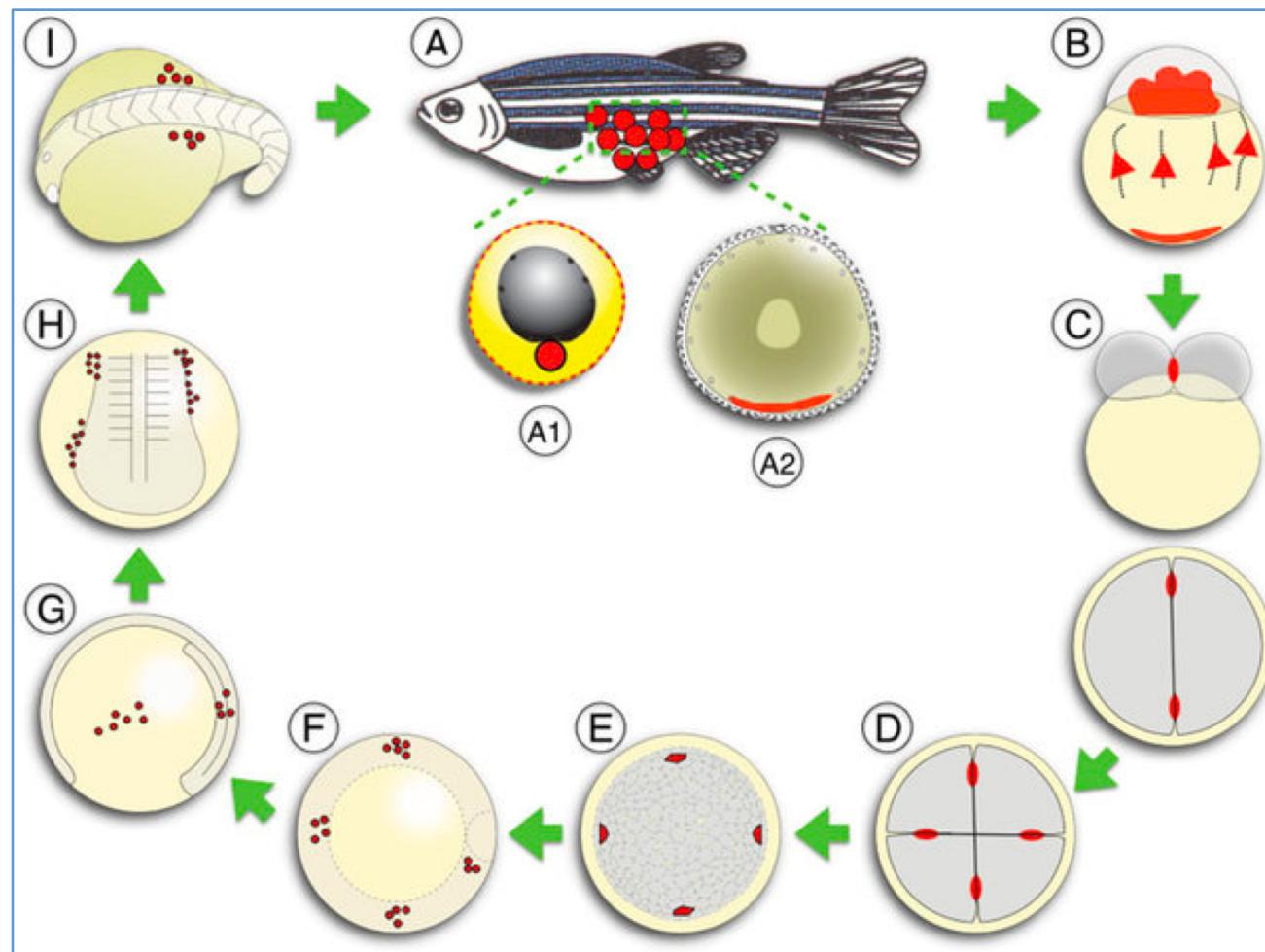
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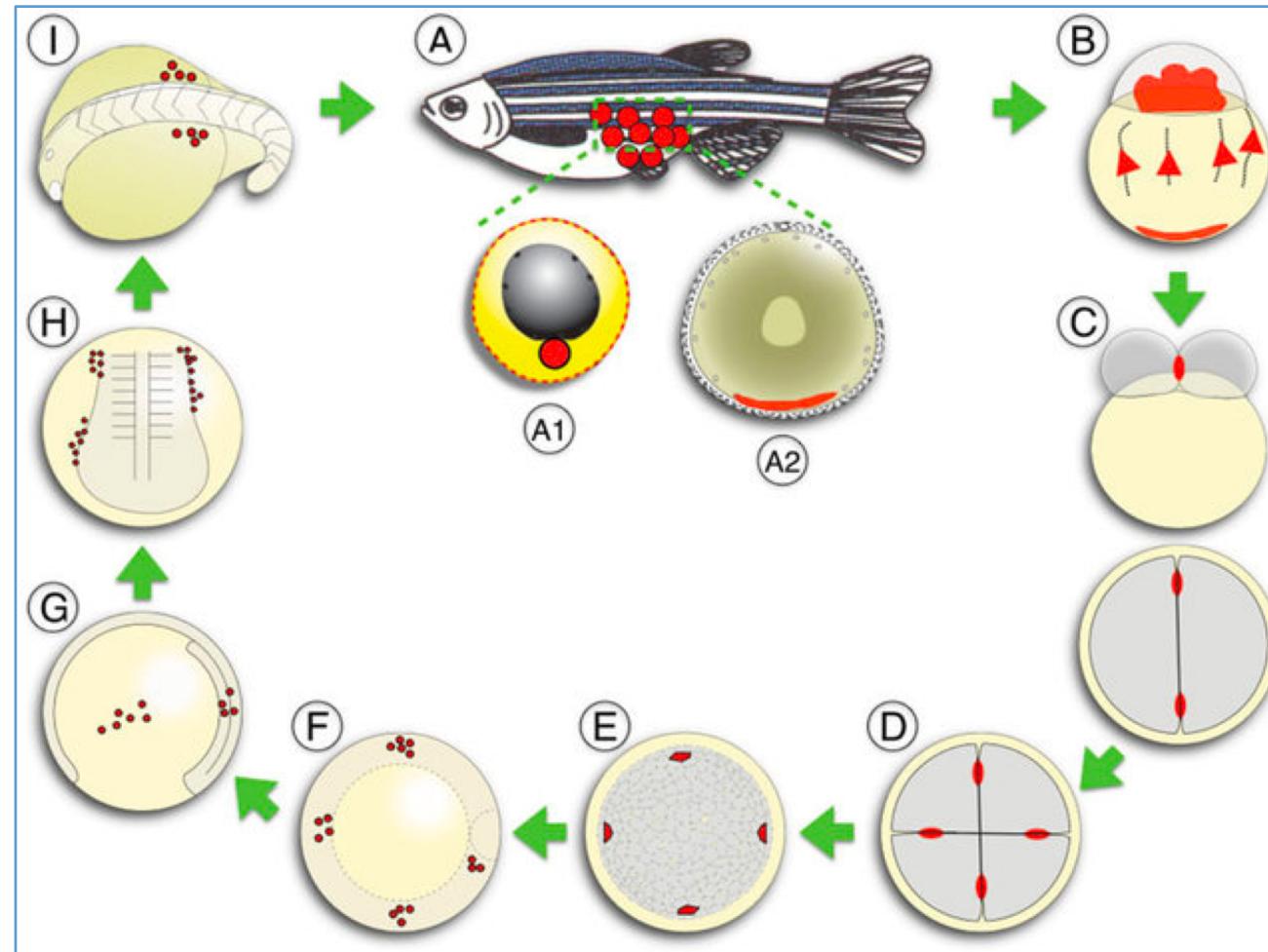
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Doesch 2014; *Critical reviews in biochemistry and molecular biology*

