Name: Asma, Amirah, Jiayi Lan, Yujie Huang, Shakera
Date: 10/01/2019

Goal:

1. Colony PCR of transformed pcb302 in A.Tume
    a. Primers GFP Fwd/Rev
2. Run gel of pcr products
3. Use the flow cytometer to take the measurements of O. Marina and Symbiodinium (including window part, hood part, transformed GFP part, and transformed RFP part).
4. Use the microscope to observe the cells states.

Name: Asma
Date: 10/01/19

Goal:

1. Colony PCR of transformed pcb302 in A.Tume

Protocol:

Colony PCR Protocol

20 µL Reaction
1. Prepare a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of the pcb gfp forward primer, and 1 µL of the pcb gfp reverse primer.
2. Add 19 µL of the concentration cocktail into a PCR tube.
3. Using a 10 µL micropipette, touch the tip onto the selected colony and swirl around in the PCR tube.
4. Place PCR tube in the thermocycler at the following generic settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. **43**° C for 1:00 minute
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

1	2	3	4	5	6	7	8	9	10	11
500ul plate 3, colony 2	500ul plate 4, colon y 1	500ul plate 3, colony 1	300 ul 2,1	200 ul 2,2	300 ul 2,2	200 ul 2,1	500ul 1,1	300 ul 1,1	200 ul 1,1	200 ul 1,2

Name: Amirah
Date: 10/01/19
Goal:

1. Run gel of pcb302 pcr products

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

1. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heated in the microwave until fully dissolved (usually about 45 seconds to 1 minute)
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mixed
5. Inserted casting tray, made sure the rubber on the sides was not overlapping
6. Carefully poured the agarose into the tray and placed the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changed the orientation of casting tray where the rubber sides are not in contact with the sides of the system.
9. Poured in 1X TBE into the gel electrophoresis system to the fill line, being sure to submerge the gel, and removed the comb

Loading

1. Loaded ~5 µL of the ladder in the first well
2. Loaded 5 µL of DNA and load

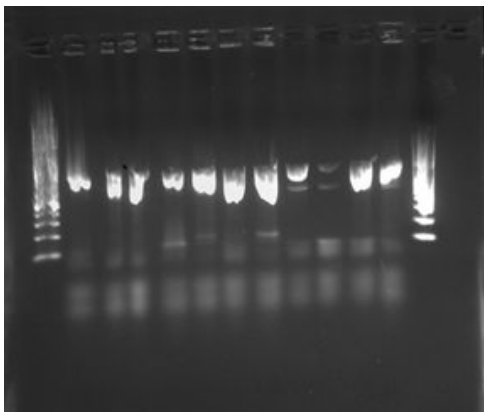
Running

1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran for about 45 minutes to an hour

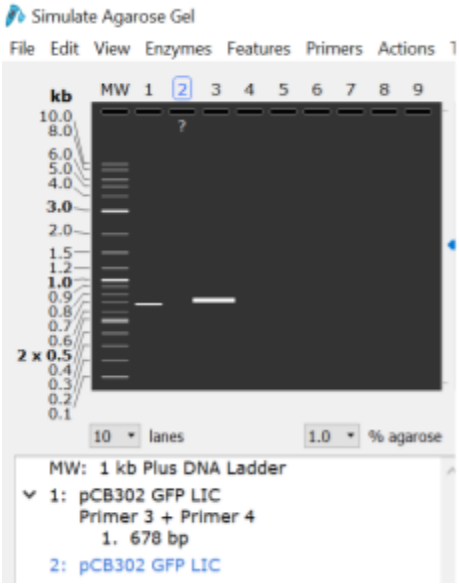
Gel key:

Lane #	1	2	3	4	5	6	7	8	9	10	11	12	13
Pcr product	1kb Plus ladder	1	2	3	4	5	6	7	8	9	10	11	100 bp ladder

Results:



Expected Results:



Primers GFP Fwd & Rev

Conclusion

The bands are approximately in the correct location. They're a little larger, but that's expected since we don't know the exact pcb302 sequence. Dimers also formed the first time we did this, so the primers aren't as efficient as we'd like them to be.

