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Development of an Alternative Sterility Method for Shellfish Aquaculture: Identification of Candidate Genes Involved in Germ Cell Specification

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Outline

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

Benefits of sterility in shellfish aquaculture



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Improved Growth



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Prevents genetic contamination



Benefits of sterility in shellfish aquaculture

Improved Growth

Prevents genetic
contamination

Year-round
Marketability



Current approach for reproductive control in oysters = triploidy

- Triploid oysters have 3 chromosome sets; effectively sterile
- ~50% of cultured Pacific oysters are triploid

Problems with triploidy:

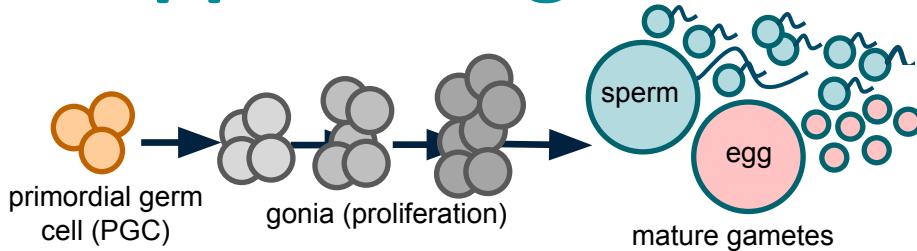


- Takes a long time to develop selected lines (10 years!)
- Can exhibit compromised performance in the field

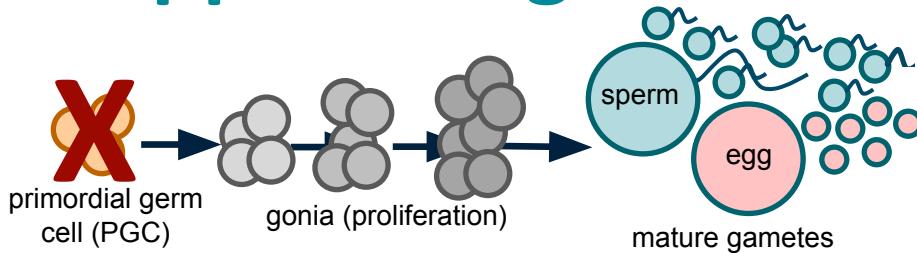
Alternative approach: germ cell elimination



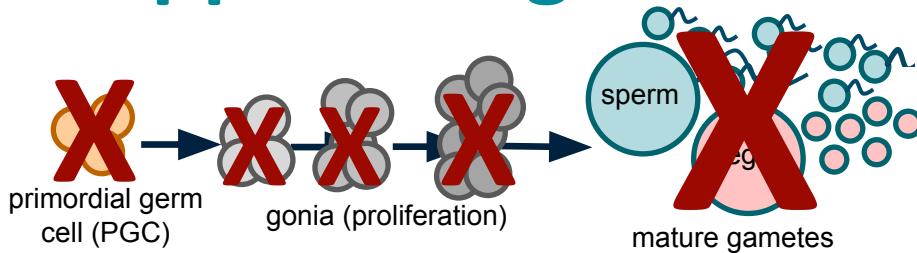
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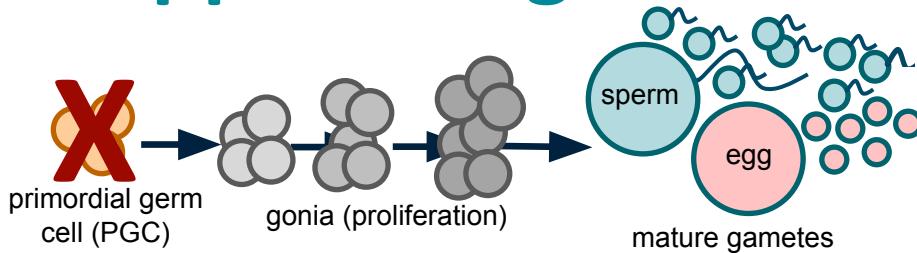
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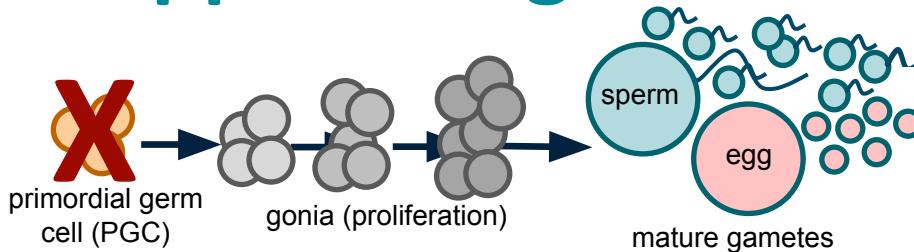
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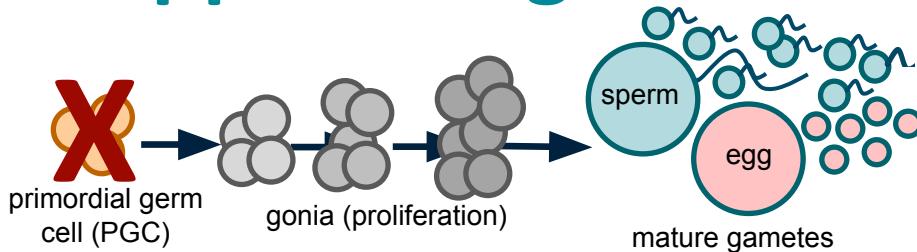