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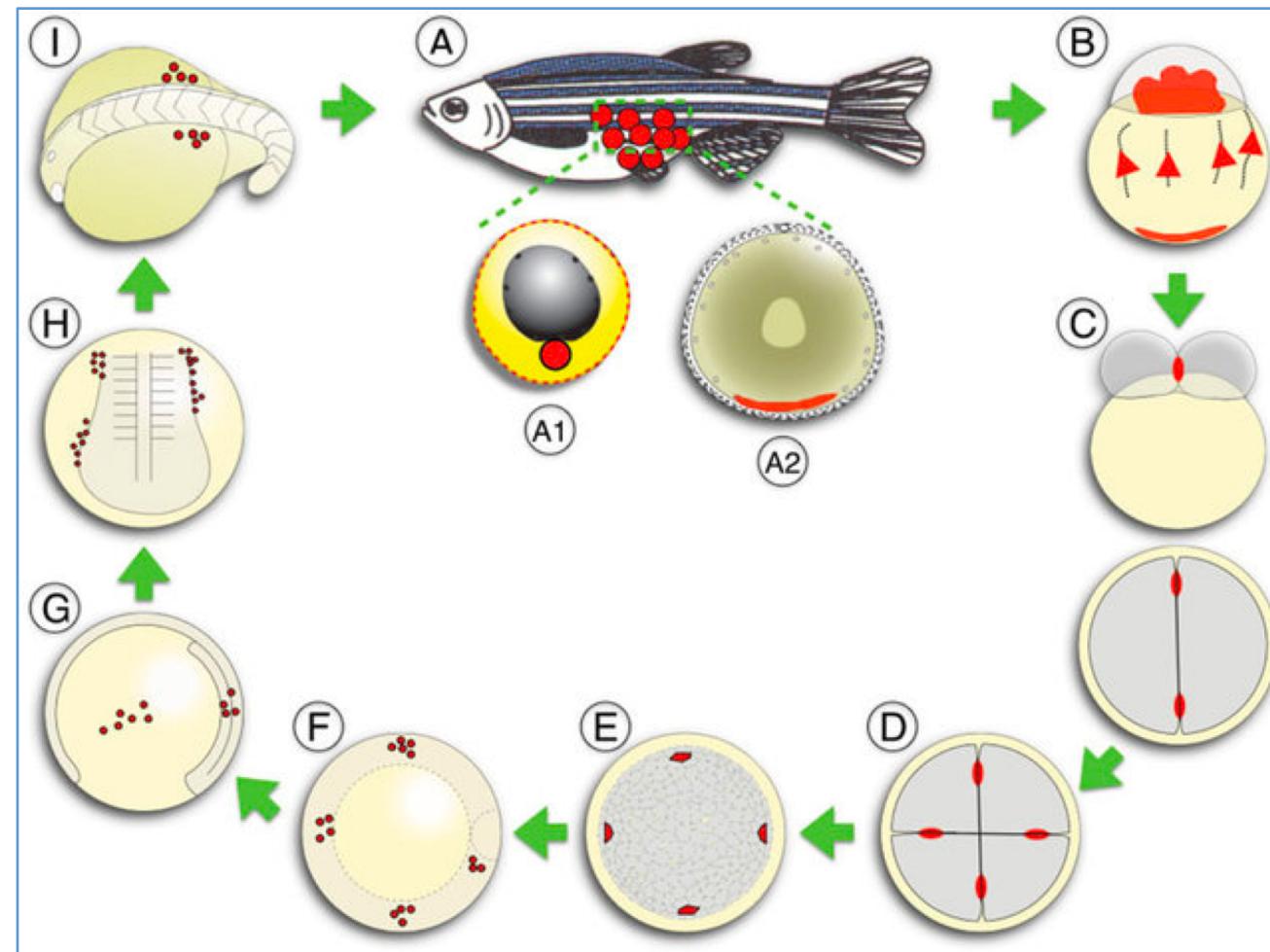


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Example: germ cell elimination in finfish

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

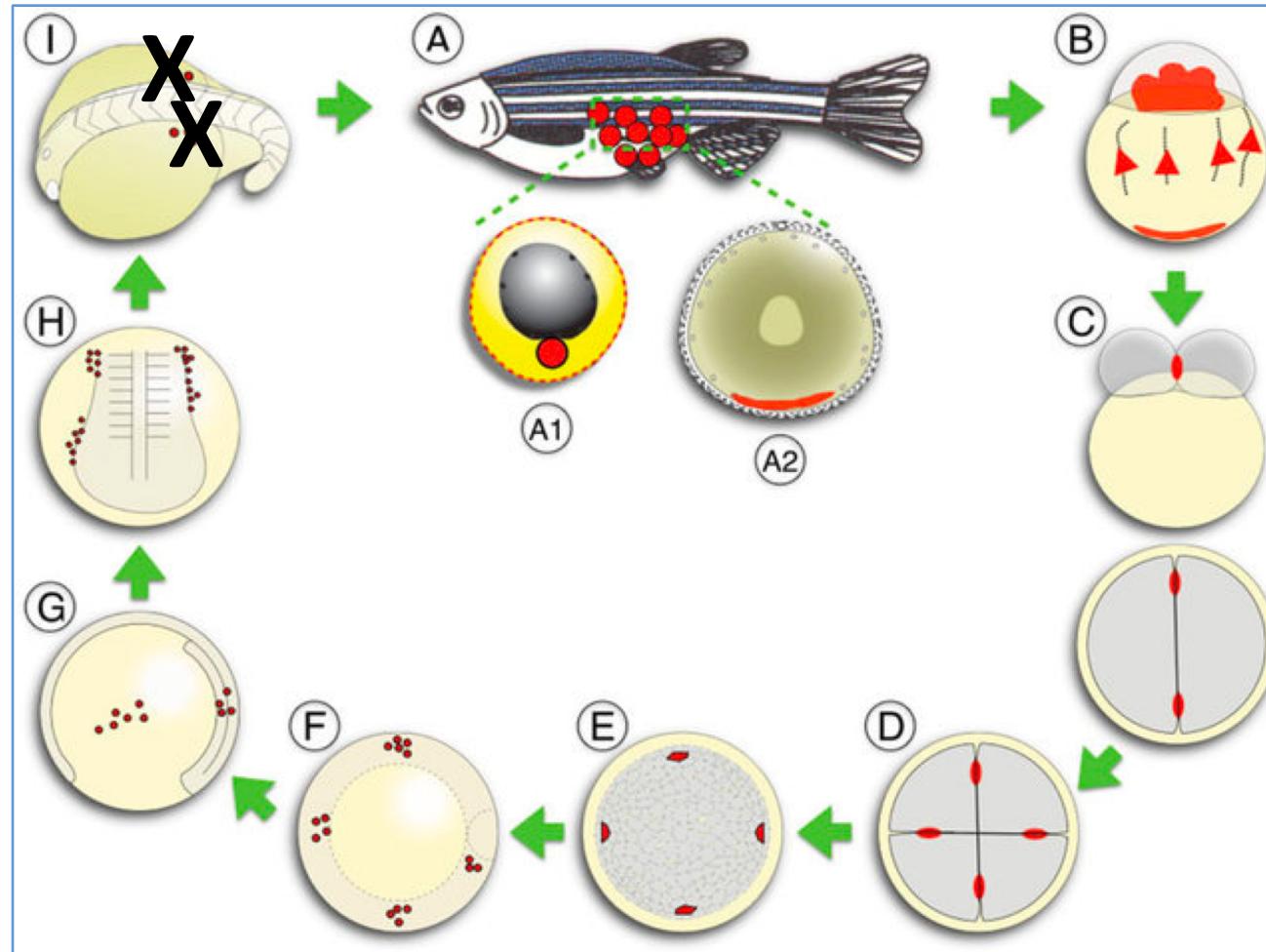
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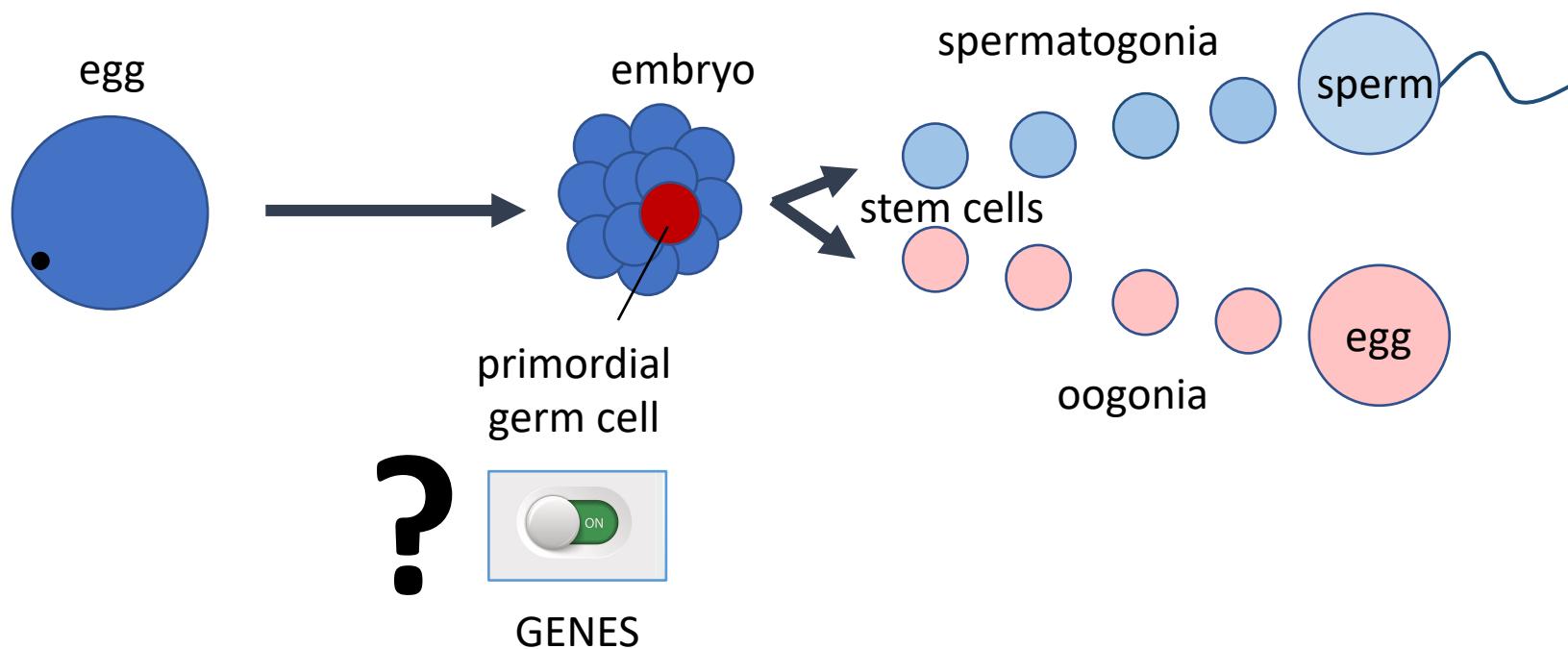
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Example: germ cell elimination in ~~finfish~~ bivalves