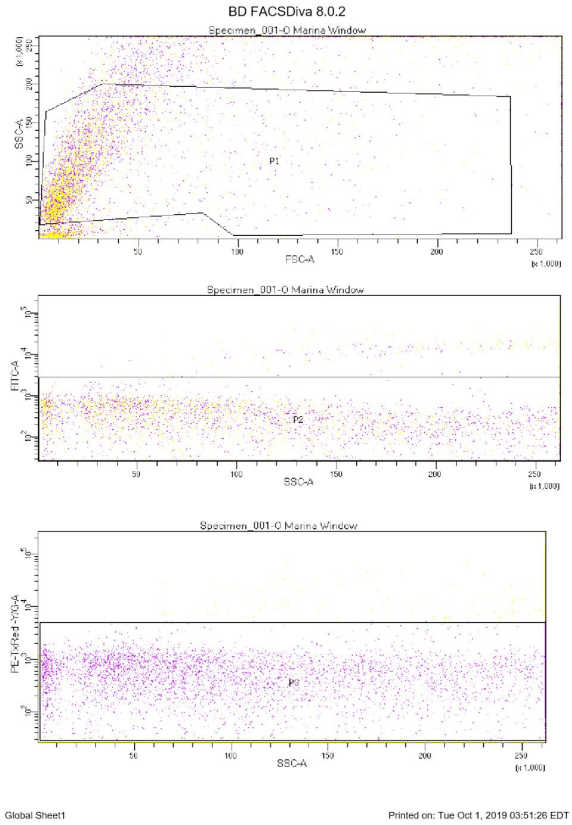
Name:Jessica, Jiayi Lan, Yujie Huang, Shakera
Date: 10/01/19
Goal:

1. Use the flow cytometer to take the measurements of O. Marina and Symbiodinium (including window part, hood part, transformed GFP part, and transformed RFP part).
2. Use the microscope to observe the cells states.

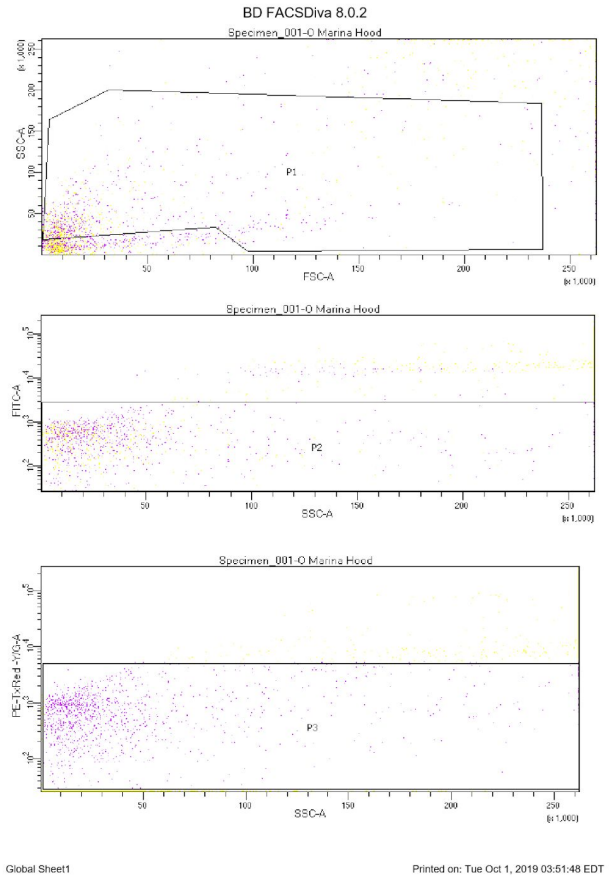
Results:

Order:

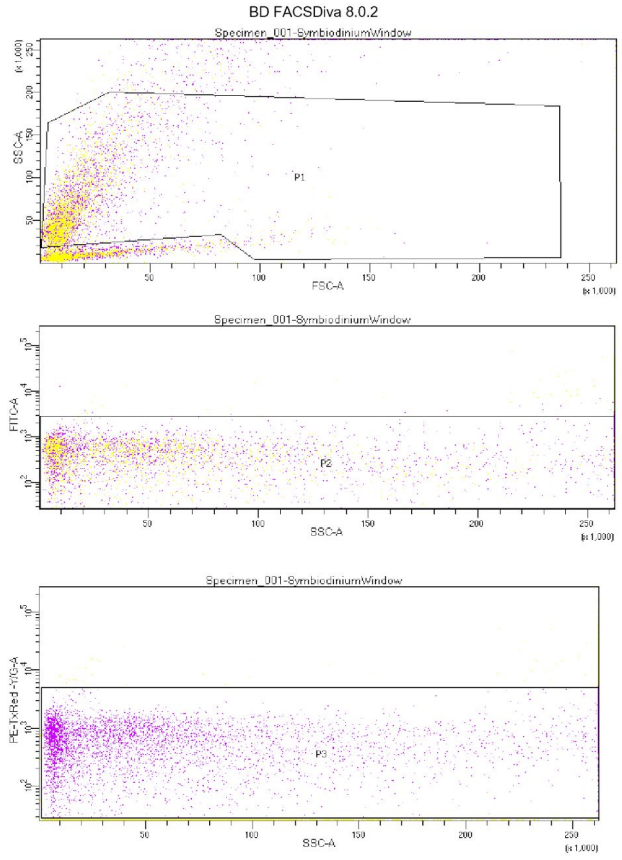
1. O.Marina incubated near the window.



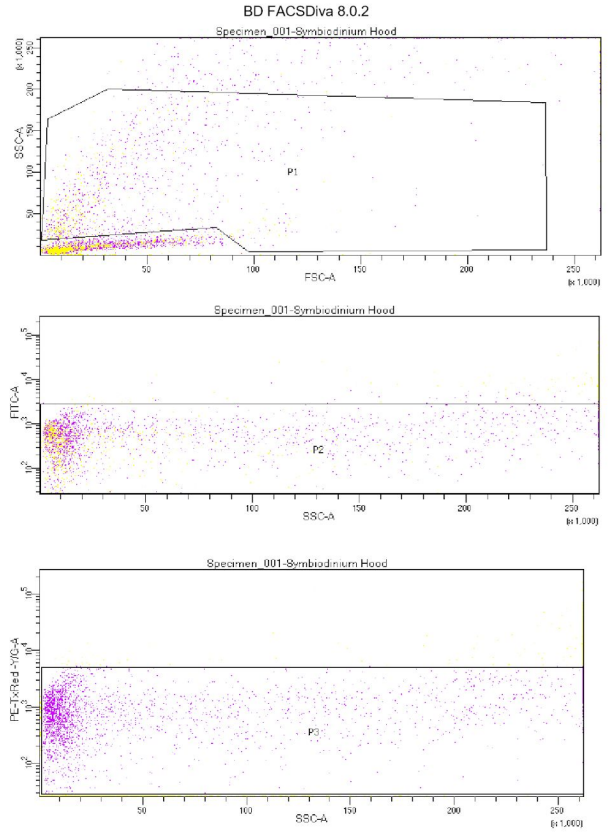
2. O.Marina incubated in the hood.



