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Supplemental Materials

for

The CURE for Cultivating Fastidious Microbes

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Table S1

Week	Spring 2016		Fall 2016	
	Topic	**Specific Activities/Assignments**	**Topic**	**Specific Activities/Assignments**
#1	Meet & greet; Basic intro to research question	Connon & Giovannoni (2002)[a] paper introduced	Meet & greet; Basic intro to research question	Connon & Giovannoni (2002)[a] paper introduced
#2	Expt. design; pipetting & sterile technique; scientific record-keeping	Dilute and inoculate seawater**	Background; How to read research paper; Expt. Design & Inoculation	(1) Discuss scientific papers (what info is in what section); (2) Dilute and inoculate seawater**; (3) Scientific papers HW assigned; (4) Assign Henson et al (2016)[c] for reading
#3	How to read scientific literature	(1) Work alongside with Connon & Giovannoni paper; (2) Work through Vartoukian et al (2010)[b] paper as a group after initial talk; (3) Scientific papers HW assigned	**Labor Day Holiday*** *Students are encouraged to observe how flow cytometry works	
#4	**Mardi Gras Holiday*** *Students are encouraged to observe how flow cytometry works		Experimental Design; Transfer and cryostock	(1) Transfer isolates that have grown**; (2) Prepare cryostocks**; (3) Understand methods used; (4) Research paper HW due
#5	Transfer positives, create cryostocks & experimental design	(1) Transfer isolates that have grown**; (2) Prepare cryostocks**; (3) Understand methods used; (4) IWA-1 assigned; (5) Research paper HW due	Transfers & Social media, the lighter side of Tumblr	(1) Transfer more isolates that have grown during the additional week; (2) Live tweet pictures of transfers; (3) Lightning talk demonstration; (4) FWA-1 assigned
#6	Scientific Writing	(1) Evaluate excerpts of good and not-so-good introduction & methods sections; (2) "Order a scientific paper" activity; (3) Sign up for weekend boat trip; (4) FWA-1 assigned; (5) IWA-1 due	Lightening talks and Live Tweeting	Student groups give 5-min summary talks on papers of their choice (chosen from a pool prepared by the instructor)
#7	DNA extraction	Perform DNA extraction from isolates**	DNA extraction	Perform DNA extraction from isolates**
#8	PCR; primer choice & design	(1) PCR of 16S rRNA genes from isolates; (2) FWA-1 due; (3) IWA-2 assigned	PCR	(1) PCR of 16S rRNA genes from isolates; (2) In-class writing 1 (create a blog post with Instagram video; based on methods)
#9	Gel electrophoresis (all sections); PCR (1 section for which PCR failed)	(1) Gel electrophoresis to confirm PCR amplicons (all sections); (2) PCR of isolates (for the section where PCR failed last week); (3) FWA-2 assigned; (4) IWA-2 due	Gel electrophoresis & poster design	(1) Gel electrophoresis to confirm PCR amplicons
#10	**Spring Break*** *Instructors confirmed PCR products from the section that did not get to perform gel electrophoresis. All successful amplicons were sequenced this week.		PCR* *PCR for all sections had failed. PCRs from this week were confirmed by instructors and sequenced.	(1) FWA-1 due; (2) FWA-2 assigned (includes reflective blog from visit to microscopy or research cruise)
#11	BLAST intro; Identify your microbe	(1) Understand how BLAST works; (2) Analyze the DNA sequence to identify your microbe; (3) HW on poster critique assigned[d]	Microscopy & BLAST	(1) Understand how BLAST works; (2) Analyze the DNA sequence to identify your microbe; (3) Microscopy center tour/view TEM of student cultures; (4) ICW-2 (blog post with primary results)
#12	Elements of poster design; Poster development & critique	(1) Design rough drafts of posters; (2) Peer poster critique session; (3) Poster critique assignment due; (4) FWA-2 due	Poster development & critique	(1) Design rough drafts of posters; (2) Peer poster critique session; (3) FWA-2 due
#13	Microscopy facility tour & Final Exam Review	(1) Microscopy center tour/view TEM of student cultures; (2) Final review Q&A session	Final Exam Review	(1) Final review Q&A session; (2) End-of-semester reflections; (3) Poster critique assignment[d] due by the end of class
#14	Final Exam		Final Exam	
#15	Poster Presentations	(1) Present your poster electronically (as a group); (2) Peer evaluation & end-of-semester reflections	Poster Presentations	(1) Present your poster electronically (as a group); (2) Peer evaluation

[a] Connon, S.A. and Giovannoni S.J., *High-Throughput Methods for Culturing Microorganisms in Very-Low-Nutrient Media Yield Diverse New Marine Isolates.* Applied And Environmental Microbiology, 2002. **68**(8): p. 3878–3885.

[b] Vartoukian, S.R., *et al*., *Strategies for culture of 'unculturable' bacteria.* Applied And Environmental Microbiology, 2010. **309**(1): p. 448-455.

[c] Henson, M.W., *et al. Artificial Seawater Media Facilitate Cultivating Members of the Microbial Majority from the Gulf of Mexico*. mSphere, 2016, **1**(2): e00028-16

[d] Details on this assignment are provided in Bakshi, A., *et al*., *A Highly Scalable General Framework for Implementing Course-based Undergraduate Research Experiences (CUREs) in Freshman Biology Labs.* American Biology Teacher, 2016. **78**(6): p. 1-7.

**BSL2 laboratory protocols required.

Abbreviations: PCR = polymerase chain reaction; HW – homework; FWA-1 & -2 = Formal Writing Assignment 1 & 2; IWA-1 & -2 = Information Writing Assignment 1 & 2; ICW-1 & -2 = In-Class Writing 1 & 2

Appendix 1. Medium recipe

The MWH1 artificial seawater medium is comprised of multiple components that are made separately and combined in the final recipe: Basic salts, Mg/Ca stock, Iron stock, P mix, AA mix, FA mix, Misc mix, Inorganic N mix, Trace metals, and Vitamins. Below we detail how to make each mix/stock, and how these are combined in the final medium. For all solutions we use acid-washed Pyrex screw-capped bottles. Note, besides the basic salts, which are made fresh for each batch of medium, the stocks and mixes can be maintained at 4˚C for additional uses. We recommend remaking the stocks and mixes every 2-3 months to avoid contamination.

Basic salts
In 948 mL deionized, MilliQ-filtered water, dissolve the following salts: NaCl (23.84 g), KCl (0.746 g), $NaHCO_3$ (0.84 g), Na_2SO_4 (4.27 g), NaBr (0.082 g), H_3BO_3 (0.026 g), $SrCl_2$ (0.014 g), and NaF (0.002 g).

Mg/Ca stock (20x)
In 100 mL deionized, MilliQ-filtered water, dissolve $MgCl_2$ x 6 H_2O (21.2 g) and $CaCl_2$ x 2 H_2O (3.04 g). Autoclave.

Iron stock (1,000x)
In 100 mL deionized, MilliQ-filtered water, dissolve $FeSO_4$ x 7 H_2O (0.0028 g) and Nitrilotriacetic acid (NTA) disodium salt (0.0081 g). Filter sterilize (0.2 µm).

P mix (1,000x)
In 100 mL deionized, MilliQ-filtered water, dissolve orthophosphate (0.0022 mL) and KH_2PO_4 (0.068 g). Filter sterilize (0.2 µm).

AA mix (50,000x)
This can be purchased from Sigma Aldrich (Cat #M5550). Filter sterilize (0.2 µm).

FA mix (2,000,000x)
Combine the following: EtOH (54.86 mL), octanoic acid (15.84 mL), decanoic acid (17.26 g), isobutyric acid (9.27 mL), butyric acid (9.14 mL), and valeric acid (10.88 mL). Filter sterilize (0.2 µm).

Misc mix (20,000x)
In 100 mL deionized, MilliQ-filtered water, dissolve L-glutamine (0.146 g), dextrose (0.180 g), D-ribose (0.150 g), sodium pyruvate (0.110 g), sodium citrate (0.294 g), oxaloacetic acid (0.132 g), Sodium acetate (0.082 g), Sodium succinate (0.162 g), alpha-ketoglutaric acid (0.168 g), urea (0.606 g), glycerol (0.074 mL), glycine betaine (0.154 g), choline (0.140 g), sodium thiosulfate (0.158 g), cyanate (0.003 g), DMSO (0.056 mL), and DMSP (0.011 g). Filter sterilize (0.2 µm).

Inorganic N mix (2,000x)
In 100 mL deionized, MilliQ-filtered water, dissolve sodium nitrate (0.646 g), sodium nitrite (0.028 g), and ammonium chloride (0.053 g). Filter sterilize (0.2 µm).

Trace metals (100,000x)
In 100 mL deionized, MilliQ-filtered water, dissolve $MnCl_2$ x 4 H_2O (0.018 g), $ZnSO_4$ x H_2O (0.002 g), $CoCl_2$ (0.001 g), Na_2MoO_4 (0.001 g), Na_2SeO_3 (0.002 g), $NiCl_2$ (0.001 g). Filter sterilize (0.2 µm).

Vitamins (100,000x)
In 100 mL deionized, MilliQ-filtered water, dissolve thiamine (1.69 g), riboflavin (0.003 g), niacin (0.985 g), pantothenate (1.013 g), pyridoxine (1.028 g), biotin (0.010 g), folic acid (0.018 g), B12 (0.010 g),

myo-inositol (0.901 g), and 4-aminobenzoic acid (0.823 g). Filter sterilize (0.2 µm). Wrap container in foil to avoid photodegradation of vitamins.

Final recipe
In a biosafety cabinet, add the following to the basic salts mix while stirring:
50 mL Mg/Ca stock
1 mL Iron stock
1 mL P mix
20 µL AA mix
0.5 µL FA mix
50 µL Misc mix
0.5 mL Inorganic N mix
10 µL Trace metals
10 µL Vitamins

While still in the biosafety cabinet, filter sterilize (0.1 µm) the entire medium into a sterile container. Check medium pH, which should be ~8.2-8.3. Wrap with foil. Store at room temperature prior to dispensing.

For additional media recipes that use modifications of the basic salts solution, see:

Henson, Michael W., V. Celeste Lanclos, Brant C. Faircloth, and J. Cameron Thrash. (2018) *Cultivation and genomics of the first freshwater SAR11 (LD12) isolate.* The ISME Journal. AOP.

Appendix 2. Flow cytometry parameters

This protocol assumes that users have received the proper training in flow cytometry and understand how to use their equipment. The following parameters are used with the Guava easyCyte 5HT (Millipore) flow cytometer for enumeration:

Gain settings

Forward scatter	1
Side scatter	2.83
Green fluorescence	4.56
Yellow fluorescence	8
Red fluorescence	8

Counting

3,000 events or 90 s.

Controls

Negative: sterile MWH1 medium
Positive: MWH1 medium with a common marine bacterial heterotroph (e.g., LSUCC0096).

Additional details and gating examples can be found in:

Thrash, J. Cameron, Jessica Lee Weckhorst, and David M. Pitre. (2015) *Cultivating Fastidious Microbes*. In Hydrocarbon and Lipid Microbiology Protocols, vol. 4 (*Cultivation*). Edited by Terry J. McGenity, Kenneth N. Timmis and Balbina Nogales.

Appendix 3.

INOCULATION PROTOCOL

Please follow BSL2 protocols and observe good sterile technique!

1. Wipe your pipets down with a small amount of bleach solution on paper towels.

2. Record your group number, plate number, and well numbers (14 total) in your lab notebook. Also be sure to record the concentration of cells in the seawater sample (*X cells/mL*)

3. Using the $C_1V_1 = C_2V_2$ equation, calculate the amount of seawater and media you must add to achieve a concentration of **1 cell/µL.**

4. Collect a tube of seawater, sterile media, and an empty tube. MAKE SURE YOU KNOW WHICH TUBE CONTAINS WHAT!!!

Each group must take turns performing steps 5-10 in the biosafety cabinet. Wear gloves when performing these steps, and keep the 96-well plate inside the cabinet. Close the plate as quickly as you can to avoid introducing contaminants.

5. Pipet out the required volume of **media**. *Close pipet tip boxes right away!*

6. Open the **empty** tube and dispense the media.

7. Pipette required volume of **seawater** into the same tube.

8. Close the top of the empty tube and shake for a few seconds.

9. Inoculate the first 7 wells (that you were assigned) in the 96-well plate with 5 µL of diluted seawater. *Use new pipet tips EACH time! Discard tips in the trash jar in the hood.*

10. Inoculate the final well you were assigned with 5 µL of sterile media. Place the cover back onto the 96-well plate.

11. Once you are done with your tubes, discard them and wipe your bench down with bleach.

CLEAN UP (check the boxes as you go):

- ☐ Gloves → biohazard autoclave bag
- ☐ Pipets → _____
- ☐ Yellow pipet tips → _____
- ☐ White pipet tips → _____
- ☐ Orange racks w/ dye → _____
- ☐ Strikers → _____

Appendix 4.

TRANSFER & PREPARE CRYOSTOCK

Please follow BSL2 protocols and observe good sterile technique!

1. Gently wipe your pipets as well with a small amount of bleach solution on paper towels.

2. Label your flasks **WITH TAPE** & cryostock tubes **WITH SHARPIE** with the LSUCC. **Record which tubes you transferred!**

Steps 3-7 must be performed within the biosafety cabinet. Read completely before proceeding!

3. Set pipette to **450 µL** (with tip!) before you open the tube containing your positive. *Close pipet tip boxes right away!*

4. Open the positive tube *inside the biosafety cabinet*. Quickly pipet out 450 µL of the culture. Close the tube.

5. Pipet the 450 µL culture into the cryostock tube. It contains 50 µL DMSO in it already. Therefore, your culture will be in 10% DMSO (optimal). **REPEAT STEPS 4-6 (make 2 cryostocks)**

6. Open the positive tube again, and quickly pipet out 200 µL of the culture. Close the tube.

7. Open the flask and pipet the culture into the flask and quickly close the flask again. *Do not touch the pipette tip to sides of flask!!!*

8. Put culture flasks in the incubator. MAKE SURE YOU LABEL THEM CORRECTLY!!!

9. Drop cryostocks tubes in the liquid nitrogen bucket. *With TAs help, place cryostock tubes in appropriate position within storage box.*

10. Wipe down your bench with bleach again and clean up as below.

CLEAN UP (check the boxes as you go):

- ☐ Gloves → biohazard autoclave bag (under the chalkboard)
  Pipets → _____
- ☐ Pipet tips → _____
  Small-tube racks → _____
- ☐ Matches → _____

Appendix 5.

DNA EXTRACTION PROTOCOL

Please wear gloves at all times. Follow BSL2 safety protocols until step 6.

1. Prepare the following tubes and label them accordingly before you begin. *If using another group's tube, also write down your own group number (along with whose sample you're using) so you remember that's yours!*
    - 5 mL bead tube (1) – *Label:* group #, sample #
    - 2 mL collection tube (5) – *Label:* group #, sample #, step # (8, 10, 13, 15,16)
    - 2 mL collection tube for DNA (1) – *Label:* group/sample # and date.

2. Filter water samples. *Perform the following steps inside the biosafety cabinet.*
    - Pull off the plunger from the syringe.
    - Using forceps, add the 0.22 μm filter on the filter house and close it off.
    - Screw on the syringe to the filter funnel.
    - Fill up the syringe to ~50 mL with culture solution. Repeat until empty.
    - Using the plunger, push the liquid through into a beaker.
    - Save filter membrane for step 2. Throw the syringe away and place filter housing in ethanol.

    What's happening: Micro-organisms should now be trapped on top of and within the filter membrane.

3. *Perform the following steps inside the biosafety cabinet.* Clean your forceps with ethanol and wipe with paper towels. Then insert the filter into the 5 ml PowerWater® Bead Tube.

    What's happening: Loosely rolling and inserting the filter membrane into the PowerWater® Bead Tube allows for efficient bead beating and homogenization in proceeding steps.

4. *Perform the following steps inside the biosafety cabinet.* Add **1 ml of Solution PW1 (after removing from 55°C water bath)** to the PowerWater® Bead Tube.

    What's happening: Solution PW1 is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic material. It is also part of the patented Inhibitor Removal Technology® (IRT). When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution PW1 should be used while it is still warm.

5. Put **5 mL tubes** in the incubator (set at **50°C**) for **5 minutes**.

6. Then **vortex** for **5 minutes**.

    What's happening: The mechanical action of bead beating will break apart the surface of the

filter membrane that contains trapped cells and aids in cell lysis.

7. Centrifuge the tubes **≤ 4000 x *g*** for **1 minute** at **room temperature**.

8. Transfer the **supernatant** to a **clean 2 ml Collection Tube**. Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.

   What's happening: The cells have now broken apart and all their contents are floating in solution. The supernatant contains your DNA and all other cell components. By pipetting it off, the supernatant is separated and removed from the filter membrane and beads at this step.

9. Centrifuge at **13,000 x *g*** for **1 minute**.

   What's happening: Any remaining beads, proteins, and cell debris are removed at this step. This step is important for removal of any remaining contaminating non-DNA organic and inorganic matter that may reduce the DNA purity and inhibit downstream DNA applications.