GENE

MOLECULE

DELIVERY

Alternative approach: germ cell elimination



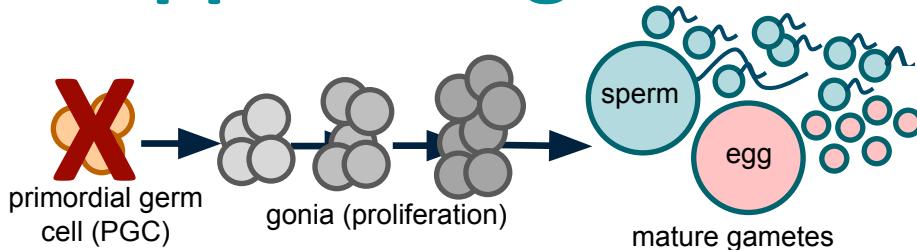
GENE

“dead end”
(dnd)

MOLECULE

DELIVERY

Alternative approach: germ cell elimination



GENE

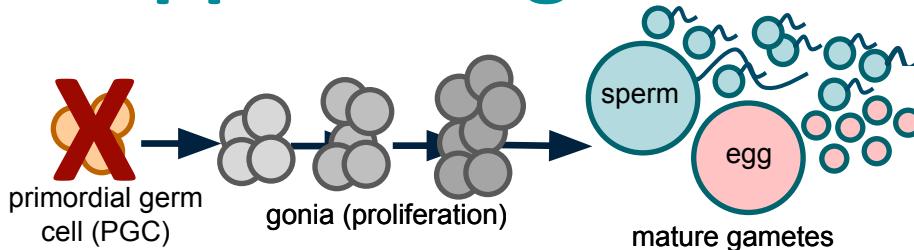
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DELIVERY

Alternative approach: germ cell elimination



GENE

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MOLECULE



DELIVERY

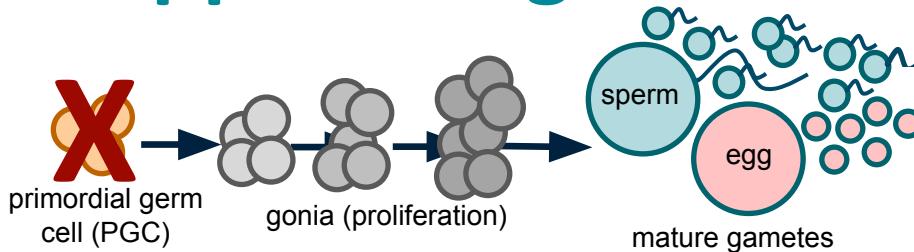


transporter
mediated



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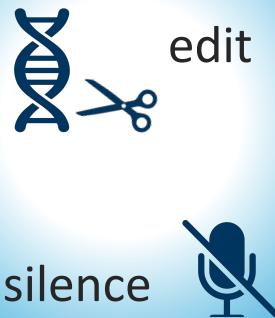
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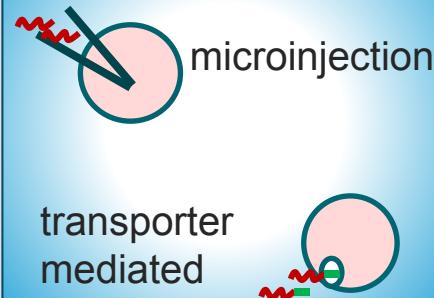
GENE

"dead end"
(DNA)
?

