2·19·29

6:15 am - 1st of 3 ♀s eggs hydrating (In 100ml seawater). Liberated eggs from gonad sample w/ lab spatula in 20 ml seawater. Filtered 90→20μm + screened eggs off the 20 into a 400 ml beaker. All 3 ♀s complete @ 6:35. Eggs very tear drop shaped

7:30am · @ 7:15 checked rounding of eggs + all looked good. Activated sperm in seawater + checked motility. Male #2 looked much better than others. Fertilized w/ 400ml sperm ♂2 @ 7:30.
Obs maybe 5 sperm/egg. Added an additional 400ml sperm. Female 1 does not look as good as others. Female 3 looks best quality.

8:15am · Screened fert eggs over 20μm to remove extra sperm vol. up to ~50ml in aet. seawater.

8:50am - few polar lobes on any of 3 females

10:45 - F#3 subsample ~4 cell stage 20-30% unfertilized or pre-cleavage. ♀1+2

12:00 - F#3 subsample ~16 cell stage

12:15 - 16 cell stage cell dissociation

12:45 - F#3 subsample ~32 cell

1st dissociation

16 cell 73 (12p)

① 1 mL embryos

② 3 rinses - 1+2 in art. seawater, 3 in dPBS

④ 15 min @ 15 - pipetting every 2 min ~~10x~~ (30sec, P200)

⑤ 5 min P200 pipetting

⑥ Filter

30μm — 300mL

10μm — split in 2 tubes, rinse through, ~~105~~ 150mL Ca/Mg free seawater 0.1% BSA

2nd dissociation 2:45

① 3 @ 1 mL spin 2500 1 min

② 1 rinse seawater

③ 1 rinse dPBS

④ 15 min 10 mg/mL protease - mech pipette every few min

⑤ ① pipette 15s
② 22G 15s
③ 27G 15s (5-7 times)

obs (pre filter)
some clumps
very few clumps
also very few clumps

⑥ filter each 20μm screen

⑦ spin 600 rcf for 5 min

⑧ add 250mL seawater + 0.1% BSA

post filter
few ~~some~~ clumps
about the same as above
lost sample

3ed dissociation

① 2 x 1mL 4:30pm (73 blastula all spinning)

② Rinse 1x in 1mL Seawater - decant vol. + remove residual w/ P200 (otherwise 12ez. cells w/ P1000)

② Rinse 1x in 1mL dPBS - " - Still observed loss, mybe 2.5min here, def be gentle

③ 500µL 10 mg/mL Cold Protease (warmed to RT @ ~15min + mixer)

④ 15 min @ RT pipetting every few min

⑤ 27G syringe 15sec - 5-7 times, watch no nice bubbles

⑥ pre wet a 20 + 30µm filter (1 each)
* Check + make sure Flow through ~500mL
obs: 400-500mL

⑦ Spin 5min @ 600 rcf

⑤ remove supe w/ P200 ~~decant~~ gently

⑧⑨ resuspend in 200µL art SW (Mg/Ca free) + 0.1% BSA

obs: 20µm filter fewer clumps, a few doublets triplets in both - maybe 10%? because to check digestion before filtering to check yourself on the digestion

ID	side 1 count	side 2 count	# Square per slide	average counts per square	(1x10^4) hemocytometer ct	DF	cells/mL	Volume	total cells
(1) ♀3 feet eggs	11	10	9	10.5/9 =	11,666	2	23,333		
1st dissociation 20µm screen	11	18	9	14.5/9 =	16,111	2	32,222	0.3	9,666
1st dissociation 10µm screen	20	30	9	25/9 =	27,777	2	55,555	0.15	8333
(1) 2nd dissociation 20µm screen									
(1) 2nd dissociation [illegible]						2			
3rd diss ~~20µm~~ 20µm > 2 cell	14		4			2			
3rd diss 20µm singles	16.5		4						

clump

(1) Only ♀ cog counts @ 1:2 dilution in trypan blue - pics of blue eggs on phone. Lots of scramble obs. Stains Red