10. Avoiding the pellet, transfer the supernatant to a **clean 2 ml Collection Tube**.

11. Add **200 µl of Solution PW2** and vortex briefly to mix. Incubate at **4°C** for **5 minutes**.

    What's happening: Solution PW2 is another part of the patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

12. Centrifuge the tubes at **13,000 x g** for **1 minute**.

13. **Avoiding the pellet**, transfer the supernatant to a **clean 2 ml Collection Tube**.

    What's happening: The pellet at this point contains additional non-DNA organic and inorganic material (cell debris and junk). For best DNA yields and quality, avoid transferring any of the pellet.

14. Add **650 µl of Solution PW3** and **vortex** briefly to mix.

    What's happening: Solution PW3 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations this will adjust the DNA solution salt concentration to allow binding of the DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the spin filter.

15. Prepare a **clean 2 mL Collection Tube** with a **Spin Filter** inside it. Load **650 µl** of supernatant onto a **Spin Filter**. Centrifuge at **13,000 x *g*** for **1 minute**. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.

    What's happening: The DNA is selectively bound to the silica membrane in the Spin Filter basket and the flow through containing non-DNA components is discarded.

16. Place the Spin Filter basket into a **clean 2 ml Collection Tube**.

    What's happening: Due to the high concentration of salt in solution PW3, it is important to place the Spin Filter basket into a clean 2 ml Collection Tube to aid in the subsequent wash steps and improve the DNA purity and yield.

17. Shake to mix Solution PW4 before use. Add **650 µl of Solution PW4** and centrifuge at **13,000 x *g*** for **1 minute**.

    What's happening: Solution PW4 is an alcohol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica membrane.

18. Discard the flow through and place spin filter back in the tube. Add **650 µl of Solution PW5** and centrifuge at **13,000 x *g*** for **1 minute**.

    What's happening: Solution PW5 ensures complete removal of Solution PW4 which will result in higher DNA purity and yield.

19. Discard the flow through and place spin filter back in the tube. Centrifuge again at **13,000 x *g*** for **2 minutes** to remove residual wash.

    What's happening: The second spin removes residual Solution PW5. It is critical to remove all traces of wash solution because the ethanol in Solution PW5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

20. Place the Spin Filter basket into a **clean 2 ml Collection Tube**.
    - This is the tube where your DNA will be stored. Label it appropriately with the group/sample number and date. If you're using another group's number, also put your group number on it so you remember that's yours!

21. Add **50 µl of Solution PW6** to the center of the white filter membrane.

    What's happening: Placing Solution PW6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution PW6 passes through the silica membrane, the DNA that was bound in the presence of high salt is selectively released by Solution PW6 (10 mM Tris) which lacks salt.

22. Centrifuge at **13,000 x *g*** for **1 minute**.

23. Discard the Spin Filter basket. The DNA is now ready for any downstream application.

24. Place your tube of DNA into a rack (back of the room) to keep frozen until we use it next week.

“What’s happening” steps above taken from the MoBio package insert (https://mobio.com/media/wysiwyg/pdfs/protocols/14900-S.pdf)

Appendix 6. PCR protocol

Excepting the Taq, thaw all components and place on ice once thawed (keep Taq on ice for the entire process). Assemble a master mix (all components in the table below except the template DNA), scaled according to the number of PCR wells that will be utilized, keeping the master mix tube on ice. Dispense 49 µL master mix per PCR tube. Add 1 µL template DNA solution (see DNA extraction protocol), snap lids in place, flick gently to mix, vortex briefly, and place in the thermocycler.

Component	Volume (µL)	Final concentration
10X PCR buffer	5	1X
50 mM $MgCl_2$	1.5	1.5 mM
10 mM dNTPs	1	0.2 mM each
10µM forward primer	2.5	0.5 µM
10µM reverse primer	2.5	0.5 µM
Template DNA	1	Depends on initial DNA concentration
Taq DNA polymerase (5U/µL)	0.2	1U* *U = "unit": a special unit for describing concentration of enzymes
Distilled water	36.3	
Total volume	**50**	

PCR thermocycler parameters assuming the 27F/1492R primers:

1. 94˚C for 3 minutes
2. 35 cycles of:
   - 94˚C for 30 seconds
   - 50.8˚C for 30 seconds
   - 72˚C for 2 minutes
3. 72˚C for 10 minutes,
4. Hold at 10˚C.

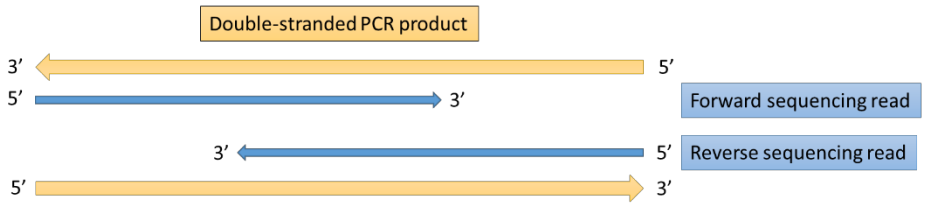
Remove and store tubes at 4˚C until imaging with gel electrophoresis.

Appendix 7.

SEQUENCE ANALYSIS USING BLAST

I. Retrieve the forward and reverse sequencing reads.

Sanger sequencing works on both DNA strands going in anti-parallel directions. Therefore you get two sequencing reads from sequencing both strands.

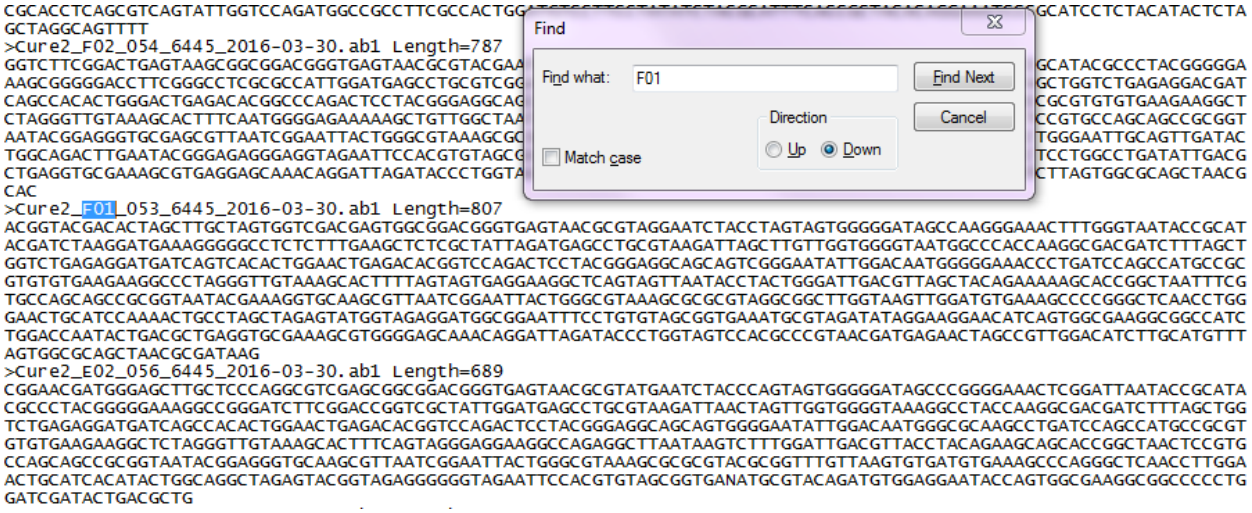


The read that goes 5'→ 3' in a left → right direction is called the forward read. The one that goes 5' → 3' in a right → left direction is called the reverse read.

A. Find the labels for the forward and reverse sequencing reads for your sample (based on LSUCC#) from the "Sequence Sheet" excel file.
I have used LSUCC3021 (highlighted) as an example for the demo.

LSUCC#	Forward Read	Reverse Read	Closest BLAST hit	Top hit % ID	Gen Bank #
3005	A01	A03			
3011	B01	B03			
3012	C01	C03			
3016	D01	D03			
3018	E01	E03			
3021	F01	F03			
3025	G01	G03			
3027	H01	H03			
3028	A02	A04			
3030	B02	B04			
3033	C02	C04			
3040	D02	D04			
3042	E02	E04			
3040	F02	F04			

B. Search (Ctrl+F on Windows or Command+F on Mac) the label (F01 and F03, individually) in the "CURE1208Seqs.txt" file.



C. Copy and paste the forward and reverse reads into a separate text file along with the header line for that sequence (found above the sequence itself) that starts with a ">" symbol. *The header lines are highlight for the sequences below.*

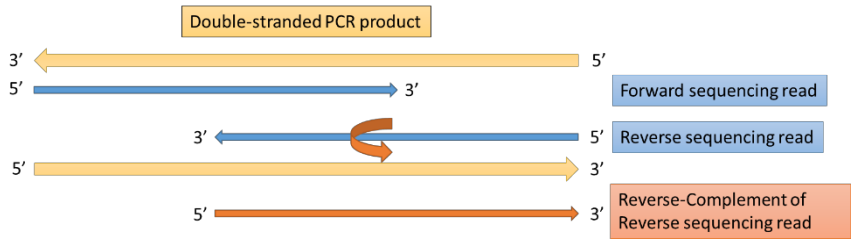
```
>Cure2_F01_053_6445_2016-03-30.ab1 Length=807

ACGGTACGACACTAGCTTGCTAGTGGTCGACGAGTGGCGGACGGGTGAGTAACGCGTAGGAATCTACCTAGTAGTGG
GGGATAGCCAAGGGAAACTTTGGGTAATACCGCATACGATCTAAGGATGAAAGGGGGCCTCTCTTTGAAGCTCTCGC
TATTAGATGAGCCTGCGTAAGATTAGCTTGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCTTTAGCTGGTCTGA
GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTCGGGAATATTGGACAAT
GGGGGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCCTAGGGTTGTAAAGCACTTTTAGTAGTGAGGAA
GGCTCAGTAGTTAATACCTACTGGGATTGACGTTAGCTACAGAAAAAGCACCGGCTAATTTCGTGCCAGCAGCCGCG
GTAATACGAAAGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGCTTGGTAAGTTGGATGTG
AAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGCCTAGCTAGAGTATGGTAGAGGATGGCGGAATTTCCTG
TGTAGCGGTGAAATGCGTAGATATAGGAAGGAACATCAGTGGCGAAGGCGGCCATCTGGACCAATACTGACGCTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCGTAACGATGAGAACTAGCCGTTGGA
CATCTTGCATGTTTAGTGGCGCAGCTAACGCGATAAG


>Cure2_F03_069_6445_2016-03-30.ab1 Length=797

CCACTTCTGGTGCACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTC
TGATTCACGATTACTAGCGATTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGGACTACGACCGGCTTTCTC
GGATTAGCTTCCCCTCGCGGGTTCGCAACCGTTTGTACCGGCCATTGTAGCACGTGTGTAGCCCTACACGTAAGGGC
CATGATGACTTGACGTCGTCCCCACCTTCCTCCGATTTGTCATCGGCAGTCTCCTTAGAGTTCCCGGCCTAACCGCT
GGCAATTAAGGATAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGC
AGCACCTGTCACTCGGTTCCCGAAGGCACCAATCTATCTCTAGAAAGTTCCGAGGATGTCAAGTGTAGGTAAGGTTC
TTCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTG
CGGCCGTACTCCCCAGGCGGAGAACTTATCGCGTTAGCTGCGCCACTAAACATGCAAGATGTCCAACGGCTAGTTCT
CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATTG
GTCCAGATGGCCGCCTTCGCCACTGGATGTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATCCCGCATC
CTCTACATACTCTAGCTAGGCAGTTTT
```

D. Rename the headers to "Forward" and "Reverse" for the correct sequences. *Do NOT lose the ">" symbol that the header line starts with!* Use the "Sequence Sheet" excel file to determine which labels correspond to the forward and reverse reads. *The renamed header lines are highlight below.*

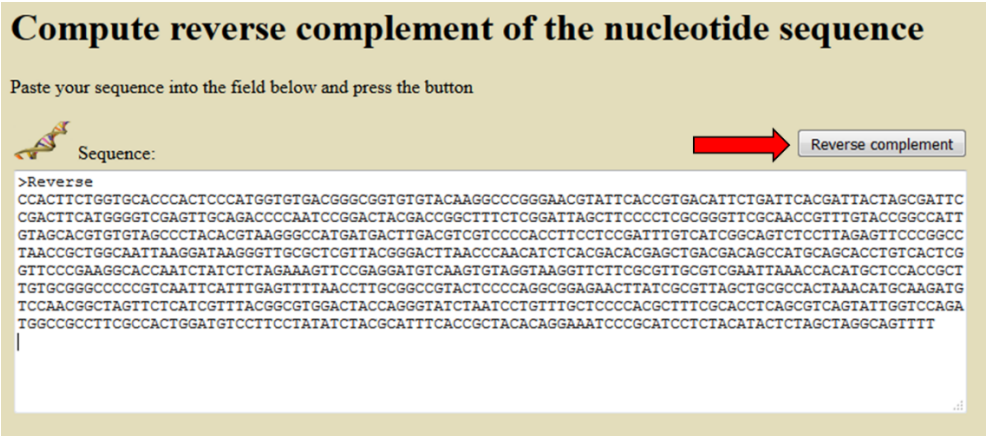
```
>Forward

ACGGTACGACACTAGCTTGCTAGTGGTCGACGAGTGGCGGACGGGTGAGTAACGCGTAGGAATCTACCTAGTAGTGG
GGGATAGCCAAGGGAAACTTTGGGTAATACCGCATACGATCTAAGGATGAAAGGGGGCCTCTCTTTGAAGCTCTCGC
TATTAGATGAGCCTGCGTAAGATTAGCTTGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCTTTAGCTGGTCTGA
GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTCGGGAATATTGGACAAT
GGGGGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCCTAGGGTTGTAAAGCACTTTTAGTAGTGAGGAA
GGCTCAGTAGTTAATACCTACTGGGATTGACGTTAGCTACAGAAAAAGCACCGGCTAATTTCGTGCCAGCAGCCGCG
GTAATACGAAAGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGCTTGGTAAGTTGGATGTG
AAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGCCTAGCTAGAGTATGGTAGAGGATGGCGGAATTTCCTG
TGTAGCGGTGAAATGCGTAGATATAGGAAGGAACATCAGTGGCGAAGGCGGCCATCTGGACCAATACTGACGCTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCGTAACGATGAGAACTAGCCGTTGGA
CATCTTGCATGTTTAGTGGCGCAGCTAACGCGATAAG


>Reverse

CCACTTCTGGTGCACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTC
TGATTCACGATTACTAGCGATTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGGACTACGACCGGCTTTCTC
GGATTAGCTTCCCCTCGCGGGTTCGCAACCGTTTGTACCGGCCATTGTAGCACGTGTGTAGCCCTACACGTAAGGGC
CATGATGACTTGACGTCGTCCCCACCTTCCTCCGATTTGTCATCGGCAGTCTCCTTAGAGTTCCCGGCCTAACCGCT
GGCAATTAAGGATAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGC
AGCACCTGTCACTCGGTTCCCGAAGGCACCAATCTATCTCTAGAAAGTTCCGAGGATGTCAAGTGTAGGTAAGGTTC
TTCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTG
CGGCCGTACTCCCCAGGCGGAGAACTTATCGCGTTAGCTGCGCCACTAAACATGCAAGATGTCCAACGGCTAGTTCT
CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATTG
GTCCAGATGGCCGCCTTCGCCACTGGATGTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATCCCGCATC
CTCTACATACTCTAGCTAGGCAGTTTT
```

II. Reverse Complement the Reverse Read

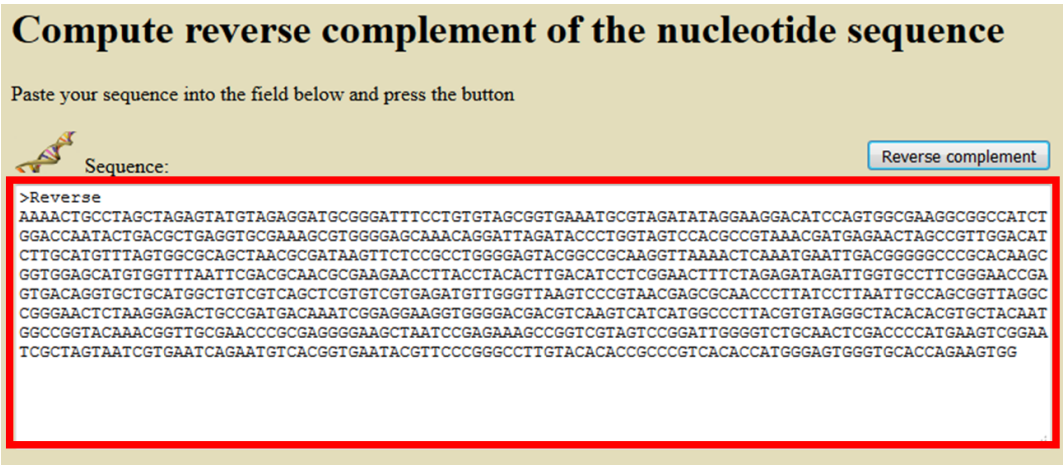
Since the reverse read is in the "opposite" direction of the forward read, they become hard to compare. Here we are trying to get the flip the directionality of the reverse read, so it goes in the same direction as the forward read (5' → 3' in a left → right manner). This makes it easier to compare the two reads.



