♀, test dissociation #1

2 cell (2×10^5 cells/mL) = 4×10^5

*4 cell (") = 8×10^5 (or 1.6×10^6)

8 cell pool (") = 1.6×10^6

35.

3.8×10^5 cell count

Should be 6×10^5 embryos - close

* this might be further along - 8 cell

(take 2mL off ice) - still cold

1. spin down 1 1mL (2500 rpm/1 min)
2. remove supe
3. add 355 mL to each of 5 tubes (Sea water ice cold) + combine
4. repeat rinse 2x (in ice cold seawater) → 3rd rinse in PBS
5. add 500 mL 10mg/mL cold protease
   - removed 20mL for a count
6. followed protocol (2 tubes of 1mL each after screening 5min spin)
7. resuspended in ~~400~~ 300 µL art. SW + ~~0.01~~ 0.1% BSA

i rinsed the screen to see if I removed clumps

2:25 pm - 1st count - viability is amazing but lots of doublets etc.

2:55 pm 2nd observations

3:25 pm - 3rd observations

will observe green @ 3:25 to see if viability of clumps increase

I put ½ vol through green 20µm screen + ½ through 30µm screen to get rid of clumps - 20µm screen still had clumps.

① Juhi says def. fewer clumps in our 20µm filtered sample count

4, test dissociation #2

(goal try to break up clumps)

① get enzyme to RT first

② more aggressive manual pipetting -
① P200 the whole time

③ directly onto 20mm filter (bypass the 40)

64 cell stage embros ← cells are even tinier
no gowth phase yet :(

① 1mL of 64 cell #1

② rinse in SW x 2 PBS + 1 (2500rpm 1min each)

③ 15 min in cold protease - inverted every few min

5min mm pipette p200 (after adding 500

④ 1mL through

⑤ spin

⑥ resuspend 150 mL art. SW + 0.1% BSA
count ~4:15

check @ 515 ▸

T3 dissociation

"blastula" 5pm #7 > ½ moving

① did 3x rinses in ice cold PBS
- pull slow when removing sype

② cold protease was on ice, warmed by hand for 5min

③ 5min P200

damn! accidentally used a 10mm

½ 50mm } pre rinse well!

½ 20mm

④ resuspend in 100ml art SW + 0.1% BSA
(10 + 20mm separate)

⑤ cont

lots of clumps even w/ 10mm filter

1.29.20

- liberate the oocytes w/ lab spatula in seawater (4C water)
- Screen on 90 → 20, rinse off the 20 in 400mL beaker
- 9:15 1st hydration notes on other sheet
- added 40mL then 400mL more the 200mL more sperm to get ~10-20 sp / egg

10:15-
10:30

- 11:15 filtered sperm from eggs + resuspended in 100mL
- performed cell counts

♀1 3500 cells/mL = 850,000
700,000
♀2
♀3 300,000

- resuspended in 50mL (rinsed on 20μM) to get them more conc.

- ♀1 200,000 200,000 cells/1mL

see counts — ♀2 205,000

- ♀3

- 12:07 - first subsample 1mL each
its a 2-4 cell sample ♀3 looked closer to mostly 4 cell
(... cell stage 2×10^5 cells/mL = 4×10^5 in 1mL
I want to know what my starting conc. needs to be to have 1million ~~2~~-4 cells (½ million embryos) in 100mL & this will make pulling stages easier)

* maybe these didnot go in fridge
- 12:40 - 2nd subsample + fixative
w/ ♀ 2 ~4 ~~8~~ cells

things we need
1 compound scope
1 spray bottle (EtOH) for slide cleaning

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♀1	♀2	♀3
20.5	12.5	2.5
2×10^5	1.2×10^5	2.5×10^4

▽ 1:20 - 8 cell sample went to ice (did a 4 cell check + they looked good

1:45 - ▽ 2:00 - 16 cell sample went to ice

→▽ 2:35 - ~32 cell sample went to ice
* also #2 ♀ to fix

3:05 - 64 cell sample

3:35 - bull of cells, difficult to tell

3:45 - did a 4 cell check + most/many moved to 8 cell ± even on ice

3:50 observed for 1st time moving embyos (ie cilinted) only for ♀1

4:30 - all embryos spinning in ♀1 40% ♀2, 60% ♀3
4c still looked 4 cell to me, but maybe like they're coming apart a bit don't look too hugging
photo ← tried, didn't work. Next time get pic of sample + sample w/ photos throughout to see if really Δing

(page 2 of 2) 4:30 ♀ 1 -> ½ are moving most still have polar body attached

CELL COUNT WORKSHEET

Sample ID	Side 1 counts (total cells 4 outer squares/4)	Side 2 counts (total cells 4 outer squares/4)	Average cell counts (average of side 1 and 2 counts)	Hemocytometer count (Hc) (average cell count * 1 x 10^4)	DF*	Cells/mL (Hc * DF)	Cells/uL (cells/mL / 1000)
2 cell stage	*121*	*144*	*132.5*	*1.3 x 10^6*	*5*	*6.625 x 10^6*	*6,625*
Fertilized eggs 1	5	12	8.5				
2	5	10	7.5				
3	3	4	3.5				
3	3	∅					
Fertilized eggs 1	17	23	20				
reduced vol 1	18	23	20.5				
50 ml 2	14	11	12.5				
3	3	2	2.5				
Pre dissociation	50	26	38				
Test 1 cell diss	479	333	406	*1 x 10^4	2		
Test 2 cell diss	1/4 sq, 2) 24/4 sq	1) 122/4 sq 2)	4/4 sq			Stand to Each Side	

Test 2 in stomp ① 135/4 sq ② 4/4 sq — Knodend

100ml Full volume

All fert. eggs spread to by 9

*Dilution factor:

① Single ② clump

Dilution factor (DF) = $\frac{Volume\ final}{Volume\ initial}$

Test 3: Side 1 ① 212/4 sq ② 44/4 sq; Side 2

Test 3: ① 210/4 sq ② 23/4 sq

DF 4

Example DF = $\frac{50uL\ cells + 200uL\ SW}{50uL\ cells} = 5\ DF$

* these clumps many > 2 for the 20 μm seemed even bigger clumps