3. Symbiodinium incubated near the window.



4. Symbiodinium incubated in the hood.



Global Sheet1

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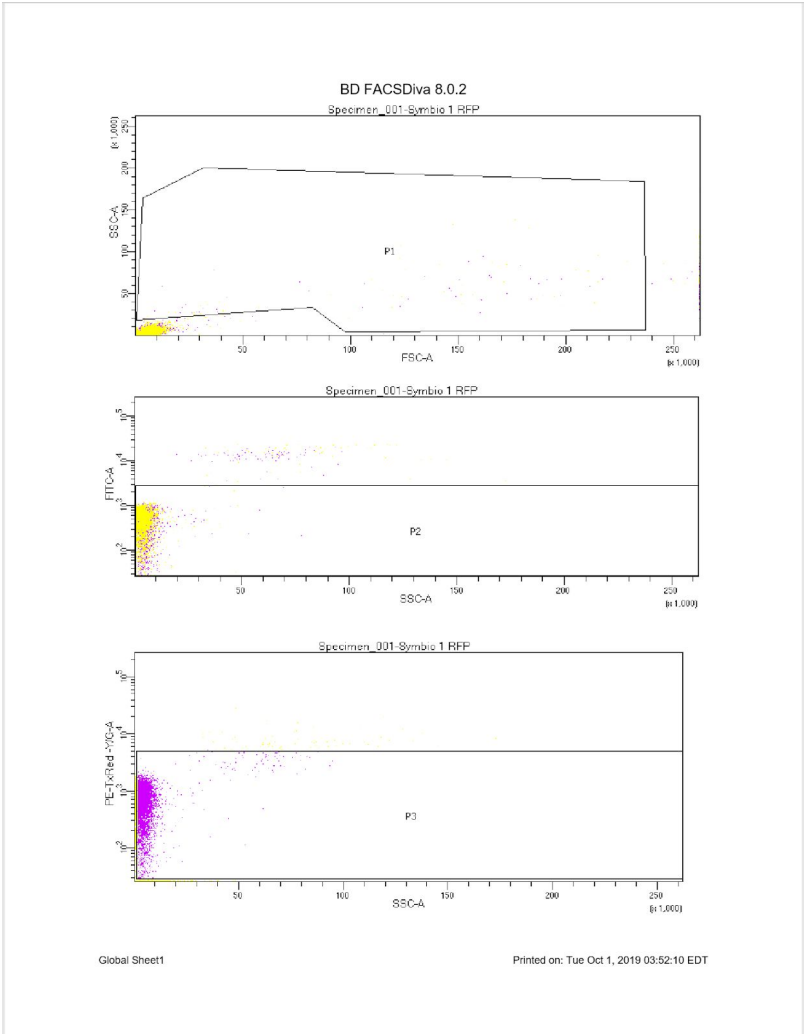