A. Go to http://reverse-complement.com/ and paste in the "Reverse" read sequence. Then hit "Reverse complement" (*red arrow in screenshot below*).



B. Once you hit, reverse complement, the output is instantaneous and shown in the same box (*red square in screenshot below*) as where you entered the original reverse sequencing read.



C. Copy and paste that output into your text file with the “Forward and “Reverse” sequences, and name this sequence “Rev_RC” (reverse complement of the reverse read). Create the header line in the same way as your other sequences – above the sequence itself and starting with a “>” sign. *See highlighted header lines in screenshot below – the original ones are highlighted yellow; the new one in green.*

>Forward

ACGGTACGACACTAGCTTGCTAGTGGTCGACGAGTGGCGGACGGGTGAGTAACGCGTAGGAATCTACCTAGTAGTGG
GGGATAGCCAAGGGAAACTTTGGGTAATACCGCATACGATCTAAGGATGAAAGGGGGCCTCTCTTTGAAGCTCTCGC
TATTAGATGAGCCTGCGTAAGATTAGCTTGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCTTTAGCTGGTCTGA
GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTCGGGAATATTGGACAAT
GGGGGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCCTAGGGTTGTAAAGCACTTTTAGTAGTGAGGAA
GGCTCAGTAGTTAATACCTACTGGGATTGACGTTAGCTACAGAAAAAGCACCGGCTAATTTCGTGCCAGCAGCCGCG
GTAATACGAAAGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGCTTGGTAAGTTGGATGTG
AAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGCCTAGCTAGAGTATGGTAGAGGATGGCGGAATTTCCTG
TGTAGCGGTGAAATGCGTAGATATAGGAAGGAACATCAGTGGCGAAGGCGGCCATCTGGACCAATACTGACGCTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCGTAACGATGAGAACTAGCCGTTGGA
CATCTTGCATGTTTAGTGGCGCAGCTAACGCGATAAG

>Reverse

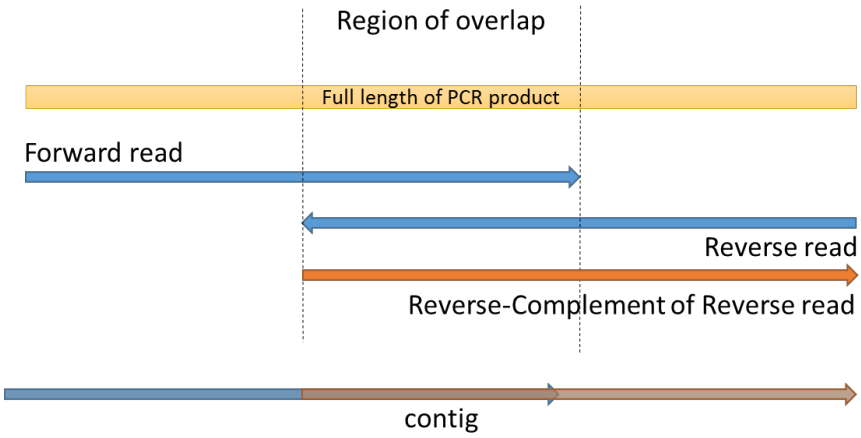
CCACTTCTGGTGCACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTC
TGATTCACGATTACTAGCGATTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGGACTACGACCGGCTTTCTC
GGATTAGCTTCCCCTCGCGGGTTCGCAACCGTTTGTACCGGCCATTGTAGCACGTGTGTAGCCCTACACGTAAGGGC
CATGATGACTTGACGTCGTCCCCACCTTCCTCCGATTTGTCATCGGCAGTCTCCTTAGAGTTCCCGGCCTAACCGCT
GGCAATTAAGGATAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGC
AGCACCTGTCACTCGGTTCCCGAAGGCACCAATCTATCTCTAGAAAGTTCCGAGGATGTCAAGTGTAGGTAAGGTTC
TTCGCGTTGCGTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTG
CGGCCGTACTCCCCAGGCGGAGAACTTATCGCGTTAGCTGCGCCACTAAACATGCAAGATGTCCAACGGCTAGTTCT
CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTATTG
GTCCAGATGGCCGCCTTCGCCACTGGATGTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATCCCGCATC
CTCTACATACTCTAGCTAGGCAGTTTT

>Rev_RC

AAAACTGCCTAGCTAGAGTATGTAGAGGATGCGGGATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGACA
TCCAGTGGCGAAGGCGGCCATCTGGACCAATACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGAGAACTAGCCGTTGGACATCTTGCATGTTTAGTGGCGCAGCTAACGCGAT
AAGTTCTCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGACGCAACGCGAAGAACCTTACCTACACTTGACATCCTCGGAACTTTCTAGAGATAGATTGGT
GCCTTCGGGAACCGAGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAA
CGAGCGCAACCCTTATCCTTAATTGCCAGCGGTTAGGCCGGGAACTCTAAGGAGACTGCCGATGACAAATCGGAGGA
AGGTGGGGACGACGTCAAGTCATCATGGCCCTTACGTGTAGGGCTACACACGTGCTACAATGGCCGGTACAAACGGT
TGCGAACCCGCGAGGGGAAGCTAATCCGAGAAAGCCGGTCGTAGTCCGGATTGGGGTCTGCAACTCGACCCCATGAA
GTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA
CCATGGGAGTGGGTGCACCAGAAGTGG

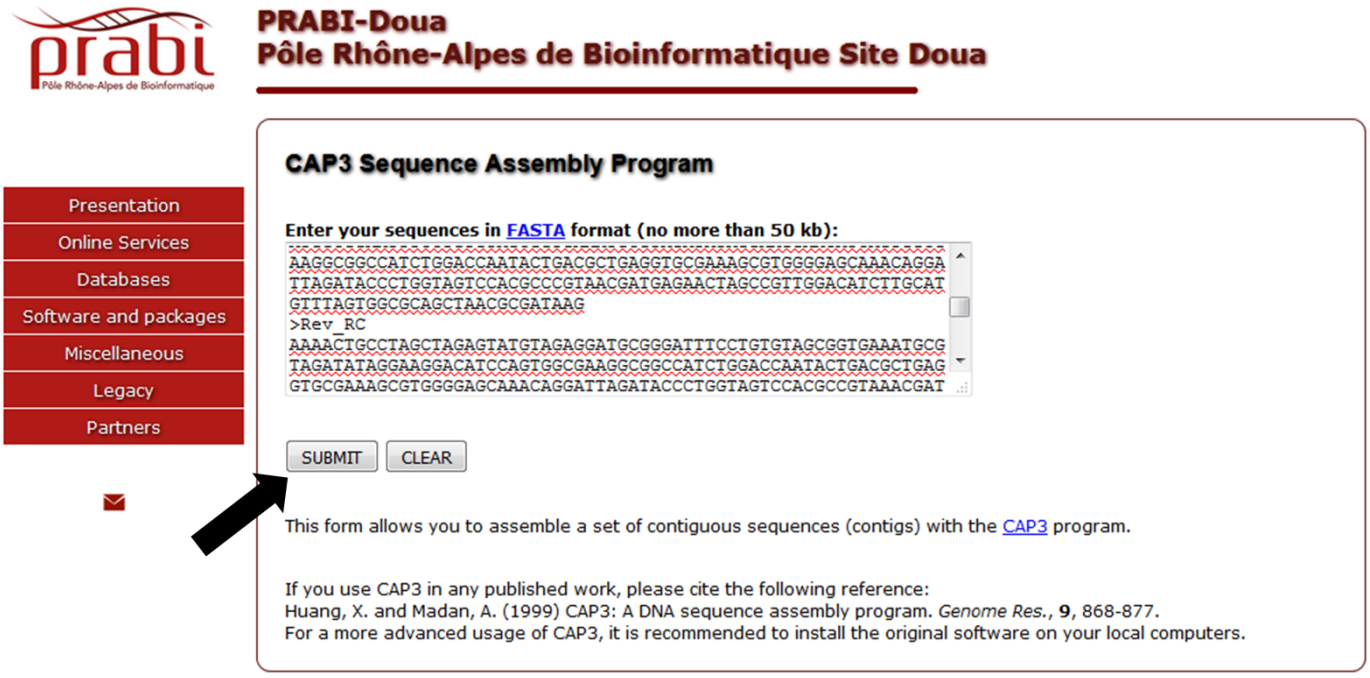
III. Create a contig

The 16s rRNA gene PCR product is 1465 bp long. Sanger sequencing can sequence 700-900 bp starting from opposite directions. If the sequences are long enough, a portion of the gene is covered by both the forward read as well as the reverse read. Due to this partial overlap, we can "join them together" to derive a longer consensus sequence that spans the entire length of the PCR product. This consensus sequence is called a ***contig.***



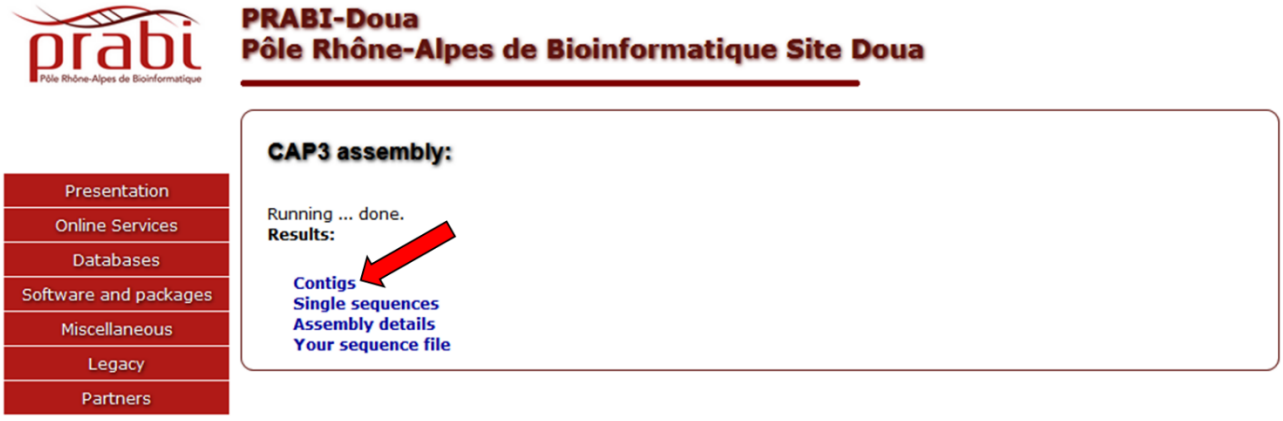
A. Go to http://doua.prabi.fr/software/cap3 (a free online contig assembly software).

B. Paste in your **forward read** and the **reverse-complement of your reverse read** along with the header lines that start with ">" and click SUBMIT.



C. Once your contigs are made, you will see the screen below. Click on "contigs" *(red arrow in screenshot below)*. Once you click it, it will give you the contig sequence with a header line that says ">Contig 1."

If you do not get a contig sequence, it is most likely because your sequencing reads were not long enough and there was not sufficient overlap to generate a contig sequence.



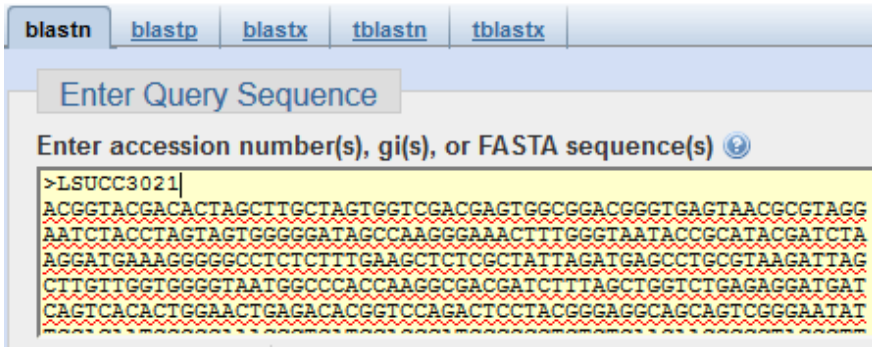
D. Copy and paste that sequence along with your forward, reverse and reverse-complement of the reverse sequences. *If you wish, you may rename the contig header with your LSUCC number. Just be sure to retain the beginning ">" symbol.*

IV. BLAST your sequences

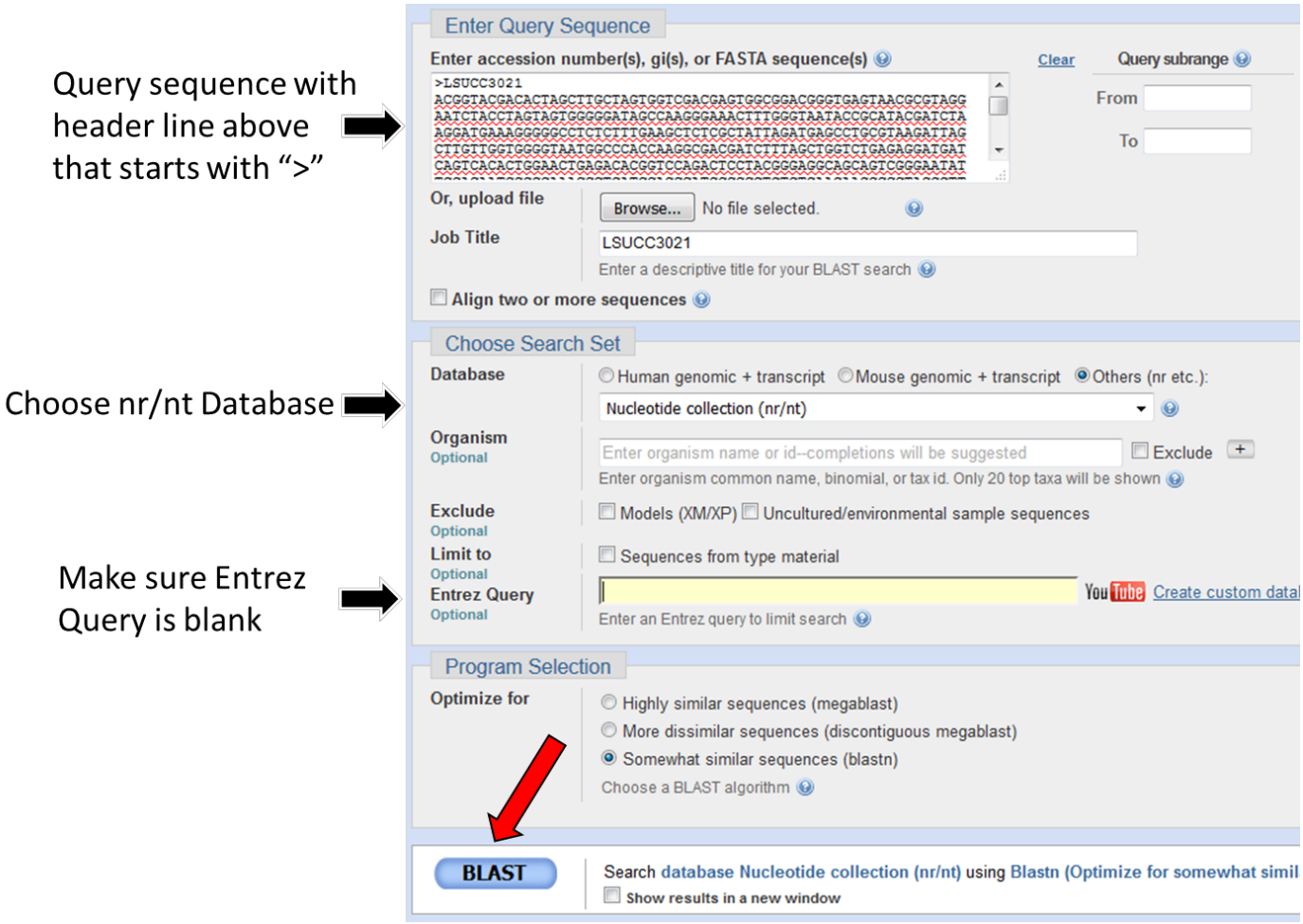
BLAST is a free online tool from NCBI that aligns your sequence to lots of other organism's sequences stored in various databases inside of it. Then it finds which organisms' sequences (in its database) match most closely to the query sequence that you entered. This is the "key" step that will help you identify your organism.

A. Go to NCBI BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Nucleotides&PROGRAM=blastn&BLAST_PROGRAMS=blastn&PAGETYPE=BlastSearch&DATABASE=refseq_rna&DESCRIPTIONS=100&EQ_TEXT=arabidopsis[orgn]&QUERY=8033).

