~~5.13.2021~~

$\frac{16}{2} = \frac{8}{9}$

$3+6 = 9 \div 2 = \frac{4.5}{9} \times 10^4$

9888 embryos

5000 embryos / mL

10,000 embryos

Geoduck Embryo Cell Dissociation Protocol

2.24.22

Equipment and Materials

Filtered Seawater
10 mg/mL Cold Protease in 0.3x ASW
20 um screens for microcentrifuge tubes
Artificial Seawater (Ca/Mg free)
Compound scope
Large centrifuge at 15C

*keep all solutions at 15C

Fertilization @ Taylor hatchery mixed parents
1:45pm 2.23.22
Kept @ 16C brought back to lab
• 4:18p feeding rate looks good many 2cell stage

Solution Prep

dPBS	dPBS	dPBS
1.25 mL ~~milliQ~~	2.5mL ~~milliQ~~	33.4 mL ~~milliQ~~
3.75 mL ASW	2.5 mL ASW	16.6 mL ASW
5 mL @ 0.75x ASW	5mL @ 0.5xASW	50 mL at 0.33x ASW

1 mL FBS	10uL BSA (20mg/mL =2%)
9 mL 0.3x ASW	1.99mL 0.3x ASW
10 mL at 10%FBS in 0.3x ASW	2 mL 0.3x ASW/0.01% BSA

Procedure

~~2222 cells/mL x 9mL~~ = 20k embryos/tube

7:20 am
- [ ] 1. Prepare embryos in duplicate 15 mL conical tubes (~25k embryos/tube) 20k embryos total 10k/tube
- [ ] 2. Spin for 1 min at 2500 rcf (~4000rpm)
- [ ] 3. Decant (pour off and remove residual liquid slowly with P200) and add 1mL 0.75x ASW to rinse.
- [ ] 4. Spin for 1 min at 2500 rcf
- [ ] 5. Decant per step 3 and add 1 mL 0.5x ASW to prepare for protease digestion
- [ ] 6. Spin for 1 min at 2500 rcf
- [ ] 7. Resuspend in 1 mL of 10mg/mL Cold Protease in 0.3x ASW $\frac{60mg\ CP.}{6mL\ 0.3x\ ASW}$ = 10mg/mL in 0.3x ASW
- [ ] 8. Transfer to microcentrifuge tube for digestion

-2021

9. Incubate at 15C for 15 min. Pipette with P200/P100 every few min during digestion

10. After the digestion, mix sample up and down (5 times) through a ~~27~~ 25G needle, being careful of bubbles, to break up any clumps of cells.

11. Check the digestion efficiency on microscope. – viability really good! digestion too!

12. Transfer digested cells to a 15 mL conical tube and add 3 mL ice cold 0.3x ASW with 10%FBS to inactivate protease

13. Spin the tube for 5 min at 600 rcf (1700 rpm)

14. Discard supernatant and resuspend in 2 mL 0.3x ASW with 10%FBS

15. Place pre-wetted 20um filter in a 15mL tube and filter the dissociated cells. Tap tube gently and make sure all volume is recovered.

16. Rinse filter with 2mL 0.3x ASW and transfer to labeled 15 mL tube containing cells

17. Spin the tube for 5 min at 600 rcf.

18. Resuspend with 5 mL 0.3x ASW

19. Spin the tube for 5 min at 600 rcf.

20. Decant gently with P200.

21. Resuspend in 100-500uL 0.3x ASW with 0.01% BSA.

22. Perform cell count and viability using 1:2 dilution in Trypan Blue.

23. Adjust volume if necessary.

9am cells to Dana

- 8:15 all but beads

$\frac{145}{4}$ sq $\times 2 \times 1 \times 10^4 = 725{,}000$ cells/mL

diluted

$\frac{141}{9} \times 2 \times 1 \times 10^4 = 313{,}333$ cells/mL

313 cells/mL

33 mL cells } targeting
10.2 mL water } 6000 cells/lib