Geoduck planning:

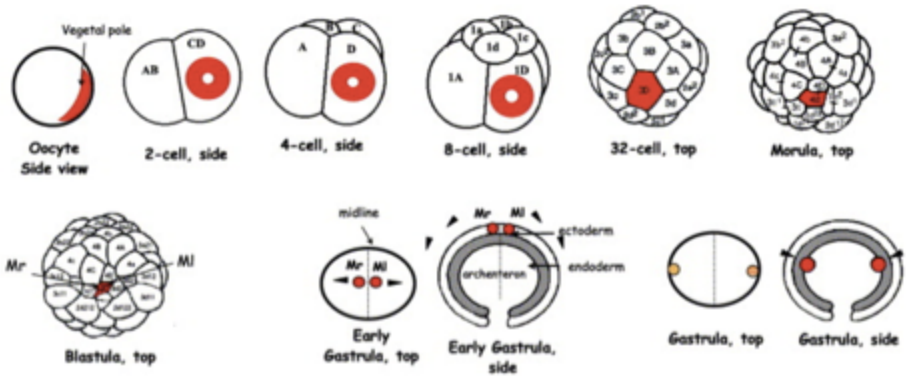
1. How to pool?

https://docs.google.com/spreadsheets/d/1x4gLI04pZNdKjggileJ2Iq-CMz6PaB6G9AIXp86an4c/edit?usp=sharing

Brent 5:07 PM

How about this as a starting point for equalizing cell numbers per stage

	#_cells per embryo	#_embryos	#_cells
4	2500	10000	
8	1250	10000	
16	625	10000	
32	312.5	10000	
64	156.25	10000	
128	78.125	10000	
256	39.0625	10000	



Discussion 10/4/19: Pool every stage and run replicates **(if replicates needed?, ask Lauren)**. This way you don't have technical bias. However, also for blastula and gastrula looking for a very rare cell type - there's a good chance we won't get these in the pools - so run a blasula library (1 lane) and gastrula library (1 lane).

2. Where are we getting broodstock

Taylor Hatchery (or whoever gets them going first, Pt Whitney) -- Brent can test with earliest Taylor spawns at Taylor. Fertilize some the day of and monitor development. Keep some gametes cold and fertilize the next morning and monitor development. We don't need to bring those over to Seattle.

First **good** spawns are typically in January - may happen in December

Earliest is November more likely December for **test** spawns

3. **How much control do we have over spawning/can we strip spawn?**

   Need to do volitional spawns

   At 15C - 1hr first cleavage

   Highest temp we'd want to go is 18C --how do we maintain this? Set up a chiller/water bath in FTR (Chris Grue's lab space?)

   We need to see if we can store gametes overnight and fertilize the next day (Brent's note: female gametes need to be fert within 6hrs)

   Lauren, how long does library prep take? Can we be in there late at night?

4. **How many chances will we get for test spawns?**

   See above

5. **What reagents to we need to purchase?**

   Lauren, is there a list for Chromium reagents, sequencing reagents?

6. **Lab meetings with Trapnell lab?**

   Check with Lauren, although Cole's initial email had someone else cc'd. I'll check

7. **What is the status of the genome/transcriptome?**

   44k genes in transcriptome. 18 large contigs for chromosome (completeness?) annotation?

   Map to genome or transcriptome? Check with Lauren to see if preferred pipeline

8. **4 months of salary - anyone identified?**

   We need people power for transport, setting up chiller space. Testing fixatives for imaging etc. Sam, Shelly?

What information/samples do we need to collect during our experiment

- Percent fertilization
- Preserve samples for imaging?
- Read the paper Brent send about geoduck embryo to see what info is in methods (we need to be more detailed than what we've been doing for testing)

Meeting with Lauren 10/17/19

We need to filter cells because >50um will clog the fluidics of the 10x. We should keep the filtered cells (i.e. polar lobes) and lyse and freeze them because we can do a total RNA-Seq on them if we need it later. Lauren has a low input RNA-Seq protocol dialed in.

10x planning and cost: There are 8 wells on a 10x chip and each well can run between 1000-10,000 cells. It's ~ $12k for the chip (per 'well' is $1750, but you have to run a whole chip per run) and reagents and another 12k for sequencing 10,000 cells.

-Lauren said that it would make the most sense to prep the cells then hand them off to her for library prep. She did the same for her experiments because the day is too long to do your experiment and stay focused for library prep. The library prep takes 2 days total, but has stopping points. She can send us the info to purchase the correct reagents.

- She also said they have a NextSeq so they can do the sequencing in their lab.

Genome considerations

-10x does 3' sequencing. Don't get full length transcripts. Will need to think more about multimapping in downstream processing

-Lauren can help us with the bioinformatics

-10x needs a polyA (does variation in polyA length of maternal transcripts affect this?, also rDNA is polyA in oysters per Rick. That will suck up a lot of reads)

Phone call with Molly Jackson 10/28/19

Discussed timing of available gametes. Pacific oysters will come up first. The first diploid spawns will be mid January, but Molly suggests waiting until Feb. for the best spawns for the experiment. I think it makes sense to start doing trial runs in January so we're ready to hand Lauren samples in Feb. for library prep.

Purchasing reagents for 10x libraries 12/10/19

Purchased library prep for 8 rxns and the chip that's needed. Will store in Robert's lab until Lauren needs them.