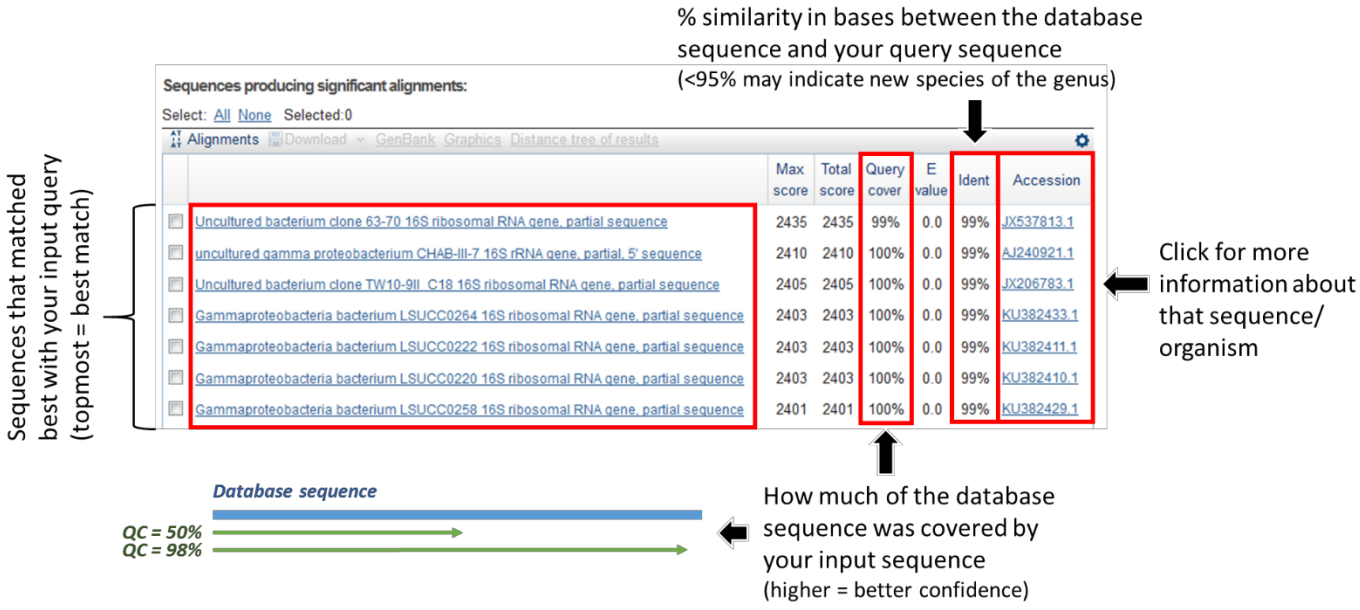
B. Delete any pre-existing value in the "ENTER QUERY SEQUENCE" box and paste in your contig sequence, along with the header line that starts with ">".



C. Choose the following settings (*see below*) and hit BLAST.

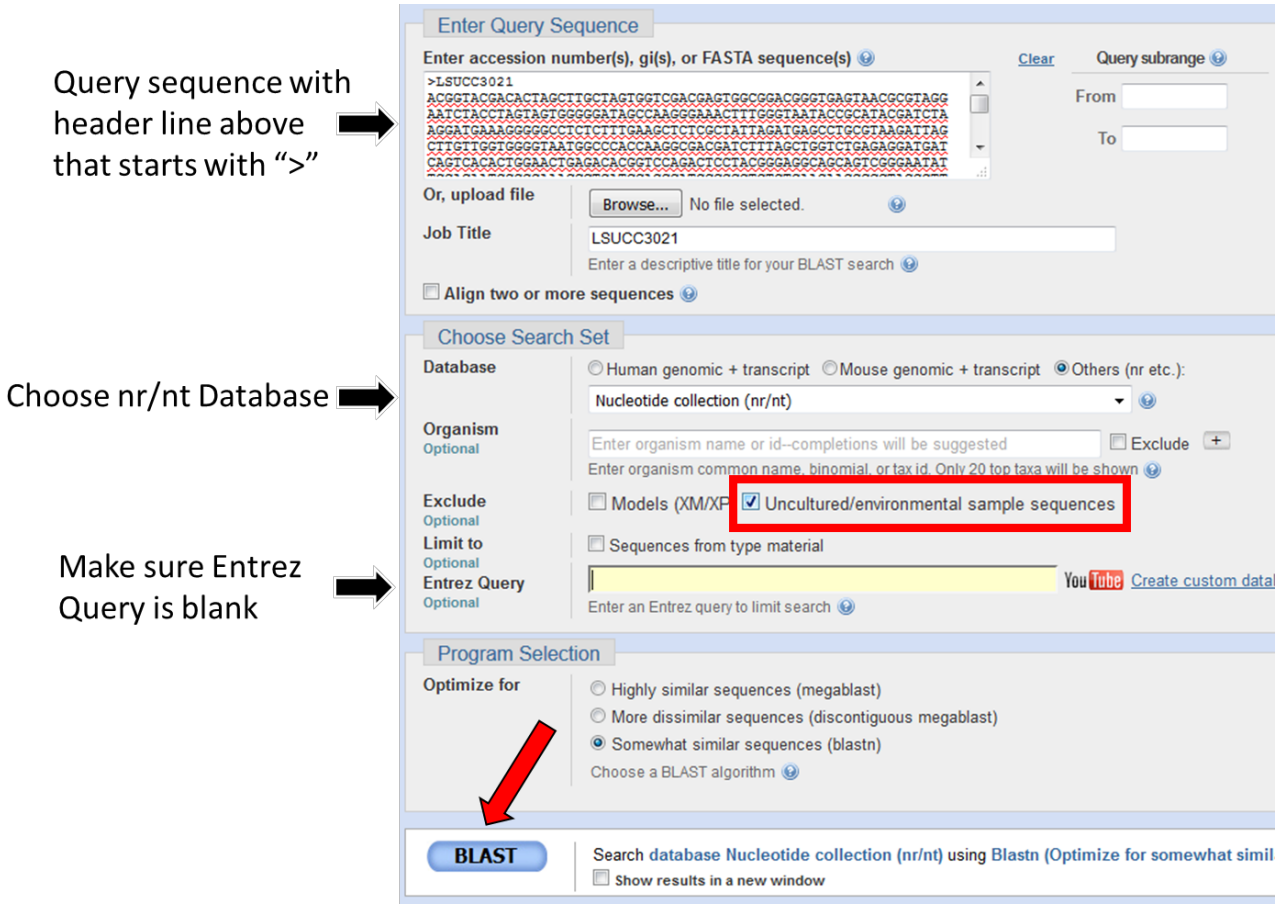


D. Interpret the BLAST output.



E. Record the top 5 BLAST hits, along with their query coverage and %ID.

F. *Sometimes, having uncultured/environmental DNA sequences can hinder our search for an actual organism match.* So, repeat steps A-E, but **EXCLUDE uncultured/ environmental samples sequences** *(red box in screenshot below)*.



G. *The contig is most likely to give us the most reliable results, since it is the longest sequence derived from "joining together" the forward and the reverse-complement of the reverse read. However, you should be able to get the same results with the forward and reverse reads as well.* So **repeat steps A-F (both with and without excluding the uncultured/environmental clones) with the Forward and Reverse reads individually**.

H. Record the top 5 BLAST hits, along with their query coverage and %ID for all 6 BLAST runs. The top hits from all of them should be relatively consistent for you to have good confidence regarding the identity of what you have cultured.

Appendix 8. Suggested scientific literature for Faculty Instructions 3; Table 1, weeks 4-5

Amon, R. M. W. and R. Benner (1996). "Bacterial utilization of different size classes of dissolved organic matter." Limnology and Oceanography **41**(1): 41-51.

Daims, H., et al. (2015). "Complete nitrification by Nitrospira bacteria." Nature **528**: 504-509.

Könneke, M., et al. (2005). "Isolation of an autotrophic ammonia-oxidizing marine archaeon." Nature **437**: 543-546.

Lønborg, C., et al. (2013). "Viral lysis of Micromonas pusilla: impacts on dissolved organic matter production and composition." Biogeochemistry **116**(1): 231-240.

Moore, L. R., et al. (2007). "Culturing the marine cyanobacterium Prochlorococcus." Limnology and Oceanography: Methods **5**(10): 353-362.

Ogawa, H., et al. (2001). "Production of Refractory Dissolved Organic Matter by Bacteria." Science **292**(5518): 917-920.

Rappé, M. S., et al. (2002). "Cultivation of the ubiquitous SAR11 marine bacterioplankton clade." Nature **418**: 630.

Simister, R. L., et al. (2016). "Examining the diversity of microbes in a deep-sea coral community impacted by the Deepwater Horizon oil spill." Deep Sea Research Part II: Topical Studies in Oceanography **129**: 157-166.

Weinbauer, M. G., et al. (2011). "Synechococcus growth in the ocean may depend on the lysis of heterotrophic bacteria." Journal of Plankton Research **33**(10): 1465-1476.

Weinstock, M. T., et al. (2016). "Vibrio natriegens as a fast-growing host for molecular biology." Nature Methods **13**: 849.

Wigington, C. H., et al. (2016). "Re-examination of the relationship between marine virus and microbial cell abundances." Nature Microbiology **1**: 15024.

Wurch, L., et al. (2016). "Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment." Nature Communications **7**: 12115.

Appendix 9.

READING GUIDE 1

DECODE

- Reading nothing but the title, try to summarize what you think this paper is going to be about (in your own "layman" words).
- If you have trouble doing it right away, identify a few keywords from the introduction, find out their meaning and try again.

UNDERSTAND

Can you identify the following from the different sections of the paper?

Introduction:

- What is the motivation for this study?
- What is the objective/goal of this study?

Abstract:

- What is the motivation for the study?
- What is the purpose for the study?
- What is the method used?
- What are the major results?
    - How was the media they used different from regular laboratory media?
    - How were they able to increase through-put rate and lower detection sensitivity?
    - How successful were their methods (in terms of numbers)?
    - Did they highlight any particular result as example of their success?
- What is the main conclusion?

Methods

- Try to identify the key methods used from the sub-section headings.
- What is DAPI used for?
- What was DMSO/glycerol used for (see Figure 1)?
- What is RFLP analysis?
- What genes were amplified by PCR? Why were these genes selected?
- What are the similarities/differences between their method and our method (to be discussed in class)
- What are the pros and cons of what they did vs. what we are doing (to be discussed in class)

Results & Discussion:

- For each figure/table in the paper, try to identify:
    - What is the main point of this figure/table? (Hint: look at the caption)
    - What is the conclusion from the figure/table?

SOLIDIFY

Try to summarize the title and the abstract *very briefly* in your own words.

Appendix 10. Order a Scientific Paper

<u>Activity:</u> The course instructor cuts the following "strips" of sentences out, which are taken from various sections of a scientific paper. The students arrange the numbered strips in the order that they believe the sentences should appear in the scientific paper.

<u>Variation #1:</u> The course instructor distributes the "strips" from different sections of the paper to different groups for them to create an order, and then have the groups come together to "complete" the paper.

1. A culturability range of 0.4 to 14.3% was calculated for the different sample collections.

2. Many microorganisms may need oligotrophic or other fastidious conditions to be successfully cultured.

3. Images were obtained with a Hamamatsu ORCA-ER cooled interline charge-coupled device camera (5Mz) mounted on a Leica DMRB microscope.

4. A cell array was made from each 48-well plate to examine wells for growth.

5. Imaging of the DAPI-stained isolates revealed unicellular organisms that were generally of small size.

6. Further innovations in the HTC approach will be needed to close the gap between culture collections and the microbial species dominating marine bacterioplankton communities.

7. The term "the great plate count anomaly" was coined by Staley and Konopka in 1985 to describe the difference in orders of magnitude between the numbers of cells from natural environments that form colonies on agar media and the numbers countable by microscopic examination.

8. 16s rRNA genes were amplified by nested PCR.

9. The cell densities of the HTCC cultures ranged from 1.3×10^3 to 1.6×10^6 cells per mL with a mean of 1.1×10^5 cells per mL.

10. Water samples for the inocula were collected on the south side of the southern jetty in Newport, Oreg.

11. Of the 47 identified cultures, 4 were *α-Proteobacteria*.

12. The use of microtiter dishes and a novel technique for making cell arrays enabled us to achieve a higher throughput rate, shorten incubation times, and raise sensitivity for the detection of cells with low growth rates relative to those in previous studies.

13. The goal of this study was to develop high-throughput culturing (HTC) methods that would enable a large number of extinction cultures to be identified so that the efficacy of this approach could be assessed with a larger sampling of isolates.

14. Culturing organisms remains and important step in the process of understanding the biology and ecology of microbial species.

SOLUTION (not to be shared with students):

Introduction: 7, 2, 13

Methods: 10, 4, 8, 3

Results: 9, 1, 5, 11

Discussion: 14, 12, 6

Note: ***The statements regarding imaging could go before or after the culturing-related statements. The main objective of this exercise is to make sure students can identify which kinds of statements belong in which section of a scientific paper, and can suggest a logical flow.***

Appendix 11. BLAST Behind the Scenes

Activity: ***The course instructor distributes copies of this activity to groups of students. Students compare the number of mismatches or "mutations" between the unknown sequence and the two sequenced (imaginary) organisms. The instructor explains that fewer mutations would be expected between related species that are more closely related. Based on this information, students decide if the unknown organism is more closely related to F. tubiflora or A. officianalis. The instructor then explains that this is basically what BLAST is doing behind the scenes but with billions of sequences, instead of just two.***

Variation #1: ***The instructor could set thresholds regarding 16S rRNA gene sequence similarity, e.g.: <95% = different genus; 95-99% = same genus, different species; ~99% = same species or different subspecies. Students then attempt to determine whether their unknown organism is a new species or potentially a new genus after comparing their unknown sequence to that of the two known species. This activity may be enhanced by giving a different "unknown" sequence to different groups, and having varying results for this analysis, that can be then shared with the class. The instructor then points out that this is how they can analyze their BLAST output to determine whether what they have cultured in a new species or not.***

	Sequence
Unknown	AGATGCTGCTAGCTAGCTGCTCGATCGCTAGCTAGCTAGC
F. tubiflora	AGATCCTGCTAGTTAGCCGCTCGGTCACTAGCTAGCTAGC
A. officianalis	AGGTGCTACATGTCAGTCGCCCGATCGTTAGCCAGCTAGC

	Number of Sequence Differences
Unknown & *A. officianalis*	
Unknown & *F. tubiflora*	

SOLUTION (not to be shared with students):

	Sequence
Unknown	AGATGCTGCTAGCTAGCTGCTCGATCGCTAGCTAGCTAGC
F. tubiflora	AGATCCTGCTAGTTAGCCGCTCGGTCACTAGCTAGCTAGC
A. officianalis	AGGTGCTACATGTCAGTCGCCCGATCGTTAGCCAGCTAGC

	Number of Sequence Differences	% Similariy
Unknown & *A. officianalis*	11	{(40-11)/40}x100 = 72.5%
Unknown & *F. tubiflora*	5	{(40-5)/40}x100 = 87.5%

- **The unknown organism is more closely related to *F. tubiflora* than *A. officianalia*.**
- **It is likely to be part of a different genus than either of these two organisms.**

FOLLOW-UP "FOOD-FOR-THOUGHT" QUESTIONS:

- **How does the length of sequence analyzed affect our conclusions?**
  - **A:** Longer the sequence analyzed, the greater the confidence we can have in our results. If the sequence analyzed is only a very short portion of a much longer gene segment (which is usually the case), we don't know if we just accidentally hit upon an unusually variable region in a much longer gene, thus skewing our % similarity calculations.
- **What kind of technical problems can affect the conclusions?**
  - **A:** Sequencing quality can greatly affect our results. If the unknown organism was not sequenced with high enough confidence, then it becomes difficult to have much confidence in the % similarity calculations. Thus, anything that can affect sequencing quality (quality of DNA, amount of DNA, technical problems in the sequencing prep steps, etc.) can all affect the conclusions.

Appendix 13. Supplemental results

Our curriculum development provided anecdotal information that generated testable hypotheses about cultivation conditions. We report these here for future reference and also as a possible starting point for comparative experiments that instructors may choose to implement.

During the first semester (fall 2015), the mCURE protocol utilized glass tubes instead of the Thermo plates indicated in the main text. This semester was also the only time we isolated numerous *Vibrio* spp., which are rarely cultivated in HTC experiments with the same media and inoculated from the same source waters (1). Although the lack of cultivation-independent data precludes us from knowing if the particular sample had a bloom of *Vibrio* spp., thus increasing the probability of their isolation, the results of this experiment present the possibility that glass preferentially selects for certain bacterioplankton. The finding corroborates our understanding of *Vibrio* spp. as commonly having a surface-associated lifestyle (2). More importantly, the hypothesis that cultivation vessel material selects for specific taxa can be tested experimentally in the mCURE setting, thus adding value to the course design.

During the fall 2016 semester, mCURE sections also experimented with using media of two different salinities for the same inoculum. Sample site salinity was 17. Students inoculated cultures in our MHW2 and MHW3 media (3) with salinities of 23.2 and 11.6, and achieved 8.3 and 15.7% cultivability, respectively. Although these results are inconclusive due to variability in student success with downstream protocol elements, future experiments of this kind will provide additional data points and eventually strengthen the relationship between media salinity, cultivability, and taxonomic identity of the isolates.

Other examples of comparative cultivation experiments easily facilitated with multiple concurrent mCURE sections include testing alternative carbon substrates and alternative incubation temperatures while using the same inoculation source. Ideally, cultivation independent data like 16S rRNA gene amplicons would also be collected to evaluate cultivation success (e.g., see (1)), but this requires additional cost and analysis expertise.