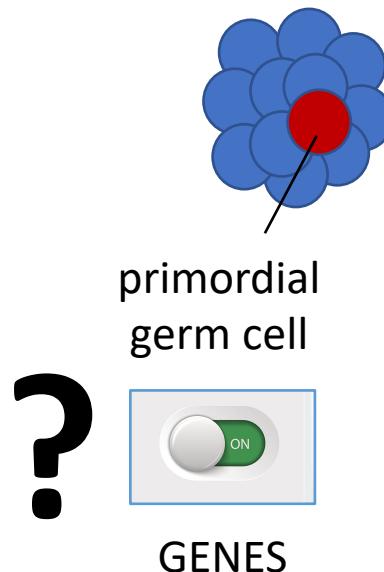
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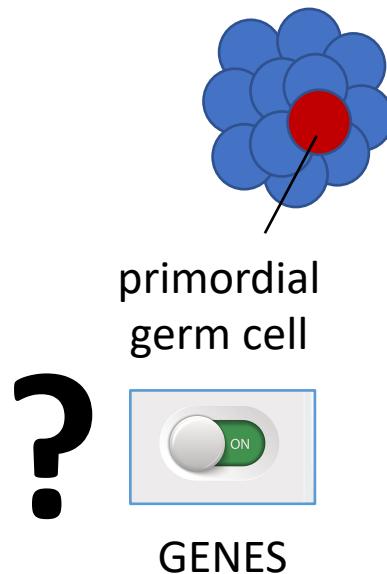
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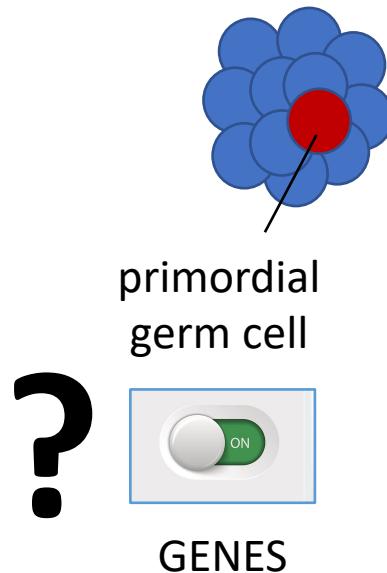


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Bulk RNA-Seq

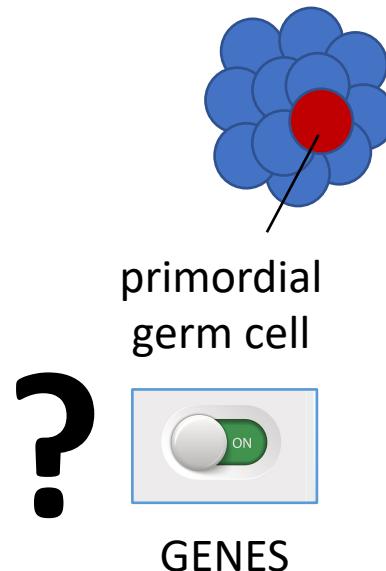


Single-cell RNA-Seq



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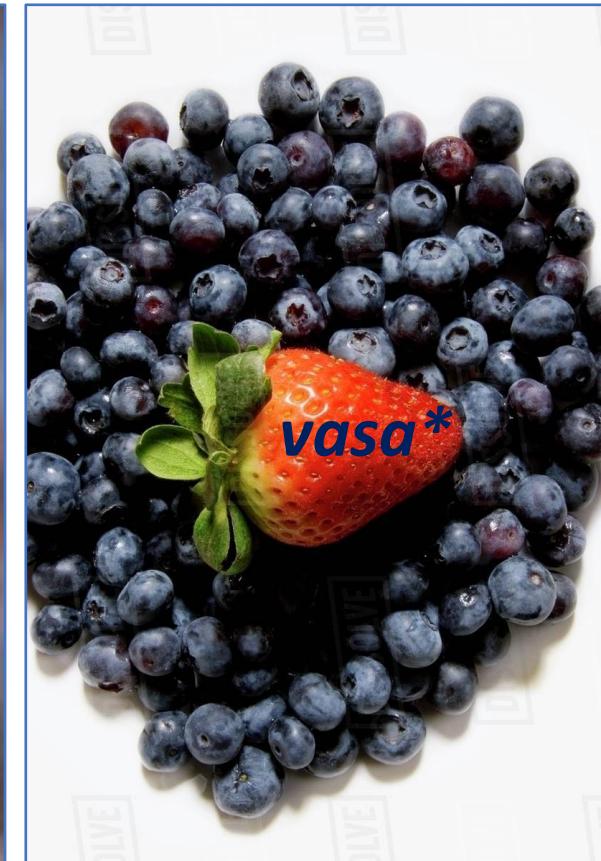
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Bulk RNA-Seq