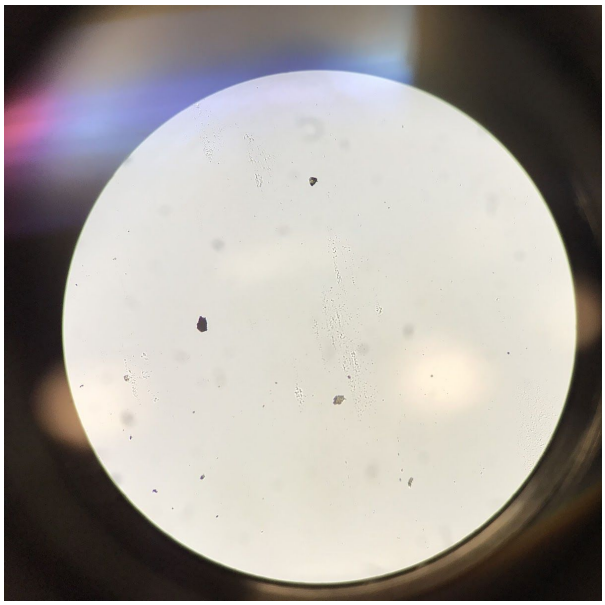
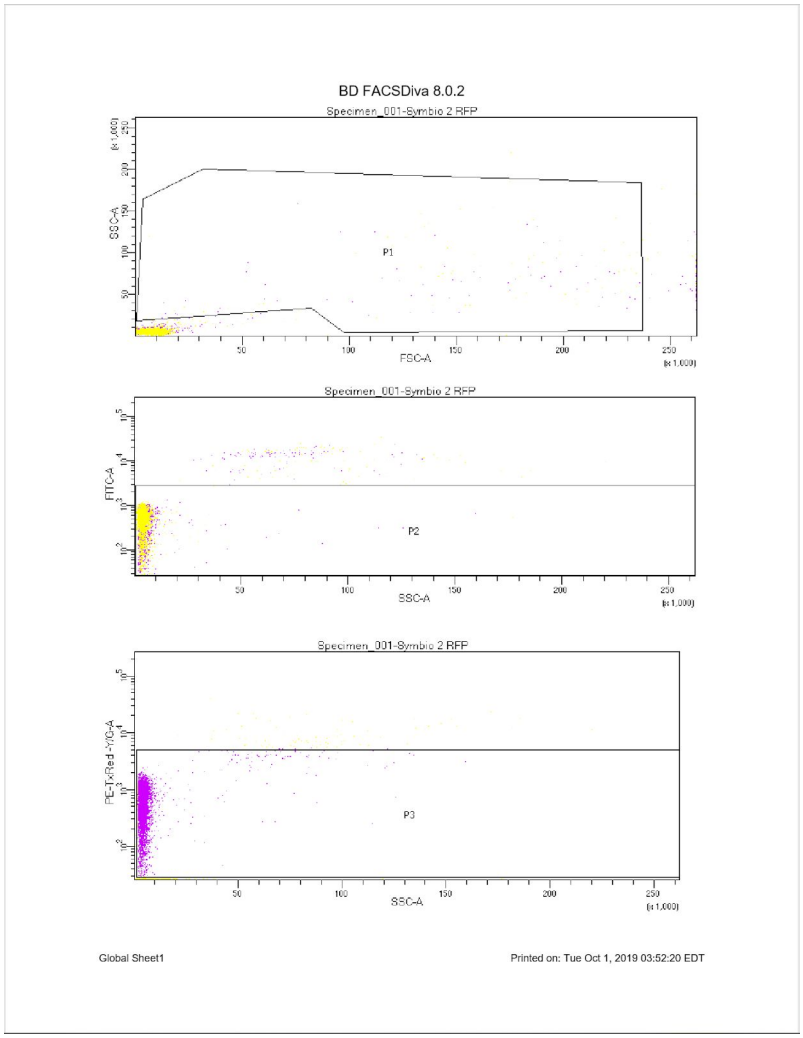
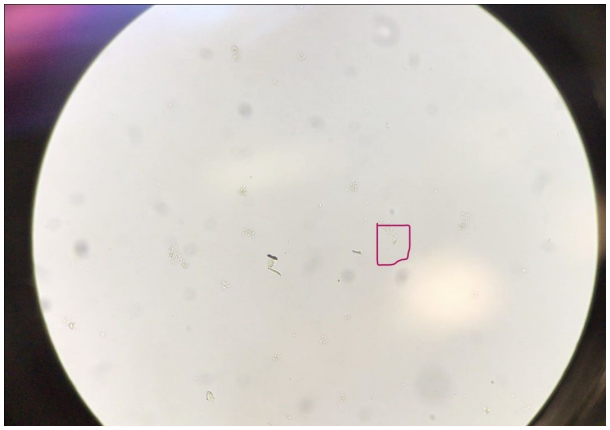
5. Symbiodinium transformed with Dino RFP-1.