References

1. Henson MW, Pitre DM, Weckhorst J, Lanclos VC, Webber AT, Thrash JC. 2016. Artificial Seawater Media Facilitate Cultivating Members of the Microbial Majority from the Gulf of Mexico. mSphere 1:e00028-16. doi:10.1128/mSphere.00028-16.
2. Dang H, Lovell CR. 2016. Microbial Surface Colonization and Biofilm Development in Marine Environments. Microbiology and Molecular Biology Reviews 80:91-138.
3. Henson MW, Lanclos VC, Faircloth BC, Thrash JC. 2018. Cultivation and genomics of the first freshwater SAR11 (LD12) isolate. The ISME Journal AOP.

Appendix 14.

INFORMAL WRITING 1

Please answer the following briefly:
½ page, typed, Times New Roman, size 12 font with 1 inch margins

Motivation: [1-2 sentences] What is the motivation for performing this experiment? **(1 pt.)**

Overall objective: [1-2 sentences] What is goal of this experiment? **(1 pt.)**

Methods: [5-6 sentences] Briefly describe the dilution, inoculation and transfer procedures that you performed. Be sure to connect it with the overall objective of the DTE method. **Mention any procedural errors when performing the steps**. Diagrams may be helpful and OK to draw, but does not replace the writing. **(5 pts.)**

A few tips:

- *General:* State volumes, not pipet sizes. Do not describe the methods step-by-step, provide a concise summary. Do not describe sterile technique, but state that it was observed or that the procedures were conducted in a biosafety cabinet, where necessary.

- *Dilution*: State where the water was from (Calcasieu Jetties, Cameron, LA), initial concentration, final concentration, what solvent did you use to perform the dilution.

- *Inoculations*: State the volume of diluted sea-water inoculated, number of wells inoculated, any controls performed, characteristics of the growth medium, total incubation time and temperature. What technique was used to identify the positives?

- *Transfers/Cryostocks*: What did you do with the wells that showed growth? What were the cryostocks made in? Incubation times & temperature for culture flasks.

Results: [2-3 sentences] How many of your 8 tubes were positives? Did your control show any growth? If so, what does it mean for the rest of your tubes… can you trust that any of your tubes have sea-microorganisms growing? What did you do if none of tubes showed any growth or your control showed growth? **(3 pts.)**

Appendix 15.

INFORMAL WRITING 2

Please address the following questions briefly. Your assignment must be typed and be no more than 1 page (1-side), double-spaced, Times New Roman, font size 12, with 1-inch margins all around.

DNA extraction: **(5 pts.)**

1. Briefly describe how you performed DNA extraction. *Use the 6 steps discussed in class as a guide and not the 3-page protocol you followed.*
2. Results: How many of your extractions worked? What were the concentrations of DNA extracted? *Do not forget units (ng/µL) with concentration!*
3. If not all your extractions worked, what may be some reasons for it? Do some soul/notebook-searching, because you must provide *reasonable* reasons, preferably things that truly happened.

PCR: We followed the protocol to the right when we performed PCR in class, except that you had a mastermix pre-made with everything in it except the template DNA. *Retain this information for future reference.* **(10 pts.)**

1. Briefly describe the reasons for adding each of the components of PCR.
2. Include what gene you amplified. Briefly explain the concept behind the primer design (what regions does it bind to, and what regions does it amplify).
3. The thermocycler protocol used for your reactions.
4. Any mistakes you may have made during setting up the reaction tubes.

P.S.: After your reactions were done, your PCR tubes were frozen until the next week.

Component	Volume (µL)	Final concentration
10X PCR buffer	5	1X
50 mM $MgCl_2$	1.5	1.5 mM
10 mM dNTPs	1	0.2 mM each
10µM forward primer	2.5	0.5 µM
10µM reverse primer	2.5	0.5 µM
Template DNA	1	Depends on initial DNA concentration
Taq DNA polymerase (5U/µL)	0.2	1U*
Distilled water	36.3	*U = "unit": a special unit for describing concentration of enzymes
Total volume	**50**	

Gel Electrophoresis: The gel image is the result of your PCR. **(5 pts.)**

1. Include the gel image with full annotation for what lane has what sample. *Image can be on a separate page, not included in the 1-page limit. Please print in color if needed for clarity.*
2. Be sure to have a figure caption and description describing the image.
3. Interpret the bands on the gel image gel image: How many PCRs worked? Which ones? What about the negative control?
4. What it does it mean if there is no band for your sample? Provide at least *one technical* and *one biological* reason.

Appendix 16.

FORMAL WRITING 1

INSTRUCTIONS: **(5 pts.)**

- This assignment must be typed, 2 sides of 1 page max, double-spaced, Times New Roman, font size 12, and 1-inch borders all-around.
- Reference all figures/tables where they are used in the text and place them right after with proper legends. Figures/tables are not included in the limit above.
- Please read instructions for **individually-written** assignment from Moodle. **No two assignments must show similarity in the words used!!!**
- All background information must be properly referenced.
- All references used for this paper **MUST** be primary literature sources (no websites). **Be sure to paraphrase any information you take from the papers with proper referencing and DO NOT COPY DIRECTLY even with quotes!**

Introduction **(5 pts.)**

- Background information to help the reader understand your experiment
  - What is the "great plate count anomaly"?
  - What are some reasons for it, *i.e.* what are some problems with isolating bacteria in culture? What are some ways to solve those problems?
- What is the motivation for your experiment?
- What is the goal for the project?

Methods (Dilution, Inoculations and Transfer) **(10 pts.)**

- *General instructions*: State volumes, not pipet sizes. Do not describe the methods step-by-step, provide a concise summary. Do not describe sterile technique, but state that it was observed or that the procedures were conducted near a flame, where necessary.
- *Dilution*: State where the water was from, initial concentration, final concentration, what solvent did you use to perform the dilution.
- *Inoculations*: State the volume of diluted sea-water inoculated, number of wells inoculated, any controls performed, characteristics of the growth medium, total incubation time and temperature. What technique was used to identify the positives?
- *Transfers/Cryostocks*: What did you do with the wells that showed growth? What were the cryostocks made in? Incubation times & temperature for culture flasks.
- Diagrams encouraged!! *May* be for bonus, but no promises.
- There must be **NO mention of any results** in this section!

Results and Discussion **(4 pts.)**

- How many of your original inoculation wells tested positive? What about the negative control? If your control tested negative (or positive) for growth, what does that imply for the rest of your samples?
- What did you do if none of them were positive? What could be some reasons why not all (or none) of your wells showed growth?
- Did your flasks test positive?

Future directions: What are we going to do next with our positive cultures? *Think big picture, not tiny details.* **(1 pt.)**

Appendix 17.

FORMAL WRITING 2

INSTRUCTIONS: **(5 pts.)**

- This assignment must be typed, max 3 pages, double-spaced, Times New Roman, font size 12, and 1-inch borders all-around. You may copy-and-paste text from FWA-1 where appropriate after making corrections.
- ***You must prepare a Response-to-Comments sheet*** where you list the comments I have made on FWA-1 (either on your paper and/or the common mistakes sheet) and address them point-by-point, telling me what you did about them. Attach this behind your formal writing assignment. *Failure to do so will cost you 10% of your total grade on this assignment.*
- **Reference all figures/tables where appropriate in the text**. Figures/tables are not included in the page limit (neither is the References section).
- Please read instructions for **individually-written** assignment from Moodle. **No two assignments must show similarity in the words used!!!**
- All background information must be properly referenced using correct format.
- All references used for this paper MUST be primary literature sources (NO websites). **Be sure to paraphrase any information you take from the papers with proper referencing and DO NOT COPY DIRECTLY without using quotes!** *Paraphrasing with inline referencing is preferred.*

Introduction **(5 pts.)**

- Background information to help the reader understand your experiment:
  - What is the motivation for your project, *i.e.* why should the reader care?
  - What is the "great plate count anomaly"?
  - What are some reasons for it, *i.e.* what are some problems with isolating bacteria in culture?
  - What are some ways to solve those problems?
- What is the goal for your project?
- Design the introduction like a funnel, with nice transition sentences for good flow. Go from making broad general statements, then narrow it down to your specific project objectives.

Methods **(20 pts.)**

- *General instructions*: State volumes, not pipet sizes. Do not describe the methods step-by-step, provide a concise summary. Do not describe sterile technique, but state that it was observed or that the procedures were conducted near a flame, where necessary.
- *Dilution*: State where the water was from, initial concentration, final concentration, what solvent did you use to perform the dilution.
- *Inoculations*: State the volume of diluted sea-water inoculated, number of wells inoculated, any controls performed, characteristics of the growth medium, total incubation time, and temperature. What technique was used to identify the positives?

- *Transfers/Cryostocks*: What did you do with the wells that showed growth? What were the cryostocks made in? What were the incubation times & temperature for culture flasks?
- *Molecular characterization*: Mention that you used the MoBio PowerWater® DNA Isolation Kit to extract the DNA from positive culture flasks. Do not describe the DNA isolation procedure since we used the manufacturer's published protocol. What gene did you amplify and for what overall purpose? Mention that the PCR products were sequenced, and what software you used for sequence analysis.
- **Diagram required**. All figures *must* have proper figure legend.
- There must be NO mention of any results in this section!

Results and Discussion **(15 pts.)**

- *Bacterial culturing:*
  - How many of your original inoculation tubes were positive? What about the negative control? If your control tested negative (or positive) for growth, what does that imply for the rest of your samples?
  - How many of your flasks tested positive?
- *Molecular characterization:*
  - Was your PCR successful? What was the concentration of DNA?
  - *If you ran a gel on YOUR samples:* Did you obtain the right product (of the expected size)? Include annotated **gel image** with proper figure legend.
  - What were the results of your sequence analysis? Include a figure/table from **BLAST analysis** with proper figure/table legend.
  - What is the organism you cultured?
  - Discuss your organism and include appropriate references.
- Include a **summary table** for your results (see example below). Add proper table legend!

Example 1 (assuming you were able to identify your original culture):

Isolate	**Did it transfer to a large volume?**	**PCR conc. (ng/µL)**	**Organism (*Scientific name*)**
LSUCC#	Yes	15	*Example organism*

Example 2 (assuming your flasks did not show growth):

Isolate	**Did it transfer to a large volume?**	**PCR conc. (ng/µL)**	**Organism (*Scientific name*)**
LSUCC#	No	N/A	N/A
LSUCC#	Yes	20	*Example organism*

References: **(5 pts.)**

- Full citations for inline references *must* be included here in the correct format.
- See the scientific writing guide (discussed in class) for correct referencing formats.

Note on scientific nomenclature for organisms: *Genus species* (first letter of genus capitalized but not so for species, whole thing italicized); shortened to *G. sp* (can be used after giving the full name once in the paper).

E.g.: *Escherichia coli* can be pathogenic but some lab *E. coli* strains are not harmful to humans.

Appendix 18.

BIOL 1208(R) FINAL EXAM

SPRING 2016

Name: _____ **Section: _____**

DO NOT TURN THIS PAGE UNTIL YOU ARE TOLD TO BEGIN

1. This exam has 13 pages and is composed of 2 parts: Theory (70 points) and Practical (30 points).

2. Work on the Theory part until you are told to perform the Practical part of the exam.

3. Only 1 person can be at a practical station at one time. You will have **2 minutes** at each station. Then you will be given instructions to move on. *After everyone has had a go at the practicals, you may return to any practical station and redo it as long as that station is empty.*

4. DO NOT ask your neighbor for ANYTHING, including answers, calculators, pens, pencils, erasers, rulers, etc. **NO TALKING**. Period. If you have a question, raise your hand and wait for me to come to you.

5. NO CELLPHONES, HATS OR SUNGLASSES MAY BE ON YOUR PERSON.

I have read and understood the instructions given on this page and otherwise (verbally) by my lab instructor before beginning the final exam and agree to abide by them throughout the course of this test. I understand that any indication of violation of these rules may be a violation of the LSU Code of Student Conduct and will be reported to the Office of Student Advocacy and Accountability.

Signed: _____ Date: _____

GOOD LUCK!!! ☺

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Part One: Theory – pages 3-9

Total points: _____ /70

1. The world is full of microbes, as seen under a microscope. Yet when scientists try to culture these microbes, a large majority of species fail to grow. Staley and Konopka named this phenomenon the [ ]. **(1 pt.)**

2. Answer the following questions based on the article by Connon and Giovannoni.

(a) Describe <u>one</u> reason for the phenomenon in Q #1 that *they <u>addressed</u>*. **(1 pt.)**

(b) <u>How</u> did they address the issue you described above? **(1 pt.)**

(c) How successful was their technique in terms of number of cells cultured compared to more traditional techniques? *I am looking for actual numbers here.* **(1 pt.)**

3. Describe <u>one other reason</u> for the phenomenon in Q #1, and <u>one suggested solution</u>, based on the review articles you read. *You may not use the one you described in Q #2.* **(2 pts.)**

4. Complete the table with one point of similarity, and one point of difference between the methods performed by Connon and Giovannoni and those performed by us in class. For the point of difference, mention one advantage of doing it either way. **(3 pts.)**

	Connon & Giovannoni	**BIOL 1207(R)**
Similarity		
Difference		
Advantage		

5. Clearly mark (underline & label) the **research goal** and **major conclusion** in the abstract below. **(2 pts.)**

Bacterioplankton of the SAR11 clade are the most abundant microorganisms in marine systems, usually representing 25% or more of the total bacterial cells in seawater worldwide. SAR11 is divided into subclades with distinct spatiotemporal distributions (ecotypes), some of which appear to be specific to deep water. Here we examine the genomic basis for deep ocean distribution of one SAR11 bathytype (depth-specific ecotype), subclade Ic. Four single-cell Ic genomes, with estimated completeness of 55%–86%, were isolated from 770 m at station ALOHA and compared with eight SAR11 surface genomes and metagenomic datasets. Subclade Ic genomes dominated metagenomic fragment recruitment below the euphotic zone. They had similar COG distributions, high local synteny and shared a large number (69%) of orthologous clusters with SAR11 surface genomes, yet were distinct at the 16S rRNA gene and amino-acid level, and formed a separate, monophyletic group in phylogenetic trees. Subclade Ic genomes were enriched in genes associated with membrane/cell wall/envelope biosynthesis and showed evidence of unique phage defenses. The majority of subclade Ic-specfic genes were hypothetical, and some were highly abundant in deep ocean metagenomic data, potentially masking mechanisms for niche differentiation. However, the evidence suggests these organisms have a similar metabolism to their surface counterparts, and that subclade Ic adaptations to the deep ocean do not involve large variations in gene content, but rather more subtle differences previously observed deep ocean genomic data, like preferential amino-acid substitutions, larger coding regions among SAR11 clade orthologs, larger intergenic regions and larger estimated average genome size.

Thrash et al. (2014) **Single-cell enabled comparative genomics of a deep ocean SAR11 bathytype**. *ISME Journal*.

Questions 6-26 are based on the scenario presented below.

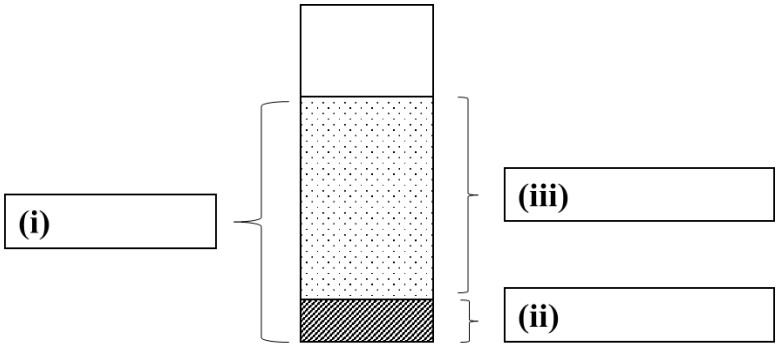
Anna wants to isolate oligotrophic bacteria from Lake Superior. However, her advisor is not around to help her. Since you have done something similar for your project, can you help her?

6. Based on your experience, what technique would you suggest Anna use to successfully isolate bacteria in pure cultures? *Give the full name, not just the abbreviation.* **(1 pt.)**

7. In accordance with the above technique, Anna wants to dilute her lake water sample to 1 cell/μL and inoculate 3 μL of the diluted water into 96 tubes with growth medium.