MOLECULE

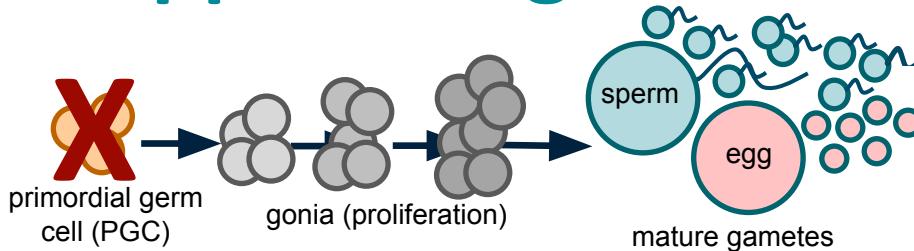


DELIVERY



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Alternative approach: germ cell elimination



GENE

"dead end"
(DNA)
?

MOLECULE



edit

silence



DELIVERY



microinjection

transporter
mediated



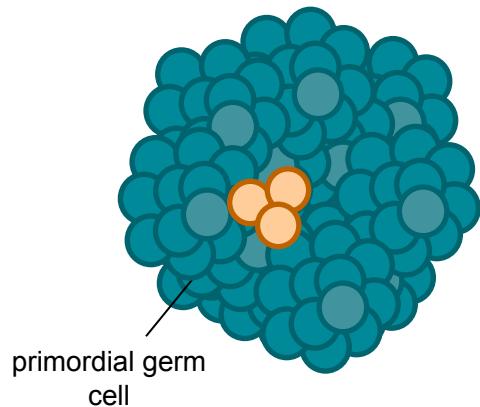
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What tool can we use to discover these genes?



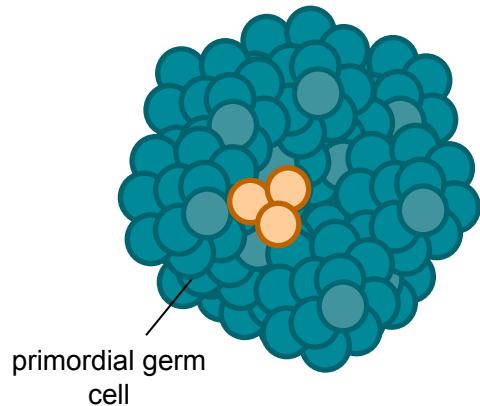
What tool can we use to discover these genes?

RNA-Seq: analyze ALL
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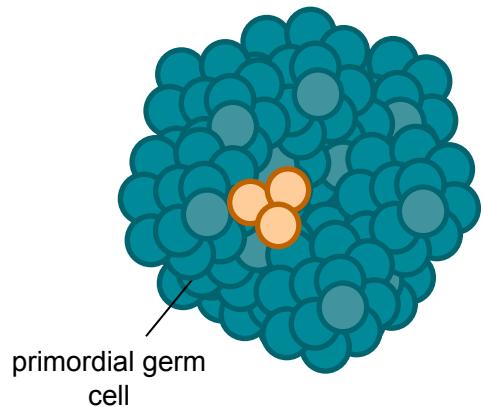
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Bulk RNA-Seq