Single-cell RNA-Seq



*germline marker gene

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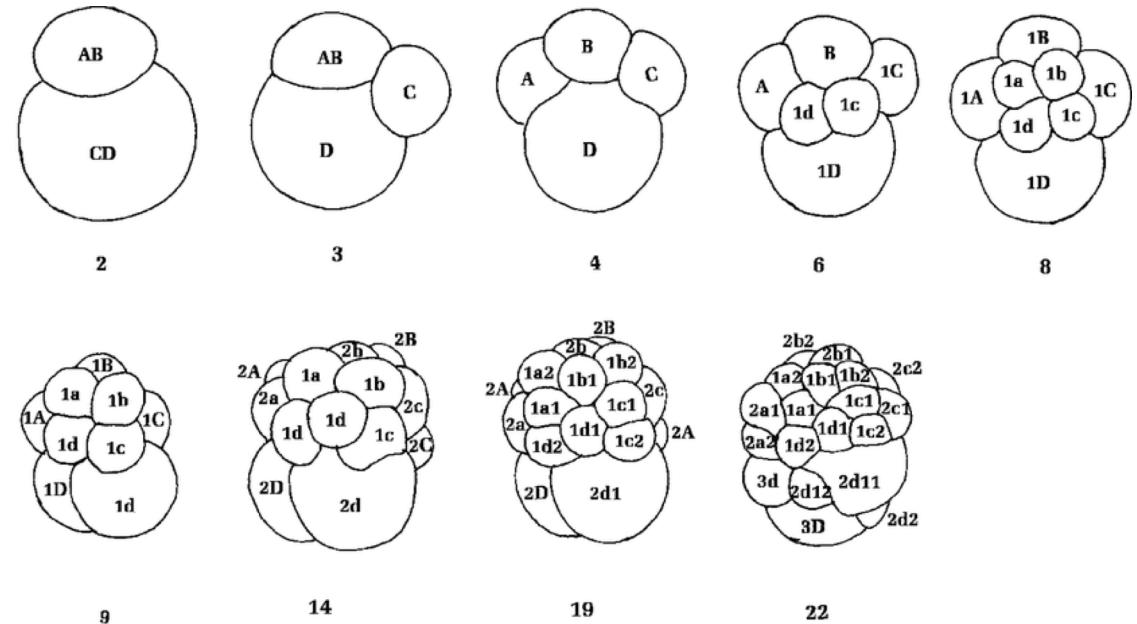
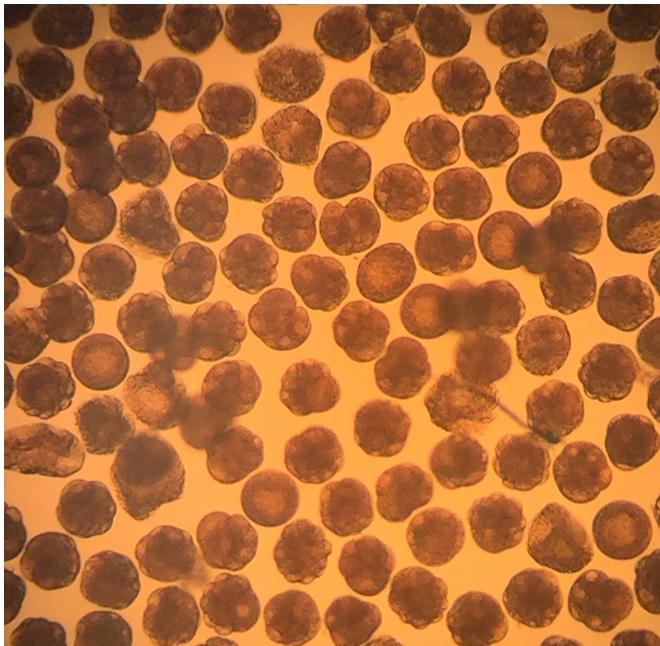


Image :G.L. Mackie, 2007; Woods, 1931; and Okada 1935

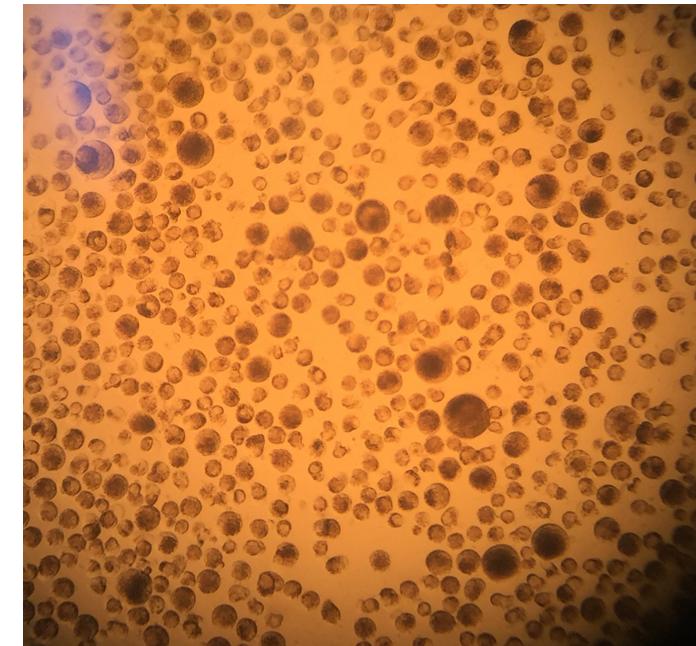
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Early cleavage embryos