(a) Mention <u>two</u> ways in which this inoculation scheme is likely to help Anna obtain <u>pure cultures</u> of <u>traditionally "unculturable"</u> microbes? **(2 pts.)**

(b) If Anna's initial water sample contained 30 cells/μL, use the information above for **Q #7** to help Anna dilute her water sample. *Fill in the blanks below* with **(i)** suggested total volume of diluted water she would need, **(ii)** volume of sea-water she needs to transfer for the dilution, and **(iii)** volume of sterile media she would have to add to perform the dilution. <u>*Provide units*</u> *and* <u>*show work*</u> *for full credit.* **(3 pts.)**



8. Considering that she is trying to grow <u>oligotrophic</u> bacteria which are known to <u>grow 2X slower</u> than those in the Gulf of Mexico, what kinds of special considerations does she need to make regarding **(a)** the growth medium, and **(b)** the *minimum* incubation time for the inoculums? **(2 pts.)**

9. To minimize contamination, Anna should use sterile technique. Mention three ways in which she can practice sterility during her inoculations. **(3 pts.)**

10. Suggest an appropriate negative control for the inoculation step for Anna. **(1 pt.)**

11. Can Anna trust her positive lake-water tubes if her control was positive for growth? Why or why not? **(2 pts.)**

12. After incubation, what method can Anna use to check for growth in the tubes? **(1 pt.)**

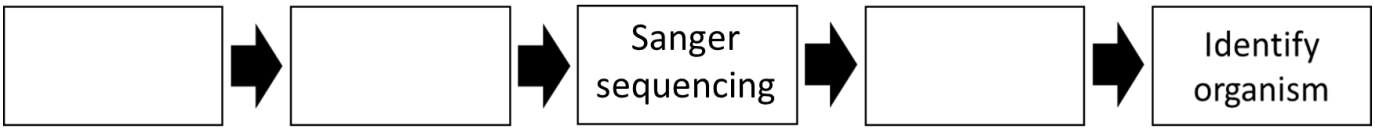
13. Despite the known introduction of skin and oral contaminants into the negative control, Anna saw no growth in the tube at the end of 2 weeks at 30°C. Two possible reasons for this may be: (1) The introduced cells could not the survive the medium conditions from the start, (2) The introduced cells initially survived and grew quickly, then ran out of nutrients and died by the end of two weeks. How can Anna test which of these two possibilities is likely to be the case. **(2 pts.)**

14. Upon checking for growth in her samples, Anna found that only 20 of her 96 tubes showed growth. The negative control showed no growth. Mention two reasons why so few of her tubes showed growth. *You may not use the reason in Q #13 above.* **(2 pts.)**

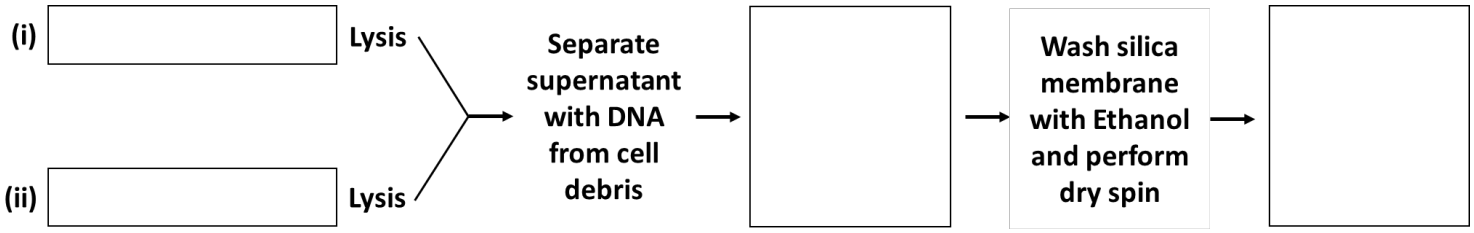
15. Suggest three ways by which she can increase her chances of seeing more positives. Indicate how each method you suggest would affect her chances of getting pure cultures, *i.e.* are they more or less likely to yield pure cultures than her current scheme. **(3 pts.)**

16. Anna wants to cryopreserve the 20 samples she has obtained as a back-up. How would you suggest she do that? **(a)** Name a cryoprotectant. **(b)** Why must it be used? **(2 pts.)**

17. Anna now wants to identify what organisms she has grown. Complete the flowchart outlining a *molecular characterization overview* scheme for Anna. **(3 pts.)**



18. Complete the flowchart with the missing steps for a successful DNA extraction. **(4 pts.)**



Indicate which of the reagents used for DNA extraction below is responsible for the types of lysis you entered for **(i)** *and* **(ii)** *above.* **(2 pts.)**

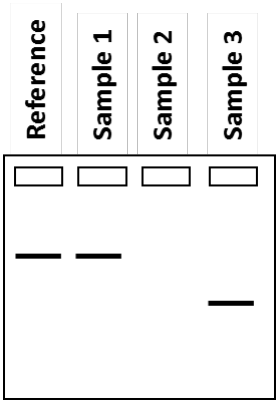
Ethanol Beads Silica membrane Detergent Filter membrane Water

19. After testing her elution product, Anna was able to detect DNA in only 5 of her 20 samples Can you think of two specific mistakes she may have made along the way? Logically explain how those mistakes would lead to the loss of DNA. **(4 pts.)**

20. Anna thinks she may have missed doing the dry spin for some of her samples during the DNA extraction process. Which step of the molecular characterization process may be inhibited by residual ethanol in DNA? **(1 pt.)**

21. What gene would you suggest Anna PCR? Clearly explain, how the architecture of the gene you recommend will help Anna amplify the DNA (without knowing which organism it is from) and help her identify the organism. *Diagrams encouraged.* **(3 pts.)**

22. Anna performed PCR on three samples and a positive control (reference band), which yielded results as shown. Answer the questions that follow based on the image to the right:



(a) She did not get a band for Sample 2. Give one *specific* reason that could have resulted in failure to amplify a PCR product. **(2 pts.)**

(b) If you assume that PCR worked for Sample 2, why does Sample 2 not show a band? **(i)** What compound is missing? **(ii)** How does this missing compound work? **(4 pts.)**

(c) Sample 3 showed a band but it was the wrong size. Anna thinks it could potentially be an archaeal species that might yield a different sized product than her bacterial control. She wants to test this possibility by analyzing her DNA sequence. *Arrange the steps (1-6) for sequence analysis in the order that they must be performed.* **(3 pts.)**

____ BLAST the contig

____ Reverse-complement the reverse sequence

____ Form contig using the forward and the reverse-complement of the reverse sequence

____ Sanger sequencing of PCR product

____ Analyze BLAST results

____ Download the forward and reverse sequences

23. Before using BLAST, Anna decided to test her hypothesis [from **Q #22(c)**] manually. Can you interpret her results below and help her decide if she cultured an archaea or bacteria? *Circle the correct choice (archaea or bacteria) and offer <u>two reasons</u> for your choice.* **(3 pts.)**

Species	Sequence
Anna's organism	AGATGCTGCTAGCTAGCTGCTCGATCGCTAGCTAGCTAGC
Bacterial sequence	AGATCCTGCTAGTTAGCCGCTCGGTCACTAGCTAGCTAGC
Archaeal sequence	----------TCGCCCGATCGTTAGCCAGCTAGC----------

24. If it turns out that Anna did not culture archaea after all, and her incorrect sized product for Sample 3 was a technical mistake, what ingredient of PCR reaction should she suspect was responsible for amplifying the wrong segment of DNA? **(1 pt.)**

25. *Anna's BLAST results are **shown on the <u>last</u> page of the exam**.*

(a) State the <u>two most likely</u> organisms this culture might be. **(2 pts.)**

(b) If you were to advise Anna, which one would <u>you</u> favor? Why? *Either of the two correct answers for above will fit so points depend on satisfactory explanation of choice.* **(1 pt.)**

26. If a sequence of sufficient length aligns almost entirely with a long enough database sequence but shows 2-5% mismatch in bases, it can potentially indicate a new strain (sub-species), whereas ≥5% can indicate a new species. Anna's results suggest that her sequences as well as the database sequences are of sufficient length. *Using this information, and Anna's results in the table above:*

Isolated Organisms	Query coverage	% ID
Bac-A	99%	97%

Has Anna isolated a known species/strain of Bac-A, or a potentially novel species, or a potentially novel strain? Provide a clear rationale for your choice. **(2 pts.)**

Part Two: Practical – pages 10-12

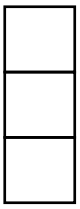
Total points: _____ /30

There are **5 stations**. You will have **2 minutes** at each station. When time is up, move to the next one. At the end of the exam, after everyone has had a turn at the Practicals, you may return to any station to redo it as long as nobody else is there.

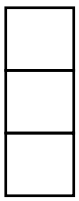
STATION 1. (7 pts. total)

1. Choose the best pipet you would use to add the following volumes. Also write what the volume readout should be set to on the appropriate pipet in order to deliver those volumes. **(4 pts.)**

a) 5 µL A / B / C



b) 100 µL A / B / C



2. What volume of liquid is pipet C set to deliver? *Include units.* **(2 pts.)**

3. Which tips (X-Z) should be used with pipet C? **(1 pt.)**

STATION 2. (FACULTY INSTRUCTIONS: Students are provided tubes labeled with various solutions needed for DNA extraction, with one of the key components missing) (3 pts. total)

1. Describe the solution that is missing in terms of its salt concentration. **(1 pt.)**

2. Why is this solution required for DNA extraction? Without this solution, where would you expect the DNA to be: bound to the column or in the flow-through? **(2 pts.)**

STATION 3. (FACULTY INSTRUCTIONS: Students are provided labeled tubes with various PCR reagents, with one of the key components missing)

(10 pts. total)

1. Name the component for PCR that is missing. **(1 pt.)**

2. Would you expect to see a PCR product using only the reagents given? **(1 pt.)**

3. Name the three major steps of PCR which correspond to the temperatures provided. Then state what happens in each of those steps. *Please be specific!* **(6 pts.)**

95°C _____

_____

50.8°C _____

_____

72°C _____

_____

4. Which step (from the ones above) is expected to be most directly affected by the absence of the missing component? **(1 pt.)**

5. Which step is magnesium required for? **(1 pt.)**

STATION 4. (5 pts. total)

1. When you make a gel for the electrophoresis of DNA, should you put the gel comb at the end of the tray or the middle? **(1 pt.)**

2. On the electrophoresis rig, should the well be at the black (negative) end or the red (positive) end? **(1 pt.)**

3. Why should it go on that end? **(1 pt.)**

4. *True or False:* DNA is separated by size and charge. *If false, correct the statement.* **(2 pts.)**

STATION 5: (FACULTY INSTRUCTIONS: The sequences provided to these students for this practical question is provided on the last page of this document. Students have access to BLAST, as well as the software for creating reverse complements and contigs) (5 pts. total)

PLEASE RETURN TO A BLANK HOME PAGE AFTER YOU FINISH

1. Can you form a contig using the sequences provided in **"Station_5.txt"**? **(1 pt.)**

2. Circle *all* aspects of sequencing reads that contribute to whether or not you can form a contig.

Length of the reads Overlap between reads **(1 pt.)**

Reads must be in same direction Reads must be in different direction

3. Use the sequences provided in **"Station_5.txt"** to answer whether the following statements are *True* or *False.* If *false*, correct the statement.

(a) A contig CANNOT be formed using the reverse sequence (Rev) and the reverse-complement of the forward sequence (Fwd_RC). **(1 pt.)**

(b) If you performed BLAST analysis with a contig made from forward sequence (Fwd) + reverse-complement of the reverse sequence (Rev_RC), and a 2nd contig made from the reverse sequence (Rev) + reverse-complement of the forward sequence (Fwd_RC), they should give you the same basic output. **(1 pt.)**

4. The two contigs described above are _____ to each other. **(1 pt.)**

Circle the correct choice to fill in the blank: **(a)** identical **(b)** reverse-complement

PLEASE RETURN TO A BLANK HOME PAGE AFTER YOU FINISH

The image below pertains to Theory Question #25.

> Forward	Max score	Total score	Query cover	E value	Ident	Accession
Congregibacter litoralis strain KT71 16S ribosomal RNA gene, complete sequence	1192	1192	99%	0.0	96%	NR_121684.1
Haliea mediterranea strain 7SM29 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	96%	NR_116976.1
Pseudohaliea rubra strain CM41_15a 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	96%	NR_044426.1
Luminiphilus syltensis strain Ivo14 16S ribosomal RNA gene, partial sequence	1166	1166	99%	0.0	96%	NR_125526.1
Halioglobus pacificus strain S1-72 16S ribosomal RNA gene, partial sequence	1158	1158	99%	0.0	96%	NR_113279.1

> Reverse	Max score	Total score	Query cover	E value	Ident	Accession
Pseudohaliea rubra strain CM41_15a 16S ribosomal RNA gene, partial sequence	1384	1384	99%	0.0	98%	NR_044426.1
Congregibacter litoralis strain KT71 16S ribosomal RNA gene, complete sequence	1362	1362	99%	0.0	98%	NR_121684.1
Halioglobus pacificus strain S1-72 16S ribosomal RNA gene, partial sequence	1262	1262	99%	0.0	96%	NR_113279.1
Chromatocurvus halotolerans strain EG19 16S ribosomal RNA gene, complete sequence	1240	1240	99%	0.0	95%	NR_115058.1
Haliea mediterranea strain 7SM29 16S ribosomal RNA gene, partial sequence	1234	1234	99%	0.0	95%	NR_116976.1

> Contig	Max score	Total score	Query cover	E value	Ident	Accession
Pseudohaliea rubra strain CM41_15a 16S ribosomal RNA gene, partial sequence	2285	2285	99%	0.0	97%	NR_044426.1
Congregibacter litoralis strain KT71 16S ribosomal RNA gene, complete sequence	2274	2274	99%	0.0	97%	NR_121684.1
Haliea mediterranea strain 7SM29 16S ribosomal RNA gene, partial sequence	2146	2146	99%	0.0	95%	NR_116976.1
Halioglobus pacificus strain S1-72 16S ribosomal RNA gene, partial sequence	2141	2141	99%	0.0	95%	NR_113279.1
Luminiphilus syltensis strain Ivo14 16S ribosomal RNA gene, partial sequence	2132	2132	99%	0.0	95%	NR_125526.1

FOR FACULTY USE ONLY – NOT FOR STUDENTS

>Fwd
AGTCGACGGCAGCACGGGTGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTATACATCGGAACGTGCCCAGTAGTGGGGGATA
GCCCGGGGAAACCCGGATTAATACCGCATACGCCCTAAGGGGGAAAGTGGGGGATCTTCGGACCTCACGCTATTGGAGCGGCCGAT
GCCTGATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCAATGCCGCGTGTGC
GAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTTTGGAAAGAAATCGTCTTTGCTAATATCAGAGACGGATGACGGTACCAATCGA
ATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCG
CAGGCGGCTATATAAGACAGATGTGAAATCCCCGGGCTCAACCTGGGAATTGCATTTGTGACTGTATGGCTAGAGTGTGTCAGAGG
GAGGTGGAATTCCACGTGTAGCAGTGAAATGCGTAGATATGTGGAGGAACACCAATGGCGAAGGCAGCCTCCTGGGATACACTGAC
GCTCATGCA

>Fwd_RC (REVERSE COMPLEMENT)
TGCATGAGCGTCAGTGTATCCCAGGAGGCTGCCTTCGCCATTGGTGTTCCTCCACATATCTACGCATTTCACTGCTACACGTGGAA
TTCCACCTCCCTCTGACACACTCTAGCCATACAGTCACAAATGCAATTCCCAGGTTGAGCCCGGGGATTTCACATCTGTCTTATAT
AGCCGCCTGCGCACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCC
GGTGCTTATTCGATTGGTACCGTCATCCGTCTCTGATATTAGCAAAGACGATTTCTTTCCAAACAAAAGTGCTTTACAACCCGAAG
GCCTTCTTCGCACACGCGGCATTGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGG
CCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTAGGCCTTTACCCTACCAACTAGC
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CCGTCGACT

>Rev
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>Rev_RC
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GAGTGGGTTCTACCAGAAGTGGCTAGT

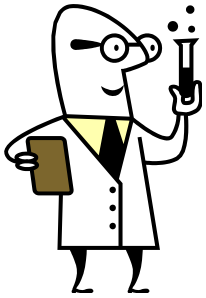
Appendix 19. Lightning Talk Rubric

Group Members: _____

Paper Presented: _____

Grade: _____/20

Section	Excellent	Average	Poor/Unacceptable	Comments
Time – 5 points	Presentation does not exceed 4 minutes **(5 pts)**	Presentation is >4 minutes or <2 minutes **(3 pts)**	Group does not present **(0 pts)**	
Methods – 3 points	Explains method efficiently and clearly **(3 pts)**	Explains some methods, but leaves out methods necessary to acquire results **(1 pts)**	Explains methods incorrectly or methods not associated with presented results **(0 pts)**	
Results – 5 points	Effectively explains results presented **(5 pts)**	Poor explanation of results **(3 pts)**	Fails to explain results **(0 pts)**	
Discussion – 7 points	Explains the "big picture" of the research article. Interprets results. Summarizes and communicates findings effectively **(7 pts)**	Summarizes results, but fails to relate the science to the "big picture" Poor interpretation of results. **(4 pts)**	No discussion. Fails to relate communicate how the paper is meaningful **(0 pts)**	