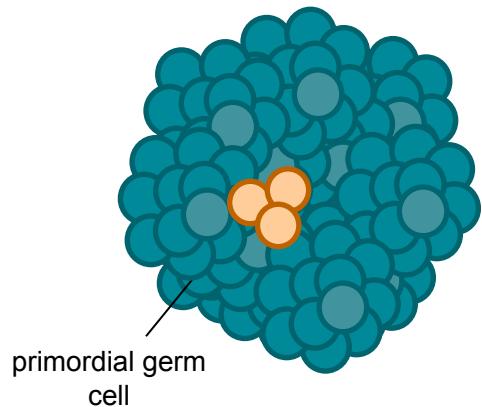


Single-cell RNA-Seq



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Single-cell RNA-Seq



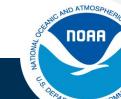
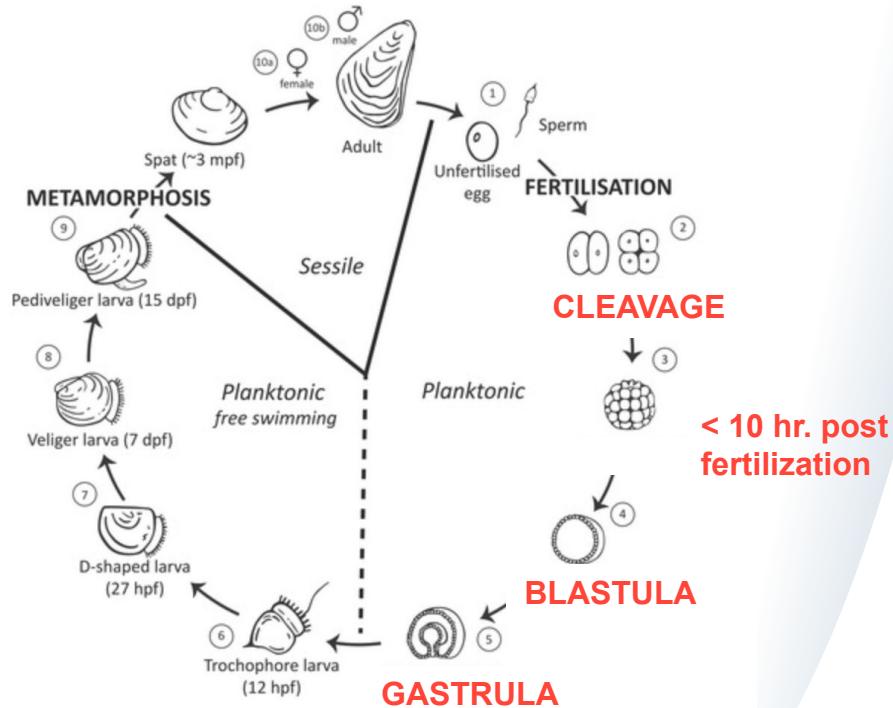
*germline marker gene

Approach: single cell RNA-Seq in bivalve embryos



Approach: single cell RNA-Seq in oyster embryos

1. Fertilize, monitor development, sample: cleavage through gastrula stage

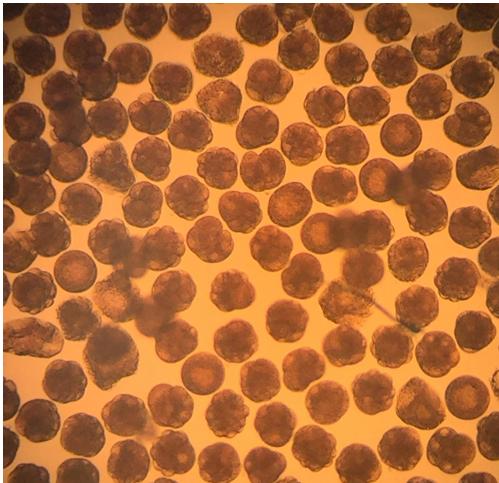


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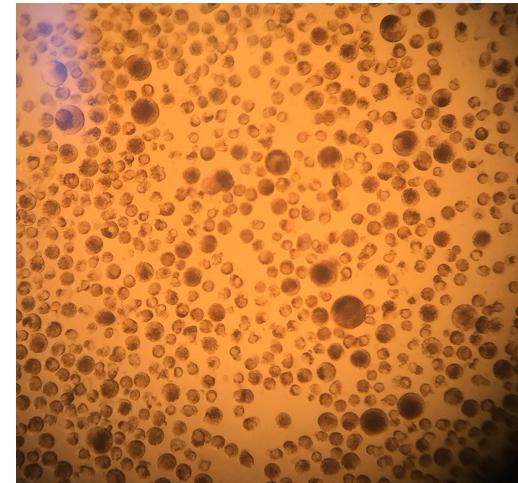
Approach: single cell RNA-Seq in oyster embryos