Post-dissociation



Approach: single cell RNA-Seq in bivalve embryos

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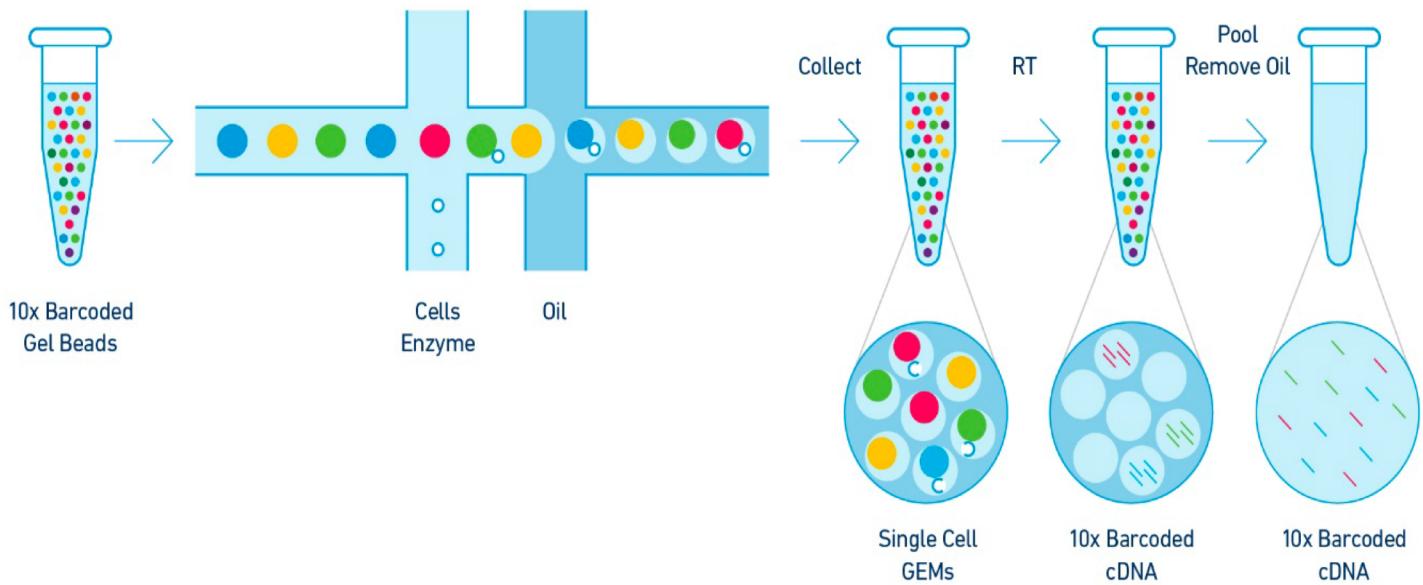
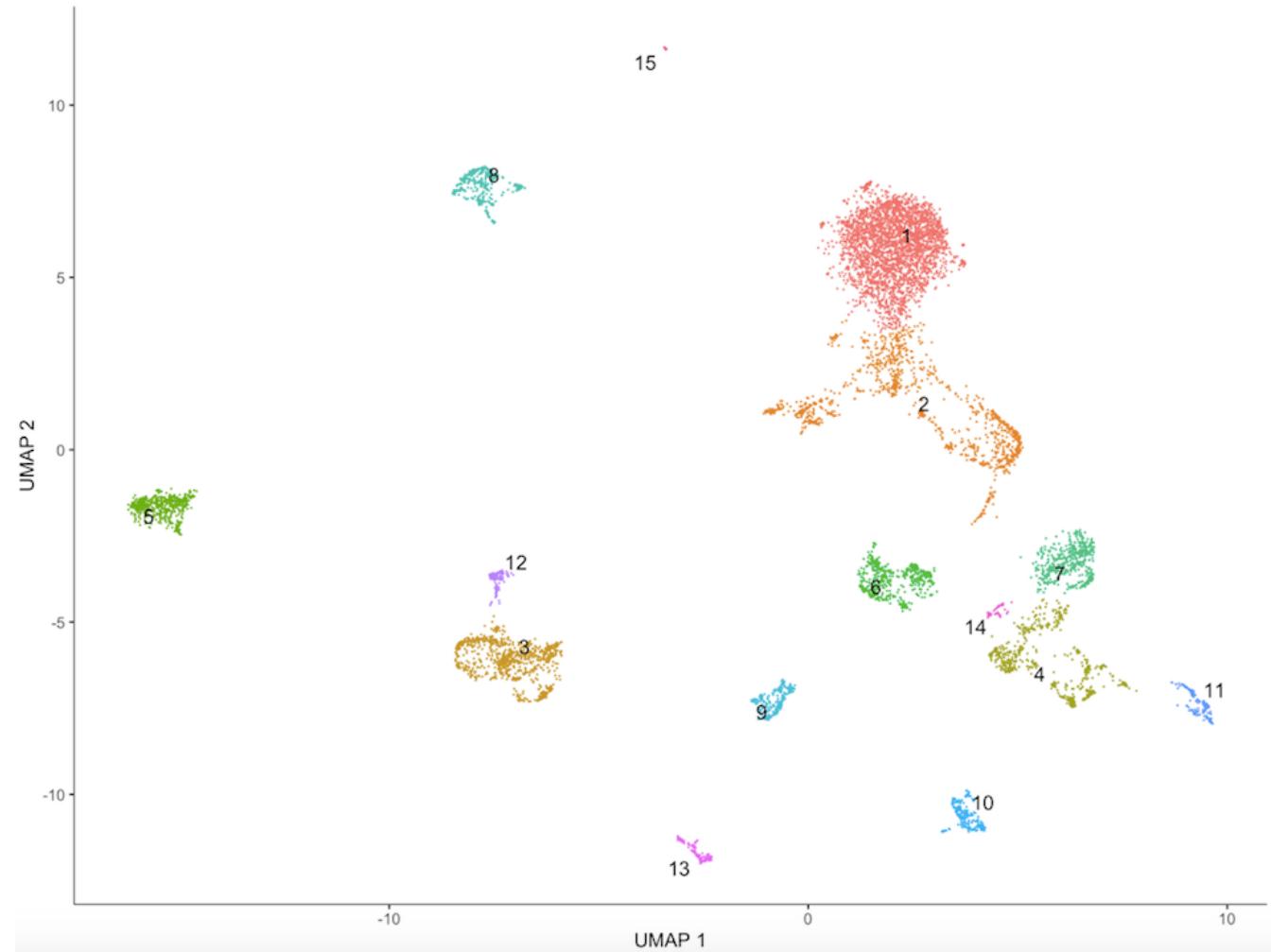