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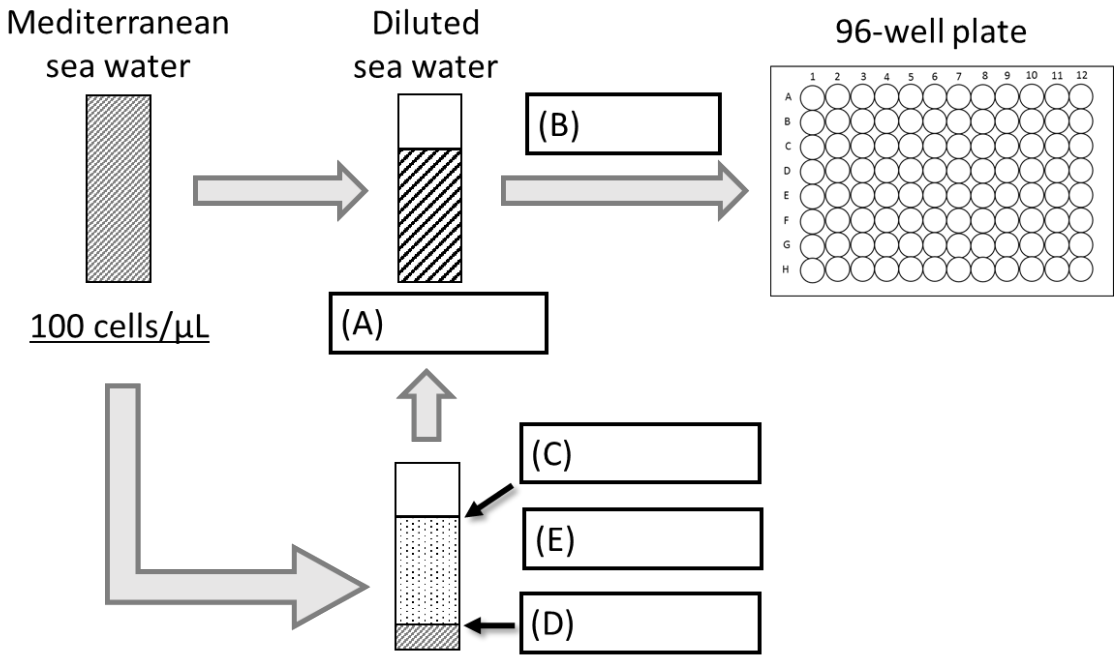
QUIZ 1

1. Complete the blanks in the diagram below based on the information given in the diagram and the questions that follow. ***Show work*** *and* ***provide units*** *wherever necessary.*



(A) Suggest a final concentration for the diluted sea-water. **(2)**

(B) What volume of the diluted sea-water must Ray inoculate to get ~5 cells/well on average? **(2)**

(C) Suggest a final volume for the diluted sea-water. *Make sure it is enough to inoculate all 96 wells!* **(2)**

(D) What volume of the concentrated sea-water must be transferred to do the dilution? **(3)**

(E) What volume of sterile media must be added to the concentrated sea-water to do the dilution? **(2)**

2. You have thee pipets – P10, P100 and P1000. *Answer the questions below.*

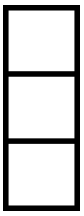
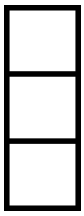
(A) Which is/are the pipet(s) that can hold the volumes of liquid you calculated in: **(2)**

(i) Question 1D: **(ii)** Question 1E:

(B) Which is the *best* pipet to transfer the volumes of liquid you calculated in: **(2)**

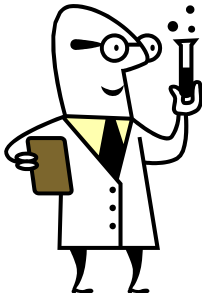
(i) Question 1D: **(ii)** Question 1E:

(C) What should the volume readout be on the *best* pipet to transfer the volumes in: **(2)**

(i) Question 1D:  **(ii)** Question 1E: 

2. Name any **three** things that we did (or precautions we took) to be sterile during the inoculation procedure. **(3)**

3. If your negative control tubes show growth of microorganisms, can you trust that any of your test samples actually have sea-water organisms growing even if they have growth? Why? *Please explain briefly.* **(Bonus: 2)**



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QUIZ 2

1) What does DTE stand for? Identify **two** ways in which DTE specifically helps us obtain pure cultures of traditionally "unculturable" marine micro-organisms. **(3 pts.)**

2) Ray is an undergraduate student working to isolate marine bacteria from the Arctic Ocean. After an initial count, she determined that the collected water has 300 cells/μL. Answer the questions that follow. *Show work and provide units for full credit.*

(a) Given what you know about DTE, suggest a final diluted concentration for Ray's water, such that she is able to inoculate ~3 cells/well. **(1 pt.)**

(b) Ray needs to inoculate ~3 cells (on average) into each well of a 96-well plate. Suggest a final volume for Ray's diluted seawater, such that she has enough to do all her inoculations. **(1 pt.)**

(c) How much of the concentrated water must Ray use to do the dilution? **(2 pts.)**

(d) How much media must Ray add to the concentrated water in order to perform the dilution? **(1 pt.)**

(e) Choose the best pipet to transfer the volumes you calculates in questions (b) and (c) above. Fill in the boxes with what the volume readout on the pipet should be set to. **(4 pts.)**

(i) Question 2(b): P10 / P100 / P1000 ☐

(ii) Question 2(c): P10 / P100 / P1000 ☐

3) As Ray was preparing to inoculate her samples, she realized that she has no extra sterile media to use as a control. Suggest **one** appropriate alternative that Ray can use for a negative control.

(1 pt.)

4) Contamination during which of the steps listed below will be reflected as growth in the negative control that **(A)** we used (sterile media), and **(B)** Ray used (your answer to Q #3)?

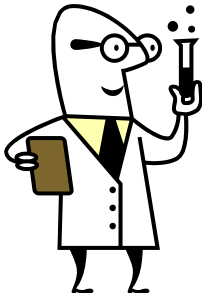
Please circle all appropriate choices in the lists below. **(4 pts.)**

(A) Our control (sterile media)	**(B) Ray's control (your answer to Q #3)**
i. Collection of sea-water	**i.** Collection of sea-water
ii. Dilution of sea-water with media	**ii.** Dilution of sea-water with media
iii. Inoculation of diluted sea-water in media	**iii.** Inoculation of diluted sea-water in media

5) Despite the known introduction of skin and oral contaminants into the negative control, there was no growth in the tube at the end of 2 weeks at 30°C. Two possible reasons for this may be: (1) The introduced cells could not the survive the medium conditions from the start, (2) The introduced cells initially survived and grew very quickly, but then ran out of nutrients and died by the end of two weeks. Suggest an experiment through which you can test which of these two possibilities is likely to be the case. **(2 pts.)**

6) Suggest **one** plausible reason why we *first* made the DMSO stock and *then* did the transfer.

(1 pt.)



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QUIZ 3

1) From the perspective of our class project, *briefly* explain why we performed DNA extraction?
(2 pts.)

2) Order the major stages of DNA extraction as we discussed them in class. **(2 pts.)**

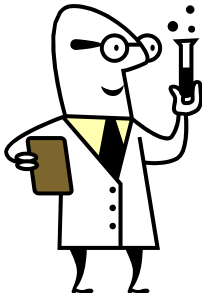
Bind DNA in supernatant to silica columns

Collect and lyse the cells

Use ethanol to clean DNA

Elute the DNA

3) A student was asked to follow the same DNA extraction protocol as what you performed in class, but this was her first time and she had to do it all on her own without supervision. Unfortunately, when she tested her eluted liquid in the end, it contained very little to no DNA. She has no idea where to even begin troubleshooting! Can you identify **any *three* (3)** things that might have gone wrong such that she experienced loss of DNA? *Please explain briefly how your responses could lead to loss of DNA.*
(6 pts.)



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QUIZ 4

1. Please choose if the following statement is *true* or *false*. If *false*, correct the statement. **(2 pts.)**

a) Polymerase Chain Reaction can amplify the whole genome in a non-selective fashion.

b) If you forgot to put in template DNA, dNTPs or primers, you will not make any PCR product.

2. Name the three major steps of PCR in order that they must be performed to be successful. Then briefly describe what happens in each of these steps. **(6 pts.)**

1st:

2nd:

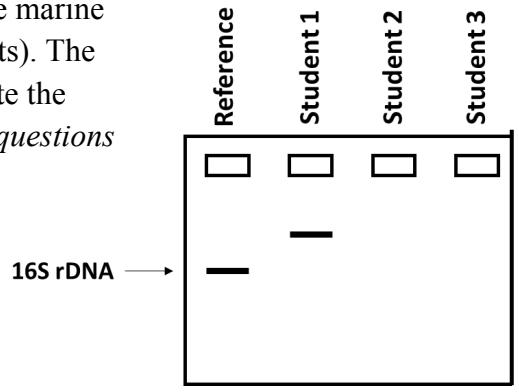
3rd:

4. Name enzyme used for the third step *and* the element/ion required for its activity. **(2 pts.)**

5. Separation of DNA fragments during gel electrophoresis is on the basis of _____. **(1 pt.)**

6. How is this different from the basis of separation for proteins? Briefly explain why. **(2 pts.)**

7. Four students are trying to use 16S rDNA to identify the marine microbe they are culturing (just as in your own experiments). The reference band is derived from a positive control to indicate the expected size of the 16S rDNA PCR product. *Answer the questions based on the gel image shown.*



(A) *Circle* the *larger* PCR product on the gel. **(1 pt.)**

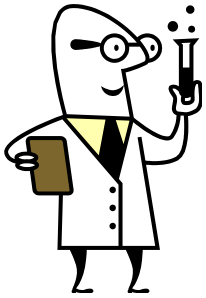
(B) Student 1 did not get the correct size product from his PCR reaction. What component of PCR should he suspect might be responsible for potentially amplifying the wrong segment of DNA? **(1 pt.)**

(C) Student 2 did not get any PCR product and upon digging further, she realized may have used the primers for a completely different gene! Explain briefly why we picked the 16S rRNA gene to identify the microbe. Which region(s) of the gene help us with the identification? **(1 pt.)**

(D) Student 3 also saw no PCR product on the gel but he is 100% positive that he added in all the right components for the PCR. So assuming his PCR worked correctly, why was he not able to visualize the DNA band on the gel? *Answer the following questions:*

(i) What reagent was missing ? **(1 pt.)**

(ii) How does this missing reagent work? **(3 pts.)**



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QUIZ 5

1. After performing PCR for 16S rRNA gene, a student got a smaller PCR product than she expected. However, she is confident that there was nothing technically incorrect about her method. She feels that she amplified 16S rRNA gene correctly and that the different size of her band is indicative of a "biological difference" of some sort in the sequence of the organism she is trying to characterize (see example in diagram). Can you suggest *one* method by which Student 2 could test this hypothesis? **(2 pts.)**

(arrows indicate primer location)

Example of expected sequence: ATGCTTCGGACTCGATTTCCCGGATGCT **(28 bp)**

Potential sequence that could lead to shorter PCR product due to deletion of sequence in grey: ATGCTTCGGCCCGGATGCT **(19 bp)**

2. *Arrange the steps (1-5) for sequence analysis in the order that they must be performed.* **(2 pts.)**

_____ BLAST the contig & analyze results

_____ Reverse-complement the reverse sequence

_____ Form contig using the forward sequence and the reverse-complement of the reverse sequence

_____ Download the forward and reverse sequences

3. *Choose whether the following statements are True or False. If false, correct the statement.* **(2 pts.)**

(a) A contig can be formed using the reverse sequence and the reverse-complement of the forward sequence.

(b) If you performed BLAST analysis using the contigs using the two methods described in **Question 2** and **Question 3(a)**, assuming the same original forward and reverse sequence, they should NOT give you essentially the same BLAST output.

4. If you had the same set of forward and reverse sequencing reads, and you formed a contig using the two methods described in **Question 2** and **Question 3(a)**, they would be _____ to each other.

Circle the correct choice to fill in the blank: **(a)** identical **(b)** reverse-complement **(1 pt.)**

5. *Align and analyze the sequences shown below to answer the questions that follow.*

Species	**Sequence**
Unknown organism	AGATGCTGCTAGCTAGCTGCTCGATCGCTAGCTAGCTAGC
Bacteria A	----------TCGCCCGATCGTTAGCCAGCTAGC----------
Bacteria B	AGATCCTGCTAGTTAGCCGCTCGGTCACTAGCTAGCTAGC

(a) Which organism (Bacteria A or Bacteria B) is the unknown organism most likely to be? **(1 pt.)**

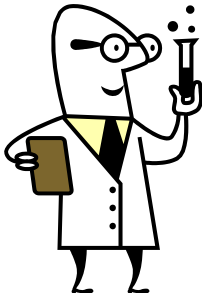
(b) Offer *two* lines of reasoning for your choice above. **(1 pt.)**

6. *Answer the question that follows based on the BLAST results shown on the right.* If a sequence of sufficient length aligns almost entirely with a long enough database sequence but shows ~2% mismatch in bases, it can potentially indicate a new strain, whereas ~5% can indicate a new species.

Isolated Organisms	**Query coverage**	**E-value**	**% ID**
F. originalis	99%	0.0	94%

Assuming that the analyzed sequence as well as the database sequences are of sufficient length, would you expected to have isolated a known species/strain of *F. originalis*, or a potentially novel species or a potentially novel strain? **(1 pt.)**

7. Take a deep breath! You have just complete the last quiz for this course. Tell me one funny incident that happened to you in lab this semester. If you can't think of anything from this lab, tell me anything funny about you or that happened to you. ☺ **(1 pt. BONUS)**



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BONUS QUIZ 1

Decide whether the following sentences from the Connon and Giovannoni (2002) paper belong to the Introduction, Methods, Results or Discussion. Write that next to the sentences. Then put them in an appropriate order (1-5) as they should appear in a scientific paper. **(5 pts.)**

Scientific paper section (Introduction, Methods, Results or Discussion)	**Sentence from Connon and Giovannoni (2002)**	**Appropriate order of appearance in a scientific paper**
Methods	A cell array was made from each 48-well plate to examine wells for growth.	2
Discussion	The use of microtiter dishes and a novel technique for making cell arrays enabled us to achieve a higher throughput rate, shorten incubation times, and raise sensitivity for the detection of cells with low growth rates relative to those in previous studies.	4
Introduction	The goal of this study was to develop high-throughput culturing (HTC) methods that would enable a large number of extinction cultures to be identified so that the efficacy of this approach could be assessed with a larger sampling of isolates.	1
Discussion	Further innovations in the HTC approach will be needed to close the gap between culture collections and the microbial species dominating marine bacterioplankton communities.	5
Results	Of the 47 identified cultures, 4 were *α-Proteobacteria*.	3

Appendix 22

Formal Writing Assignment 1

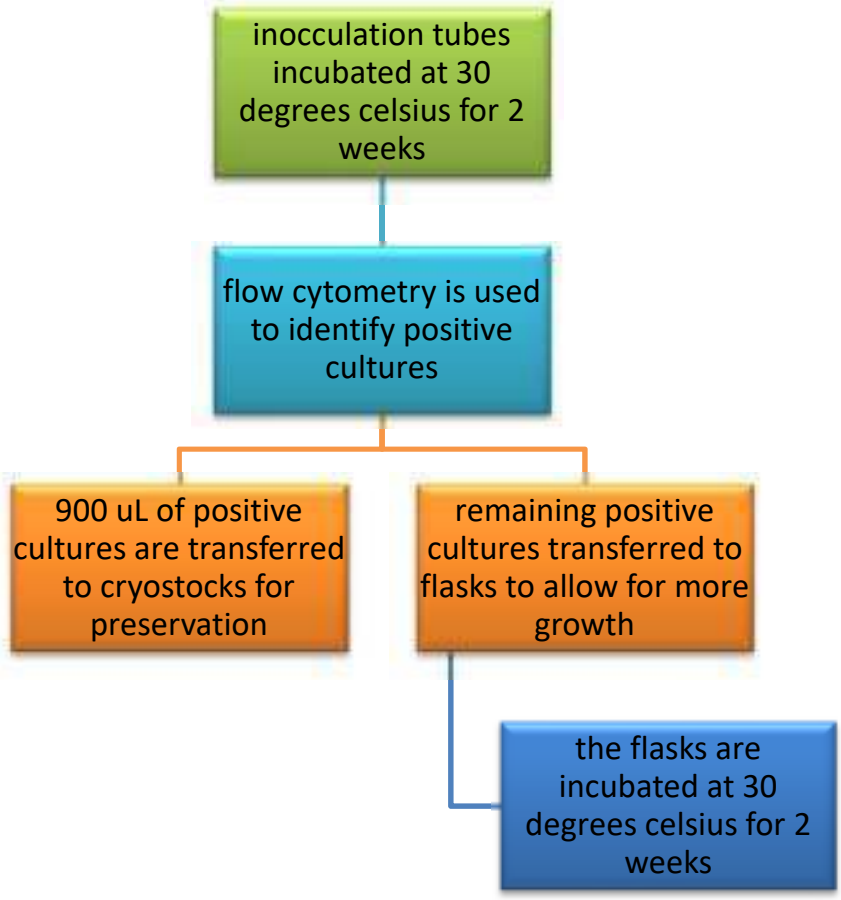
Introduction

There are about one hundred and ten octillion microbes throughout the world's oceans, however; only about seven thousand species of bacteria have been validated. Validating all of the microbes could lead to advances in the industries of food and pharmaceuticals as well as many other industries. The huge difference between validated microbes and invalidated microbes is due to what's known as the great plate count anomaly. The great plate count anomaly can be described by the difference in the amount of cells that exist and the amount that can be grown in a lab environment (Connon and Giovanni). Some reasons for this issue include competition between organisms, commensalism, and failure to mimic the correct environment. Commensalism is what is known as an association between two organisms in which one benefits and the other receives neither benefit nor harm. A few methods to these problems include dilution to extinction and high throughput culturing. Dilution to extinction, or DTE, is the process of diluting something to about one to three cells per inoculation and high throughput culturing, or HTC, is preforming the process of DTE many times. These methods help in which they decrease the chances of competition or commensalism within the cultures. They also make it easier to target a single microbe. In our classroom lab setting, we used these methods in order to try and isolate microorganisms from the Gulf of Mexico.