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2. Dissociate the embryos into a single cell suspension

Early cleavage embryos



Post-dissociation



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Approach: single cell RNA-Seq in oyster embryos

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3. Prepare the single-cell RNA-Seq
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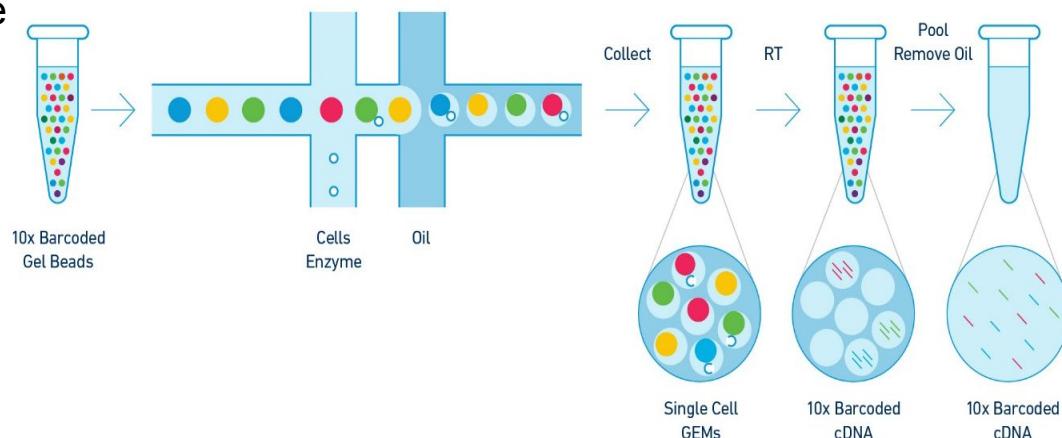


Image: 10x Genomics website



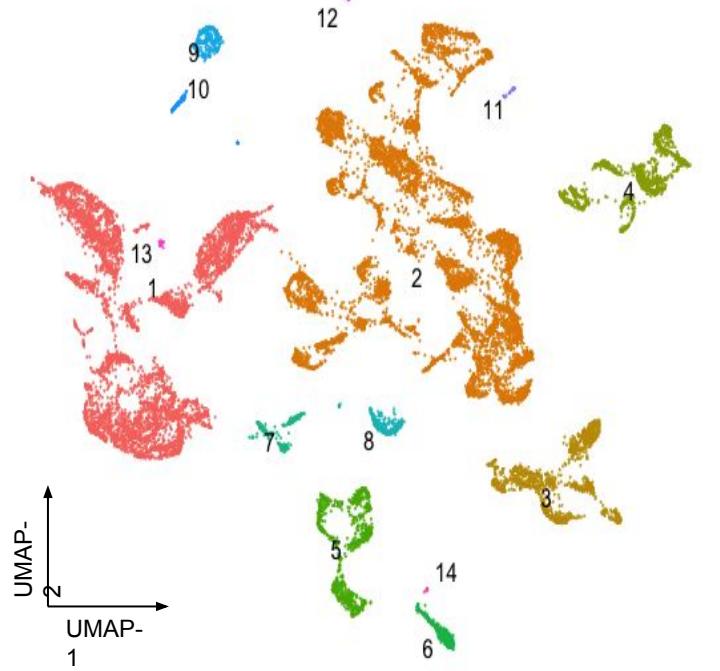
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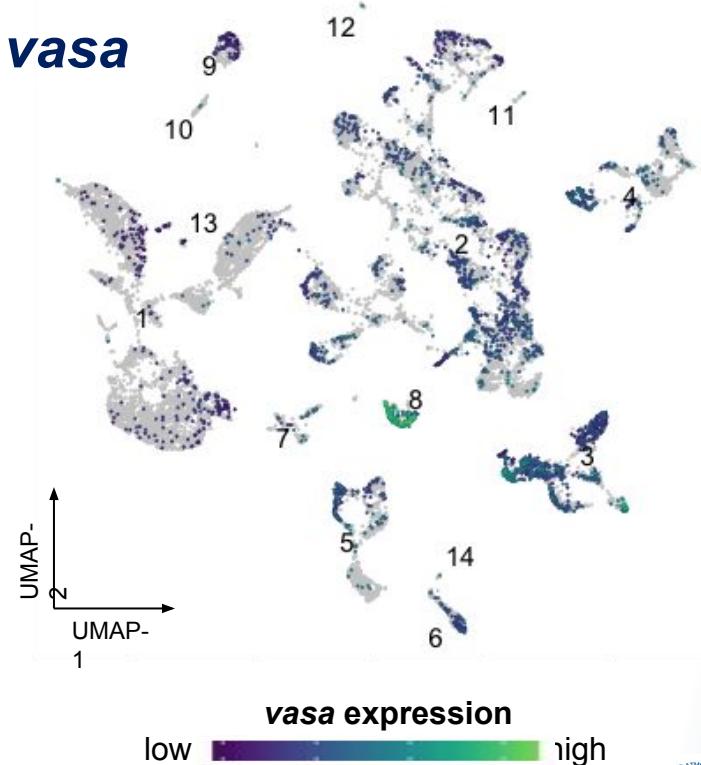
Cluster analysis of 19,000 gastrula cells (*C.gigas*)