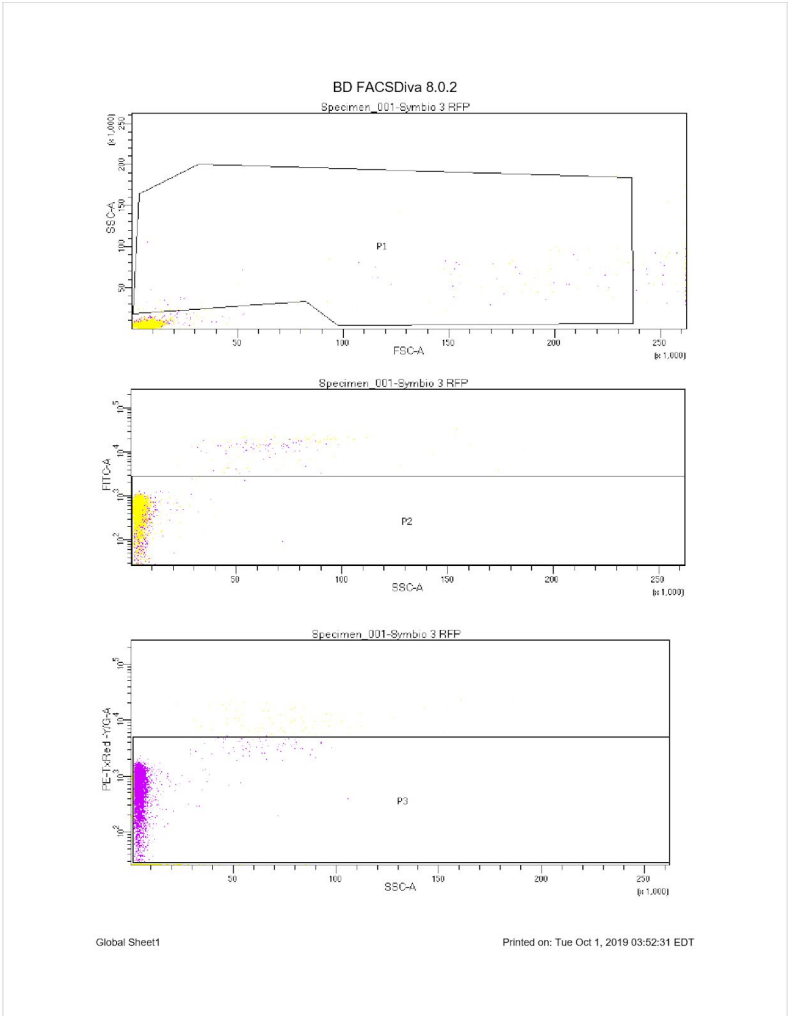


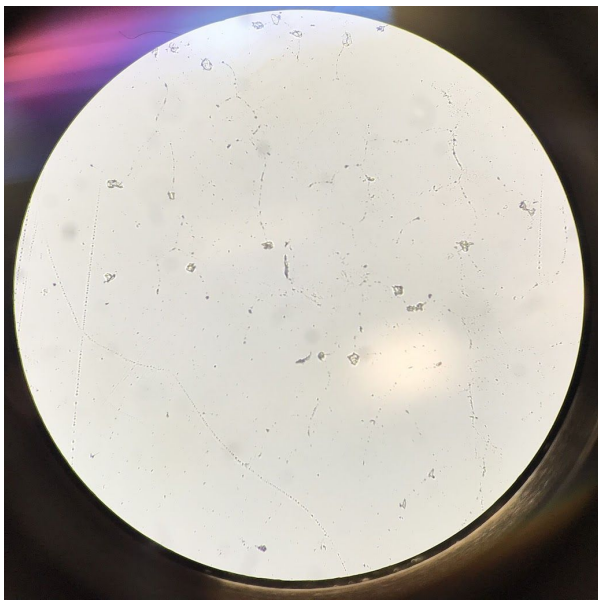
6. Symbiodinium transformed with Dino RFP-2.