Image: 10x Genomics website

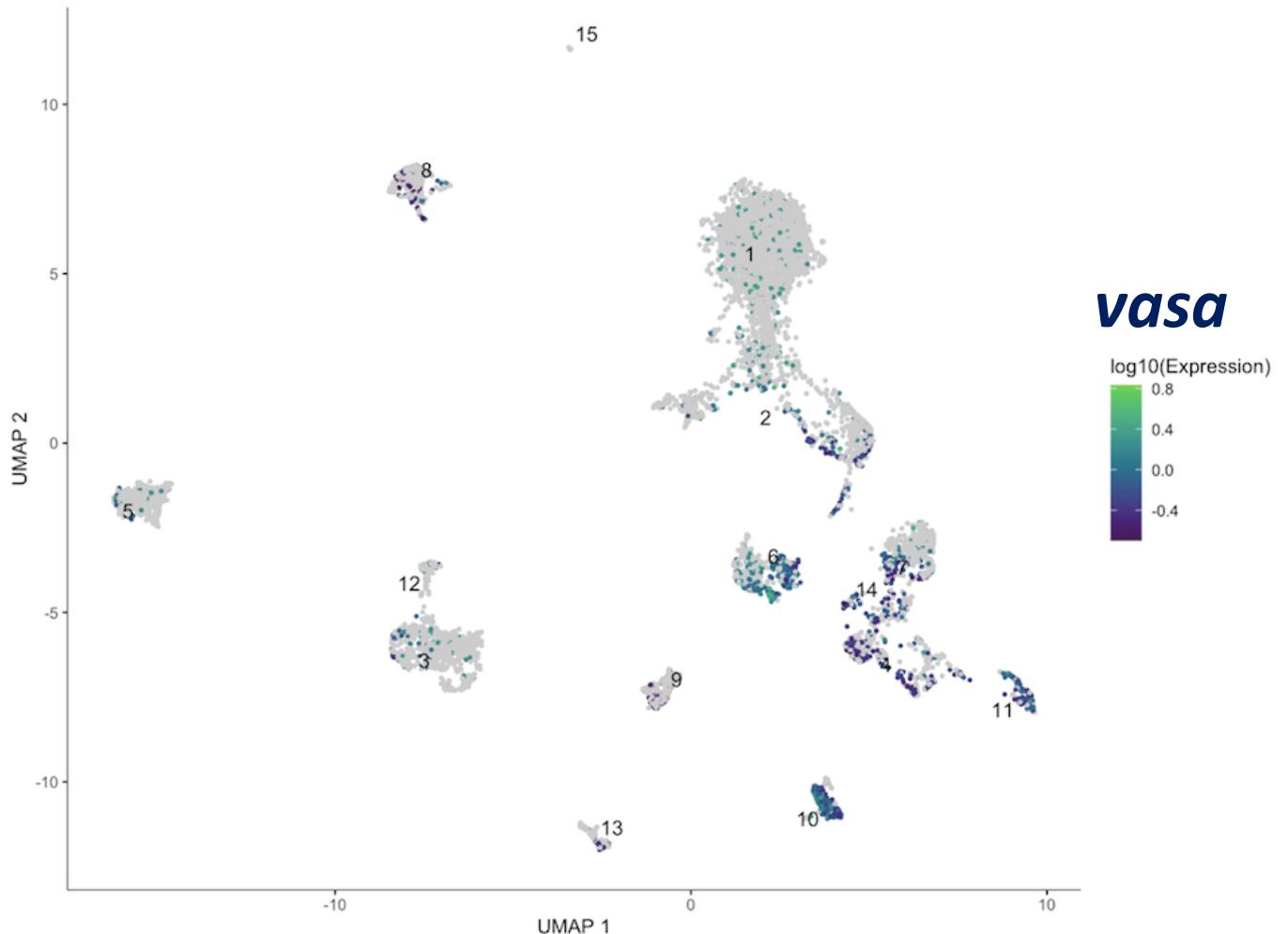
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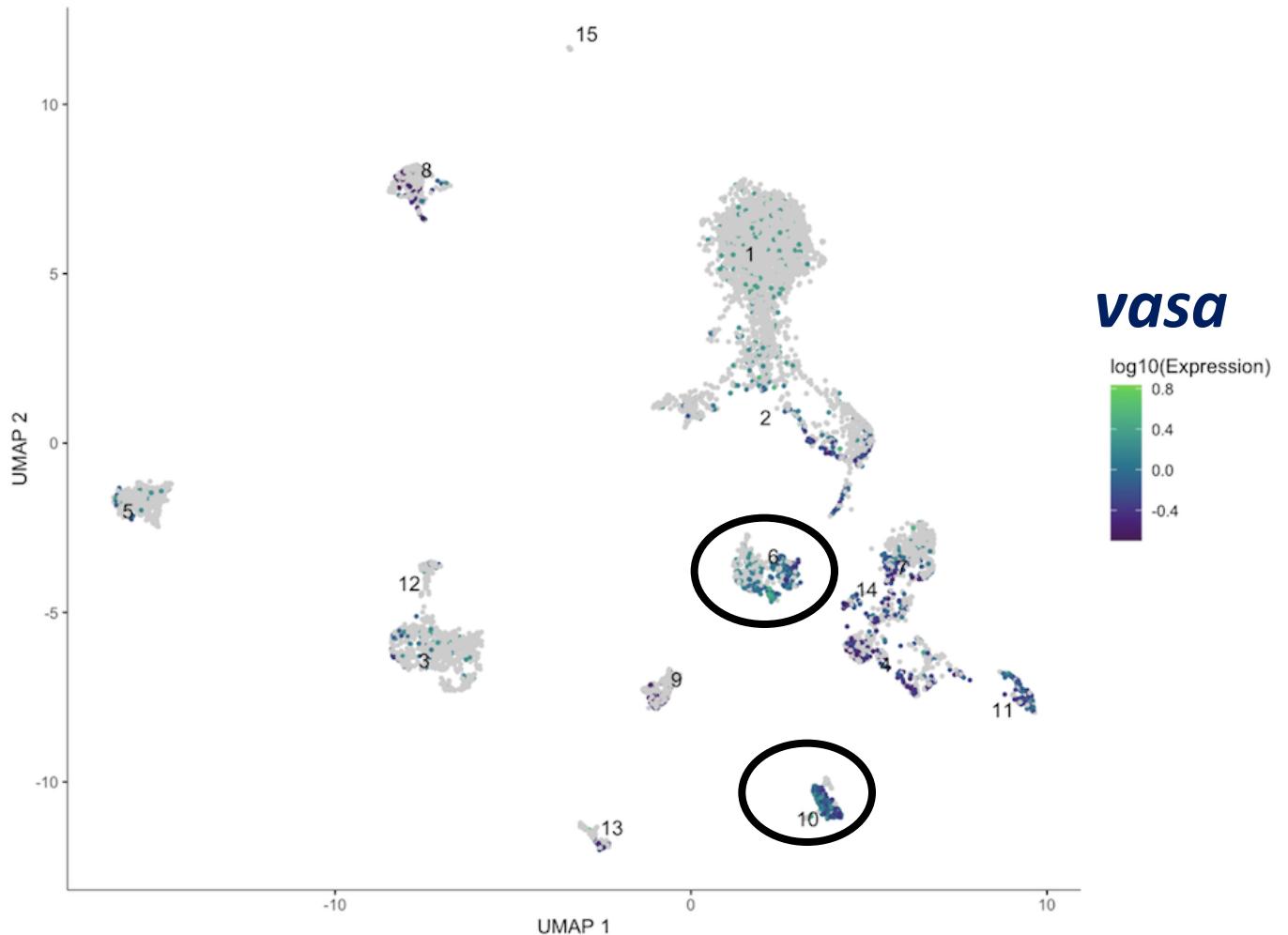
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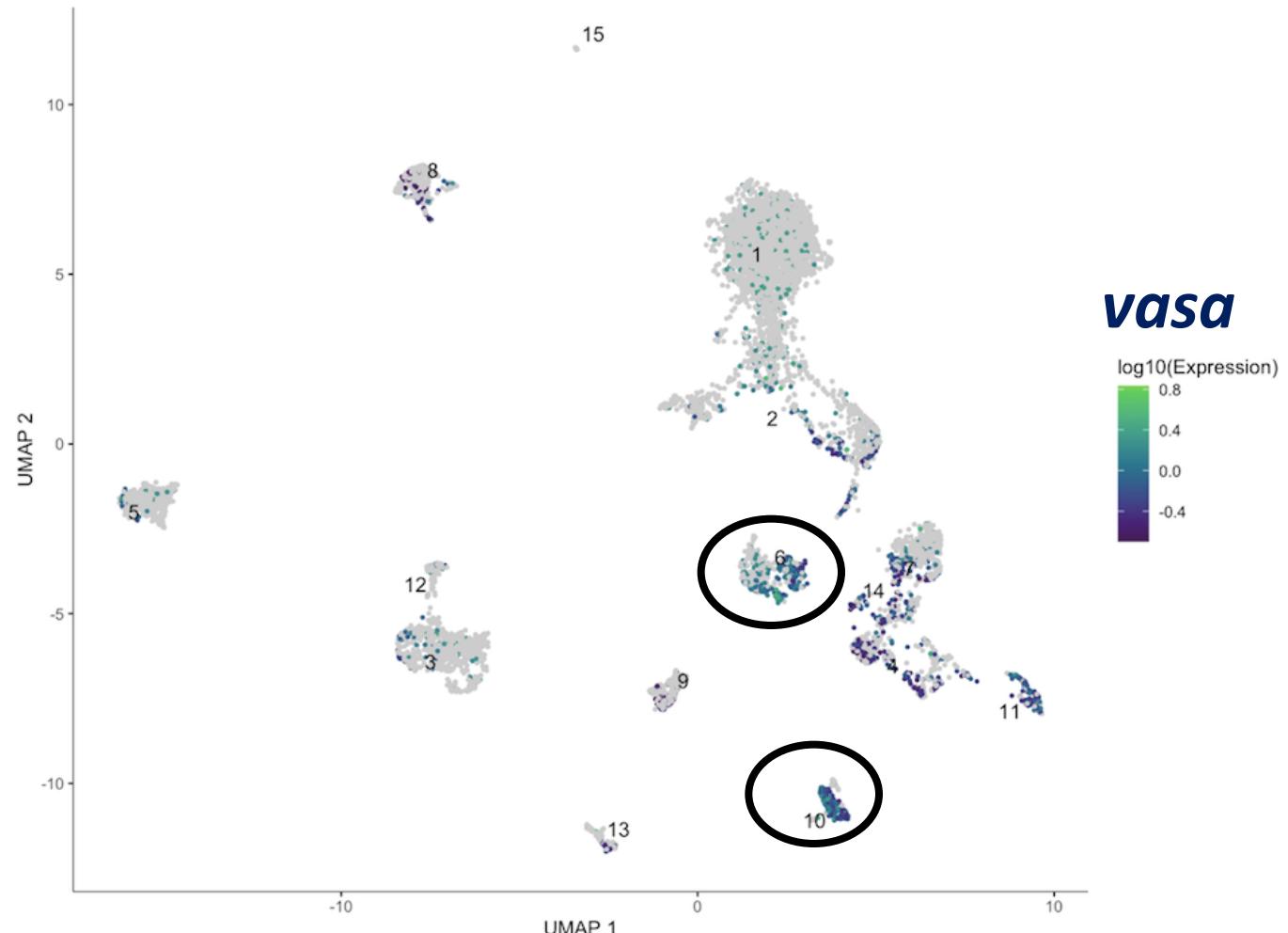
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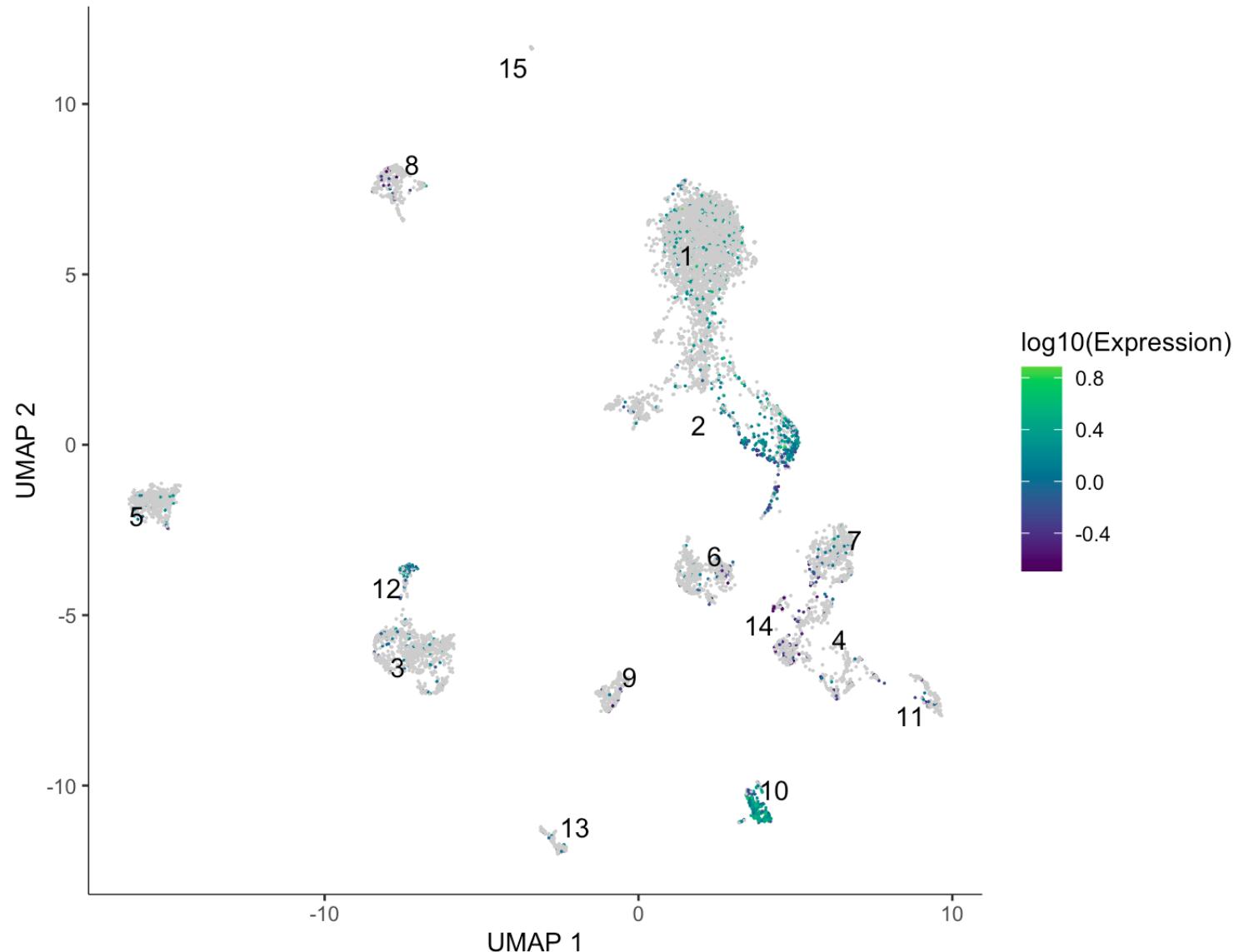
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7. Identify genes uniquely expressed in this cell type