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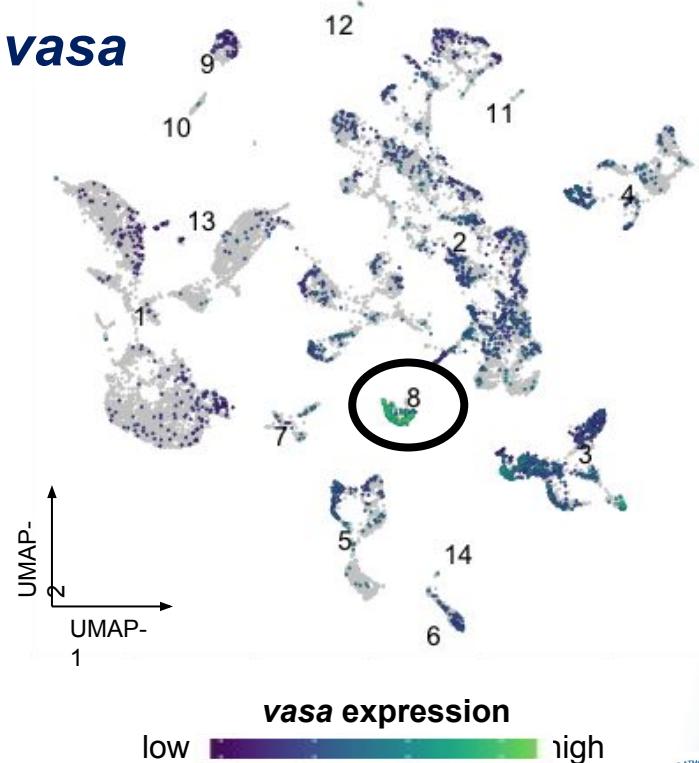
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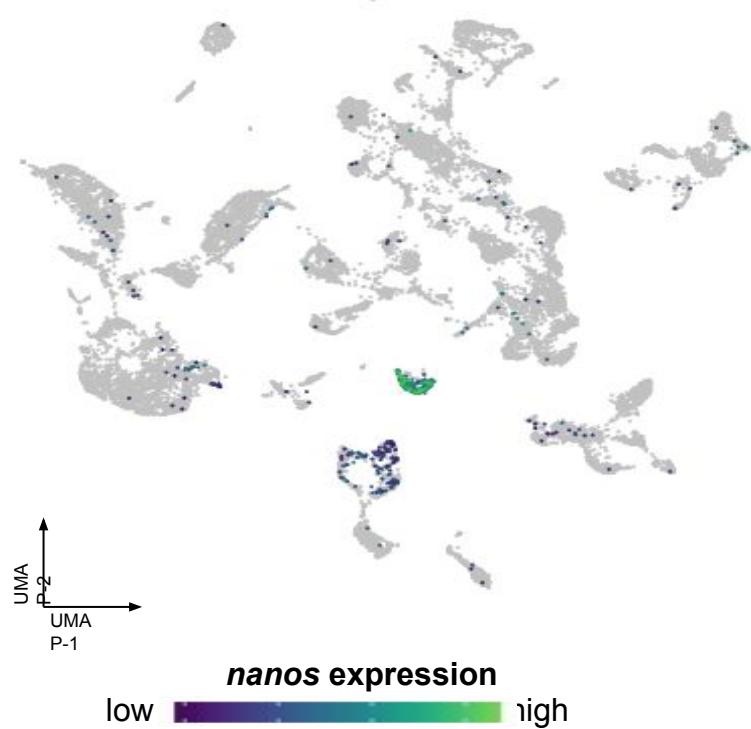