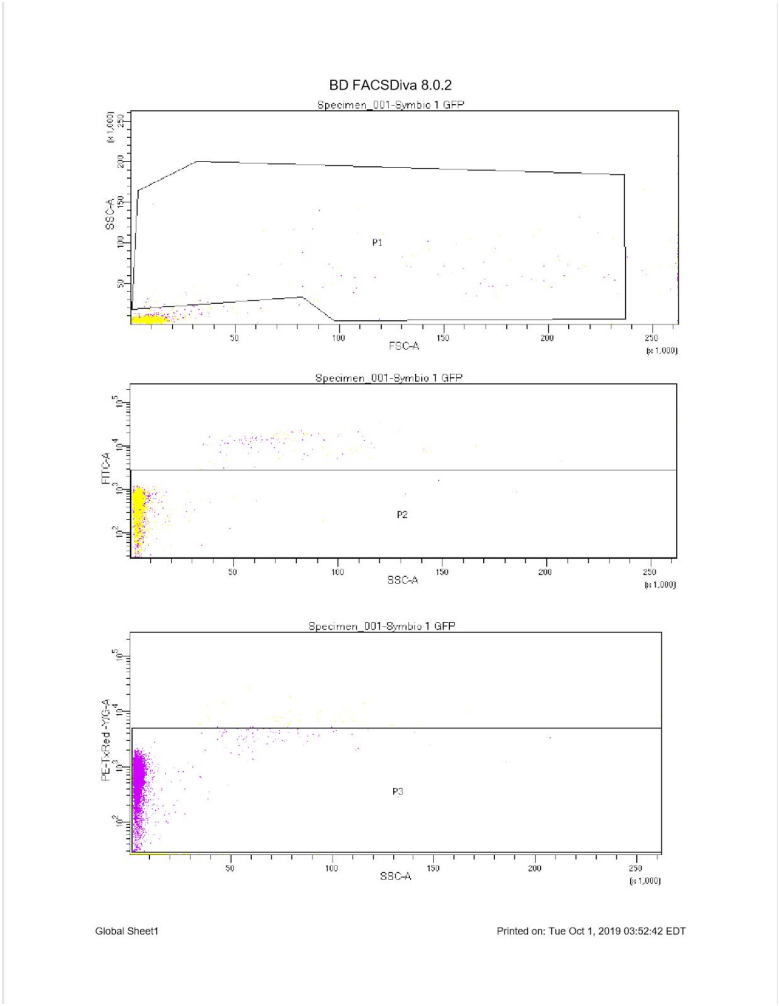


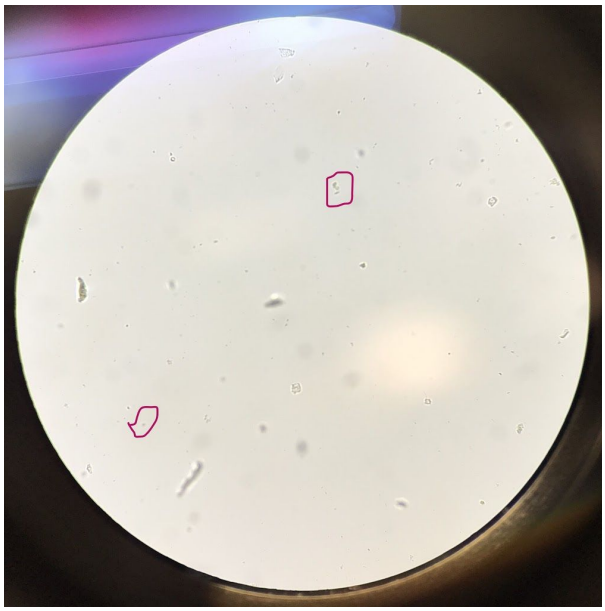
7. Symbiodinium transformed with Dino RFP-3.



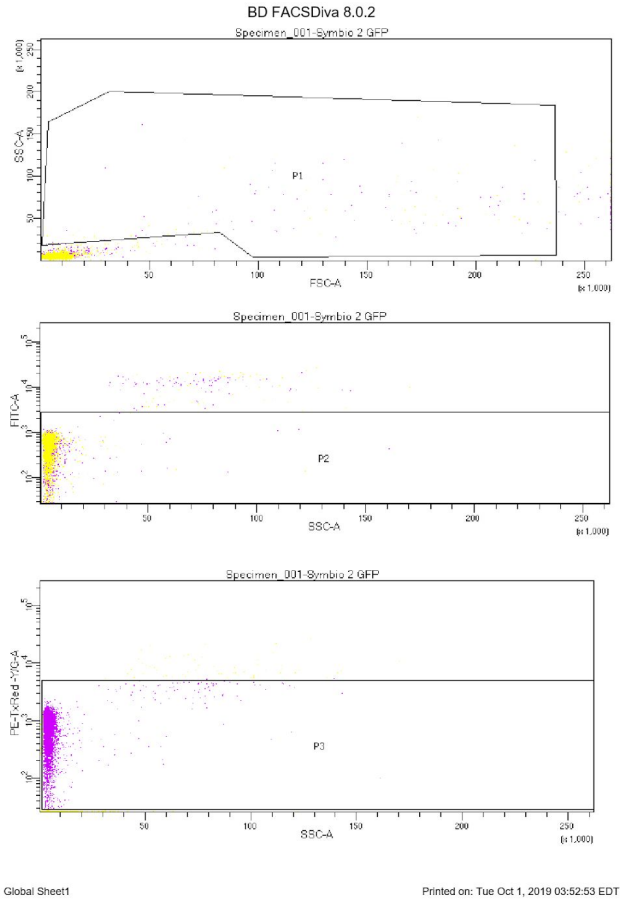


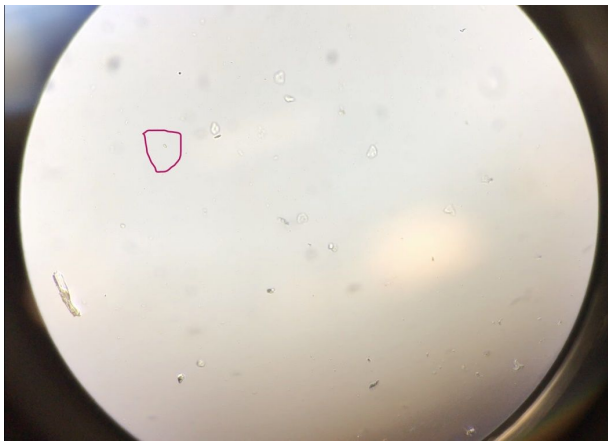
8. Symbiodinium transformed with Dino GFP-1.





9. Symbiodinium transformed with Dino GFP-2.





10. Symbiodinium transformed with Dino GFP-3.