A few candidate genes

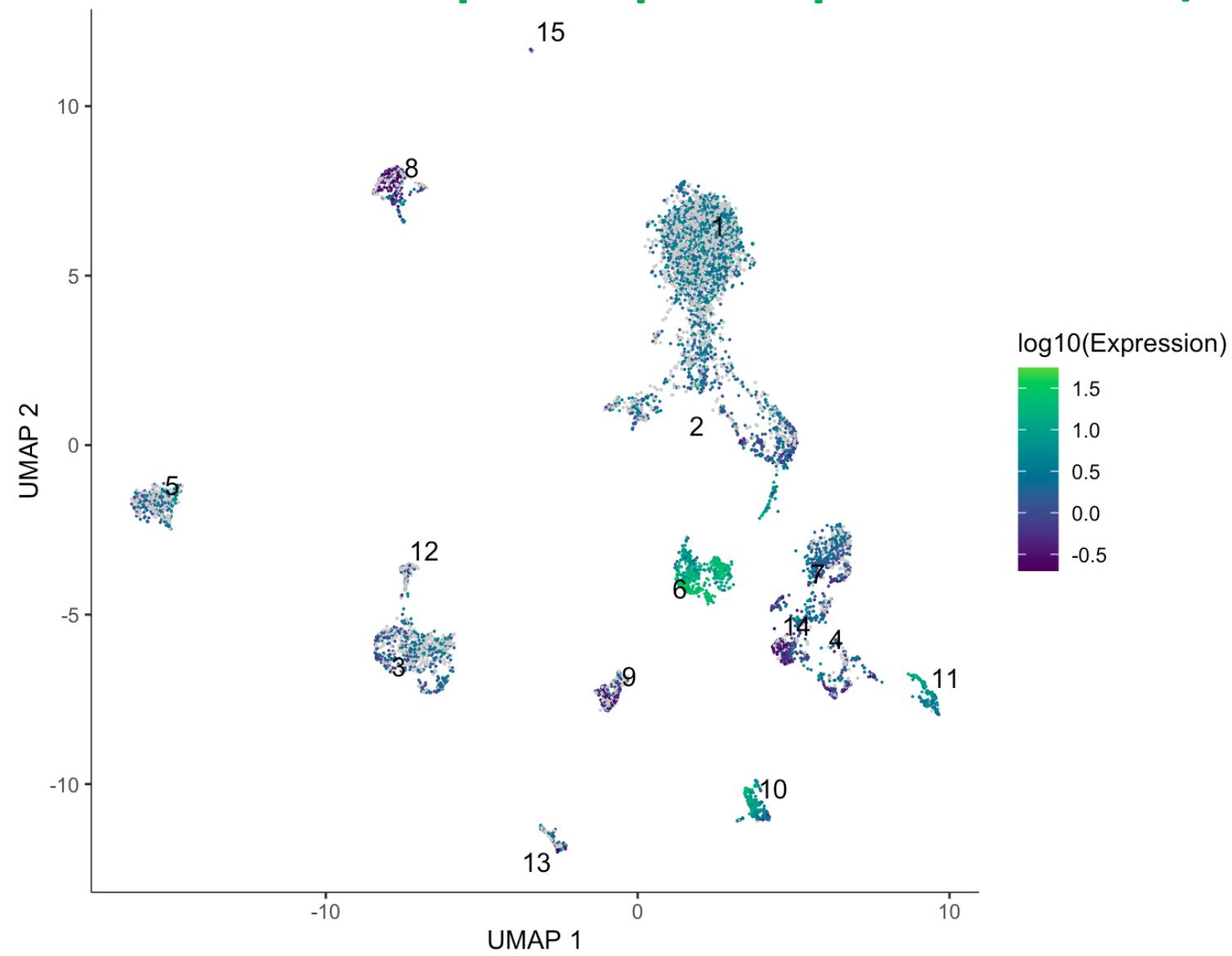
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blimp-1