Candidate genes uniquely expressed in PGC cluster



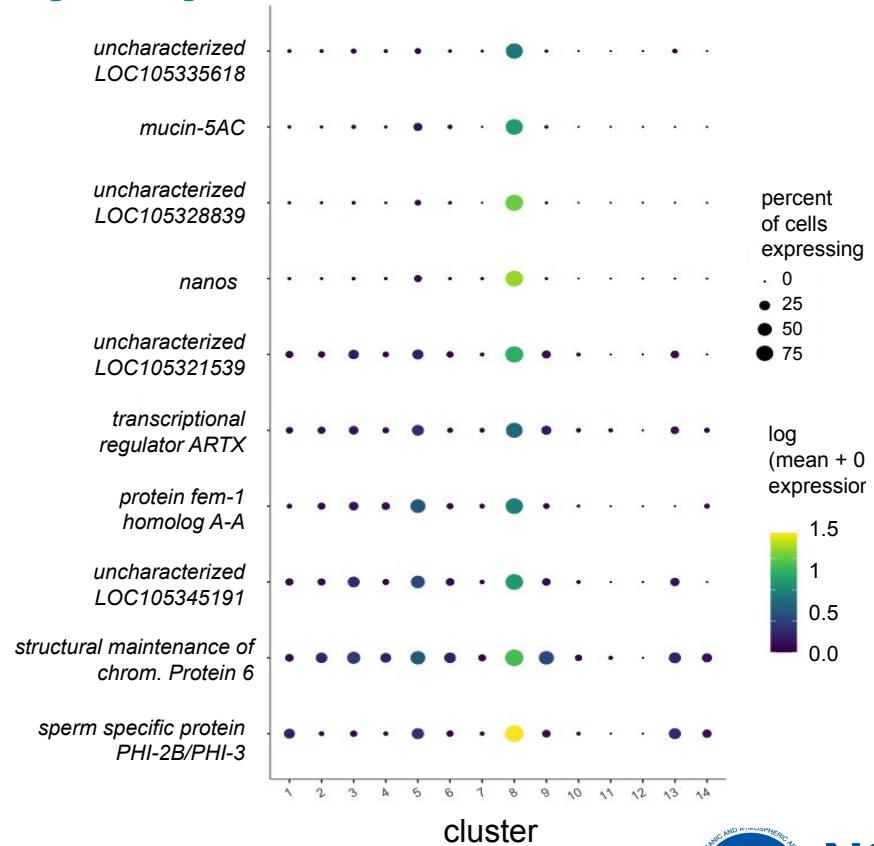
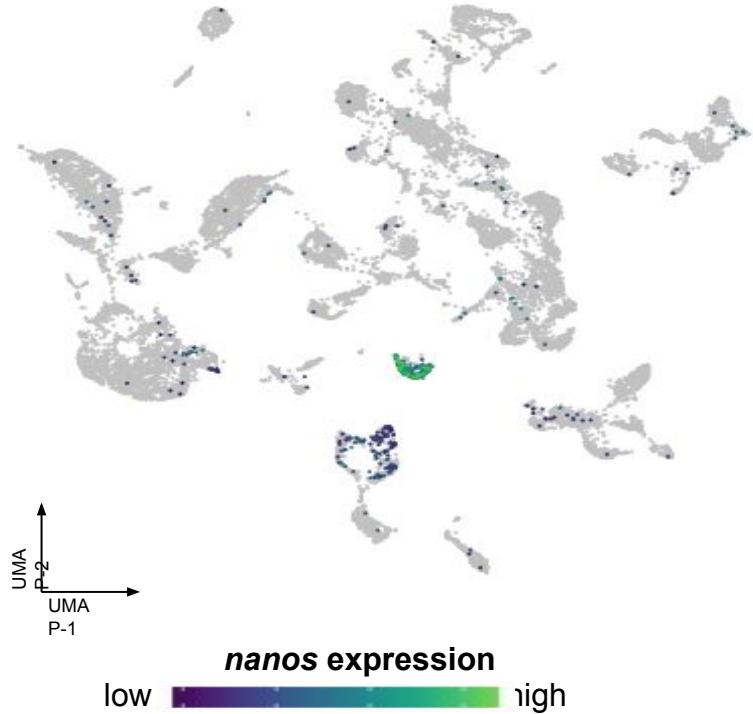
Candidate genes uniquely expressed in PGC cluster