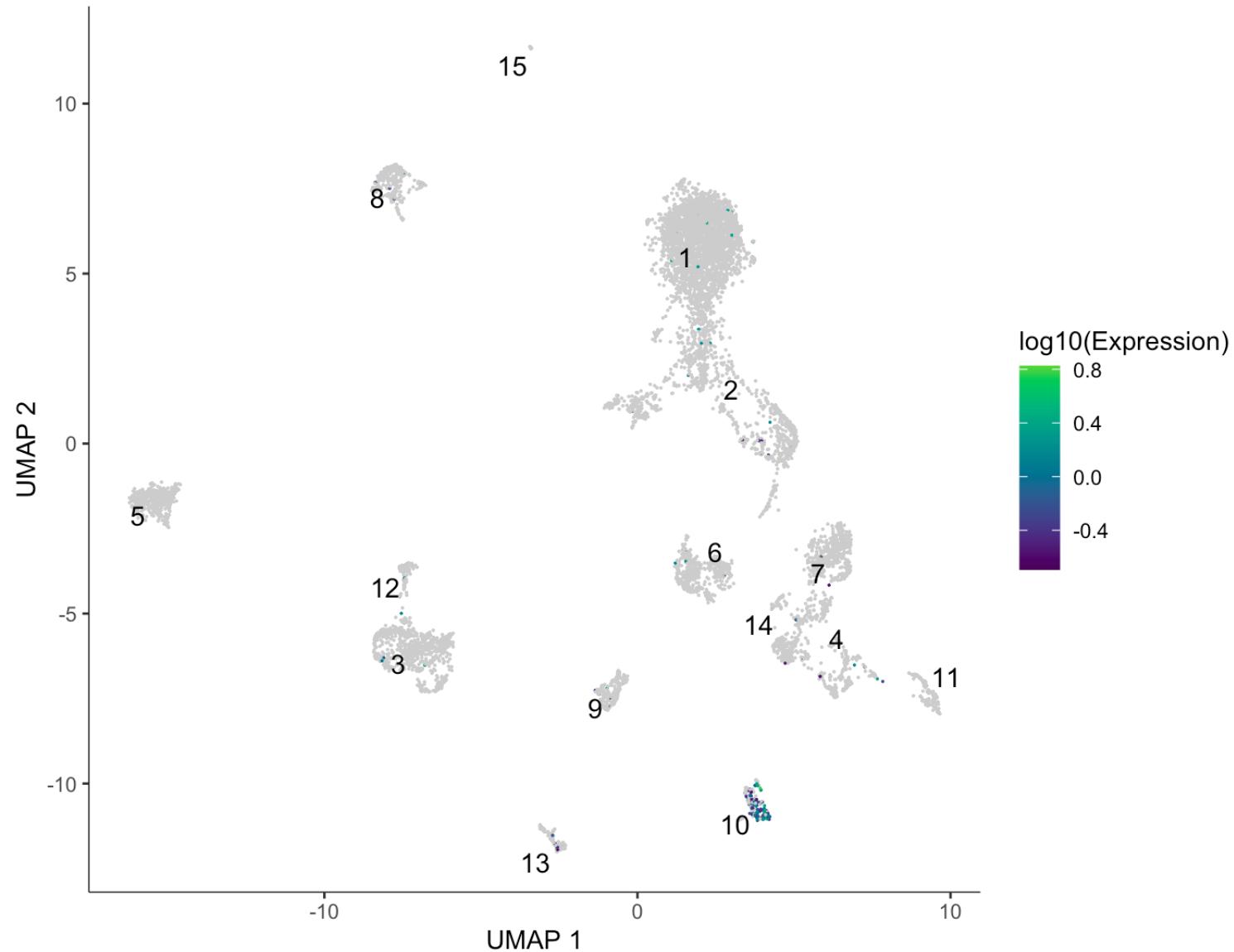
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sperm-specific protein PHI-2B/PHI-3



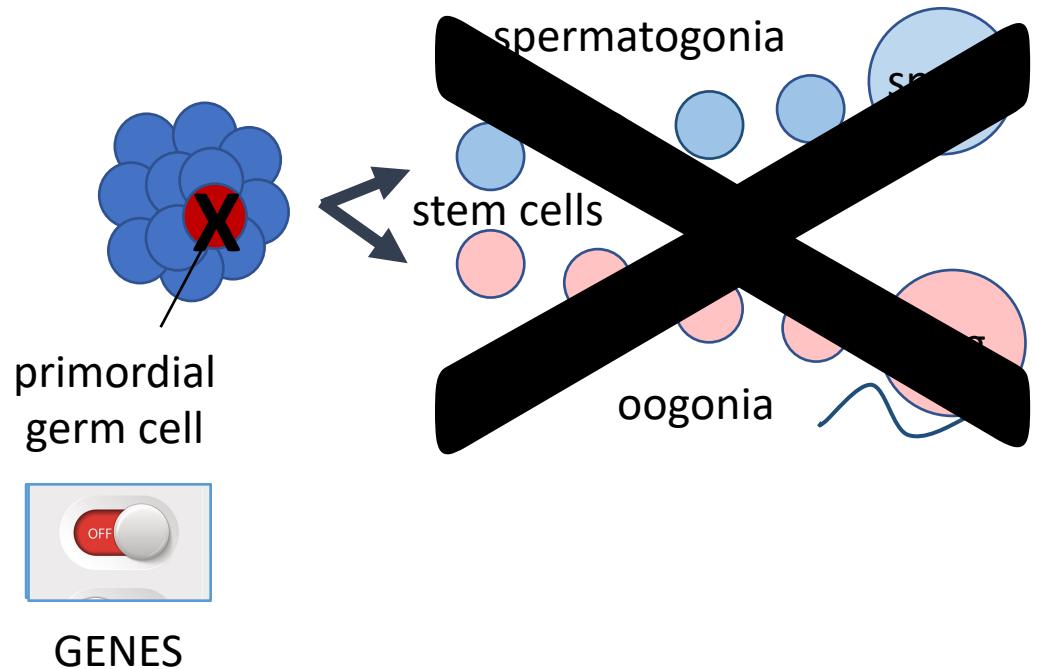
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uncharacterized **LOC105329605**



Next steps

- Narrow down 5- 10 most promising candidates genes
- Test the effect of silencing candidate genes on reproductive development
- Goal: develop protocols to induce sterility via germ cell elimination at a hatchery-scale



Acknowledgements

Taylor Shellfish Hatchery

Molly Jackson

Beniot Eudeline

Joanie Hendricks

Funding:

Pacific States Marine Fisheries Commission (Pilot Aquaculture Grant)

Washington Sea Grant

NOAA Office of Aquaculture

Bonus Slide: all cell UMAP (blastula clusters)