nanos



Candidate genes uniquely expressed in PGC cluster

nanos



Conclusions and Next Steps

- We have identified candidate genes involved in primordial germ cell development in Pacific oysters
- Next Steps:
 - Develop methods to deliver gene silencing molecules to (small) bivalve embryos
 - Evaluate the effect of silencing candidate genes on reproductive phenotypes
- Long-term Goal: work collaboratively to develop protocols to induce sterility via germ cell elimination at a hatchery-scale



Acknowledgements

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NWFSC

Krista Nichols

Funding

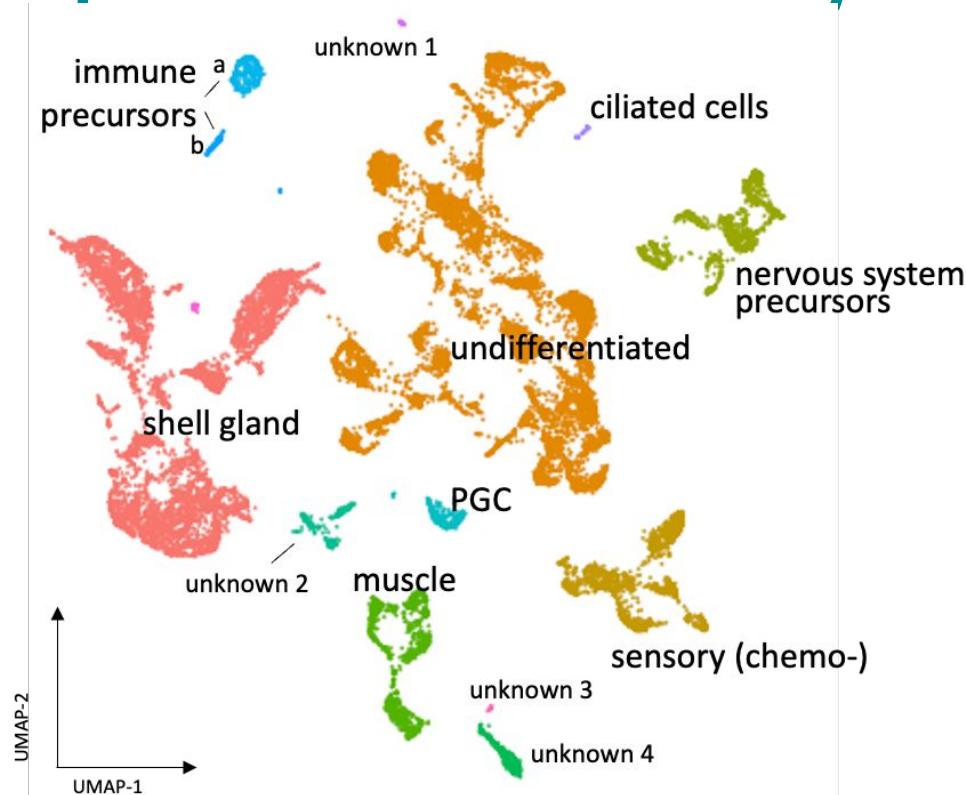
Pacific States Marine Fisheries Commission (Pilot Aquaculture Grant)

Washington Sea Grant

NOAA Office of Aquaculture - ICAF



A transcriptional “atlas” of early embryos



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