Methods

In order to reach our goal of isolating new organisms from the Gulf, we used the methods of dilution to extinction and high throughput culturing to increase our chances of successfully growing a new microbe. First the water was collected from the Gulf of Mexico and diluted using DTE. We then performed inoculations of the seawater. In order to do this, we transferred 5 uL of the seawater into a growth medium seven seperate times. We also transferred five uL of distilled

water into a growth medium as a control. We then incubated the cultures two weeks at 30° C to allow for growth. Afterwards, flow cytometry was used in order to identify which tubes showed positive growth. Once the incubation period ended, we took the positive cultures and prepared cryostocks as well as transferred them to a larger growth medium. Nine hundred uL of the positive cultures were placed into cryostocks tubes that included 100 uL of dimethyl sulfide (DMSO), a commercial solvent (Muir). We then poured the rest of the positive cultures into their own flasks and incubated at 30° for two weeks.



Results and Discussion

Out of our original inoculation tubes, five of the seven were positive for growth. Tubes number three, seven, four, six, and two showed the positive growth, and we chose numbers three and

seven to transfer to cryostocks and the flasks. However, only tube number 7 showed growth in the flask. We are not sure why number 3 did not show growth but it may be contributed to a possible human error or the conditions were not suitable to grow the organism further. Some organisms rely on commensalism to live or they may be very slow growing and therefore, difficult to grow in a lab setting.

Future Directions

Once the 2-week incubation period of the flasks is over, they will be tested for positive growth using flow cytometry. We will then extract the DNA from the cultures and use a polymerase chain reaction (PCR) to focus on the DNA and make millions of copies of it (PCR UofUtah). This will then help us try and identify which microorganisms we have grown or if we have grown one never grown before.

Works Cited

Connon, Stephanie A., and Stephen J. Giovannoni. "High-Throughput Methods for Culturing Microorganisms in Very-Low-Nutrient Media Yield Diverse New Marine Isolates." Applied and Environmental Microbiology. American Society for Microbiology, n.d. Web. 03 Oct. 2015.

Muir, Maya. "DMSO: Many Uses, Much Controversy." DMSO: Many Uses, Much Controversy. N.p., n.d. Web. 3 Oct. 2015.

"PCR." PCR. University of Utah, n.d. Web. 03 Oct. 2015.

Final Formal Writing Assignment

Sinomonas mesophila: Novel Microbes from the Dirt, Now in the Gulf

Introduction

The origins for this experiment lie in the "great plate anomaly," coined by Staley and Konopka in 1985 (1). This term refers to the event in which standard media and culturing techniques can only grow a culture of a minute range of microorganisms belonging to a natural environment, resulting in few observable microorganisms to be studied under a microscope. Most likely, these microorganisms are not adapted to standard nutrient-rich medium, such as complex carbons, compared to their original oligotrophic environments (2). Using the high-throughput culturing method (HTC) and the dilution-to-extinction method (DTE), the magnitude of culturable microorganisms can be significantly increased, possibly resulting in the discovery of novel microbes with genes that could have favorable applications to various research.

The goal of this CURE project is to discover such novel microbes, specifically any from the Gulf of Mexico, using the HTC and DTE methods. Through the help of multiple groups (this one's being Group7), the project led to multiple successes. In the case of Group 7, it led to the discovery of *Sinomonas mesophila* in Gulf coast waters, far from its original discovery in the soils of India (3).

Methods

Inoculations. Sample water was obtained from the Gulf of Mexico and diluted to a concentration of .5 cell/μL. The cells were then inoculated in a sterile environment into fourteen total wells with two control wells, six experimental wells and the control well on both Plate 1 and Plate 2. A solution for inoculating was composed of 996.3 μL of low-nutrient media designed by the Thrash lab and 3.7 μL of diluted Gulf seawater. Each well was inoculated with 5

µL of the new solution, whereas the control well was inoculated with 5 µL of media. The wells were then incubated for three weeks at 30˚C. Flow cytometry was used to check for positive growth at the end of the three weeks.

Transfers/Cryostocks. 450 µL of solution from a positive growth tube was pipetted into a cryostock tube containing 50 µL of DMSO, protecting DNA from frost and accumulation of solutes in low temperatures, for each positive result and prepared for cryostock. Another 200 µL of culture was transferred into 50 mL flasks for further incubation at 30˚C for two weeks, checked again at the end of the two weeks with flow cytometry to check for further positive growth.

DNA extraction. A syringe was prepared with approximately 40 mL of culture and filtered through a 0.22 µm filter. The filter holding the cultured microbes was then processed with the MoBio PowerWater® DNA Isolation Kit to isolate the DNA saved for PCR and sequencing.

PCR. A master mix was pre-prepared containing dNTPs, magnesium for Taq polymerase, and buffers, bringing the mixture to a PCR concentration of 14.8 µg/µL. 4.05 µL of PCR product and 6.95 µL of water were added later specifically for LSUCC 3220. 49 µL of the master mix with LSUCC 3220's specific components was added to a tube, followed by 1 µL of DNA primers specific to the conserved region of the 16SR gene sequence, and Taq polymerase. The tubes were then placed in a thermal cycler for PCR to take place.

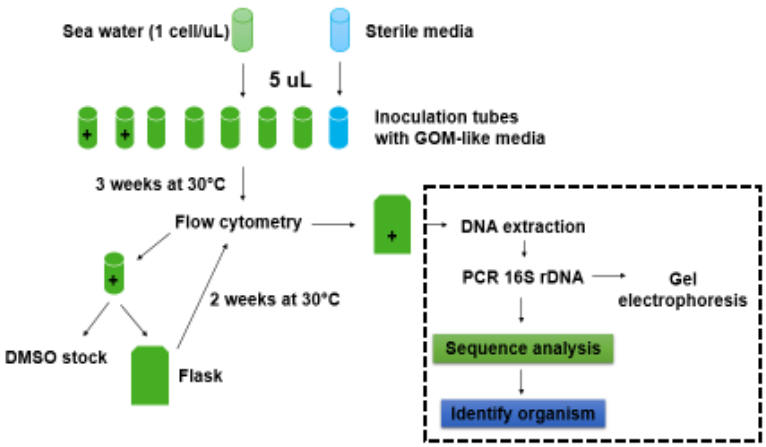


Figure 1. A pictorial summarization of methods, courtesy of Thrash Lab.

After PCR, newly amplified 16S rRNA gene sequence DNA was sent to an outside group for sequencing while some was set aside for gel electrophoresis. A BLAST analysis of the sequence was performed when results of sequencing were returned.

Results and Discussion

Toward the beginning of the project, Group 7's original wells all tested negative. This result was most likely due to contamination during inoculation. The remainder of the experiment was performed using one of Group 6's original wells, LSUCC 3110 (see Figure 2). Due to a mishap, some culture was lost during the transfer/cryostock process; volumes were changed to 350 µL per cryostock and 150 µL for the incubator flask with MWH2 media. This flask successfully came back positive and was used for DNA extraction.

Figure 3. Gel electrophoresis performed with LSUCC 3110. Only lambda ladder in leftmost well successfully spread.

In the first attempt at PCR, no DNA was amplified, discovered when no attempts at gel electrophoresis were successful (see Figure 3). The most likely cause was deteriorated chemicals and Taq polymerase from kits sitting in storage for nearly two years. PCR was repeated with new kits but did not produce successful results, requiring BLAST analysis to be performed with sequencing from LSUCC 3220 (see Figure 2), confirming the cultured microbe to be *Sinomonas mesophila* (see Figure 4). This microbe was originally denoted as strain MPKL 26, a Gram-positive, bent-rod microbe discovered in the soil of Bidar Fort of Karnataka, India (3). It originally meant the

discovery of a new species of the genus *Sinomonas* in soil with an optimum growth rate at 30˚C (3) and now gives support for viability in marine environments like the Gulf of Mexico with a similar temperature range.

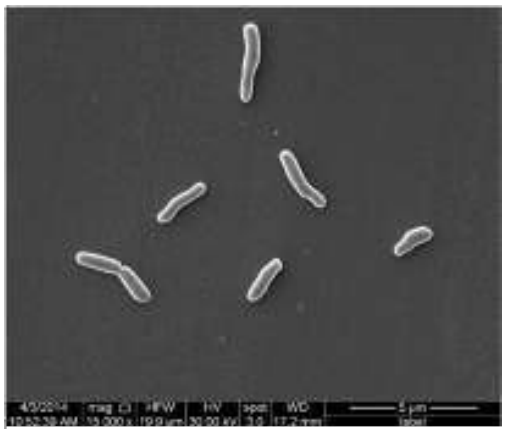


Figure 4. *Sinomonas mesophila* displays a bent-rod shape, characteristic of genus *Sinomonas.* (See reference 3)

Figure 2

LSUCC#	Did it transfer to a large volume?	PCR	PCR conc. (µg/µL)	Organism
3110	Yes	No	N/A	N/A
3220	Yes	Yes	14.8	*Sinomonas mesophila*

References

1. Staley, J. T., and A. Konopka. 1985. Measurements of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. 39:321–346.
2. Connon, S. A., and S. Giovannoni. 2002. High-Throughput Methods for Culturing Microorganisms in Very-Low-Nutrient Media Yield Diverse New Marine Isolates. Applied and Environmental Microbiology. Vol. 68, 8:3878-3885.
3. Prabhu DM, Quadri SR, Cheng J, et al. Sinomonas mesophila sp. nov., isolated from ancient fort soil. J Antibiot. 2015;68(5):318-21.

Response to Comments

Instructor Comments (TA name)

1. in few amounts … One explanation
    - In order to maintain consistency in phrasing, I rewrote the end of the first sentence and the transition into the second
2. high-throughput method (HTC)
    - I corrected the phrase to "high-throughput culturing method" to agree with the abbreviation
3. seawater concentration
    - I removed "seawater" to remove redundancy
4. No mention of flow cytometry
    - I added in the use of flow cytometry with respect to the necessity of its use
5. in low-temperature climates
    - I rephrased to "low temperature" to remove redundancy
6. Initial mention of "Group 7"
    - I added a short explanation of the multiple groups participating in the project to clarify the abrupt use of "Group 7"

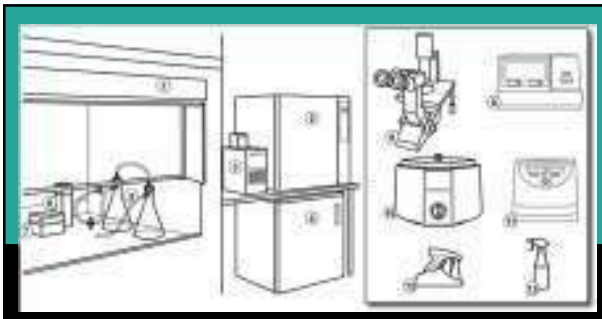
Peer Comments (peer name)

1. New paragraph
    - I transitioned to a new paragraph so that the introduction did not seem too drawn-out
2. two control well … seven experimental
    - I corrected the number of experimental and control wells to the actual amount of each used on each plate
3. Add diagram to summarize methods
    - I added a diagram from the PowerPoints, recognizing the Thrash Lab for the general summary

Production of Refractory Dissolved Organic Matter by Bacteria

Hiroshi Ogawa,[1*] Yukio Amagai,[1] Isao Koike,[1] Karl Kaiser,[2] Ronald Benner[2]

Presented by:



Methods

Culturing and Incubation: culturing seawater with bac~~te~~ria to observe the production of DOM. incubating DOM to observe rate of growth/decay

Keywords:
DOM (dissolved organic material- stabilizes fixed carbon in the ocean

Results

Stage (days)	Remineralized %	Remaining %		Decay constant (day^{-1})
		DOC	POC	
Glucose (208 ± 0 μM C)				
I (0–2)	78 ± 1	15 ± 0	7 ± 1	1.1 ± 0.0
II (2–7)	87 ± 1	8 ± 1	6 ± 1	0.13 ± 0.03
III (7–365)	95 ± 0	5 ± 0	–	0.0012 ± 0.0003
Glutamate (732 ± 2 μM C)				
I (0–2)	66 ± 5	13 ± 7	22 ± 2	1.1 ± 0.1
II (2–9)	77 ± 2	10 ± 1	13 ± 1	0.14 ± 0.08
III (9–560)	93 ± 1	7 ± 1	–	0.0023 ± 0.0003

- glucose and glutamate can be used by marine organisms to produce DOM over a long period of time.
- Both were quickly consumed, and replaced with resistant DOM.

More Results

Chemical composition	Initial substrate: Glucose, Incubation time (days)				Initial substrate: Glutamate, Incubation time (days)			
	2	4	7	365	2	4	9	560
Concentration of the bulk DOM (μM)								
DOC	30	28	26	21	28	23	24	30
DON	1.8	0.7	0.6	1.2	3.1	2.4	1.5	0.7
DOC composition (% at)								
THAS	2.5	4.0	5.9	nm	nm	nm	nm	nm
THAS	0.7	1.4	1.5	3.8	nm	nm	nm	nm
THAA	2.5	4.3	3.7	2.5	13	18*	12	5.1
Uncharacterized	94	89	89	(94)	87	82*	88	97
DON composition (% at)								
THAS	3.4	3.5	6.6	5.6	nm	nm	nm	nm
THAA	22	29	27	6.4	19	31*	33	11
Uncharacterized	75	66	67	88	80	69*	67	89
C/N molar ratio								
Bulk	32	26	28	8.9	9.1	5.7	9.3	19
Total characterized	7.5	7.9	9.2	(46)	3.5	3.1	3.5	3.6
Uncharacterized	41	37	37	(10)	5.4	6.3*	12	14
THAS composition (mole %)								
GalN	20	26	31	33	nm	nm	nm	nm
GlcN	55	50	56	67	nm	nm	nm	nm
MA	26	24	12	0	nm	nm	nm	nm
THAA composition (mole %)								
Acidic	26	27	21	30	28	12	17	28
Basic	12	10	11	11	8	6	7	8
Neutral	23	27	31	41	24	36	36	46
Hydrophobic	38	36	37	18	41	27	40	18

Preserved past 365 days, shows properties of durability to sustain fixed carbon amounts

- Molecular structure is altered to become resistant to degradation

Interpretation

DOM is used by marine organisms to produce, for example, carbon dioxide

↓

marine organisms use substrates to produce DOM

↓

Bacterial degradation alters the composition for the formation of refractory DOM

↓

Processes help preserve DOM and maintain fixed carbon in the ocean.

Sources

- Alan L. Wright and K.R. Reddy2. "Dissolved Organic Matter in Wetlands." *EDIS New Publications RSS*. Soil and Water Science, n.d. Web. 27 Sept. 2016.

Questions?

UNKNOWN MARINE MICROORGANISM FROM THE GULF OF MEXICO CULTURED THROUGH HTC

Background

The "great plate count anomaly" is a theory that there is an immense number of species of microorganisms in the ocean; however, when scientists try to grow them on a plate they are only able to obtain a few of these species (3). There are many explanations for this anomaly. Many species are not able grow *in vitro* due to a short life span, poor adaptation abilities to the culture environment, or competition with other organisms. One way to solve these potential problems is the use of dilution to extinction (DTE) (2). DTE is the severe dilution of microorganisms in order to have one to three cells per culture tube. This creates a better chance of getting cells of the same species in a tube, which increases the odds of attaining a pure culture (2). Another technique that solves these problems is High Throughput Culturing (HTC). HTC is the process of performing DTE many times. Both of these methods decrease the chances of competition within the cultures and make it easier to target a single microbe (2). The goal of our study is to obtain a pure culture and isolate a marine microorganism from the Gulf of Mexico, ideally one that has not already been cultured.

Methods

We took samples from the Gulf of Mexico that were diluted to have about 1 cell/µL and pipetted them into small culture tubes in attempt to isolate and grow microbes using the DTE method. (Fig. 1) This procedure was conducted close to a flame to help prevent contamination. First, we transferred 5 µL of the seawater into seven different inoculation tubes and 5µL of distilled water into a control inoculation tube. We incubated the tubes for two weeks at 30˚C and then, we used the technique of flow cytometry to identify positive growth in the cultures. Of the tubes that showed positive growth, we chose two of them to be transferred into larger flasks that contained more growth medium to encourage further multiplication. We incubated the flasks at 30˚C for two weeks. Sterile Technique was used for all procedures to limit the possibility of contamination. Next we used the MoBio PowerWater® DNA Isolation Kit to extract the DNA from the flasks that continued to have positive growth and performed Polymerase Chain Reaction (PCR) on the 16S rDNA in order to be able to compare the hypervariable regions of the organism's DNA to previously cultured ones. Finally, we sequenced the PCR products using Sanger Sequencing and used BLAST® in order to identify the cultured organism.

Figure 1. Locations of where LSU's Thrash Lab took samples from in the Gulf of Mexico. The red star indicates where the samples our class used for this experiment were taken from.

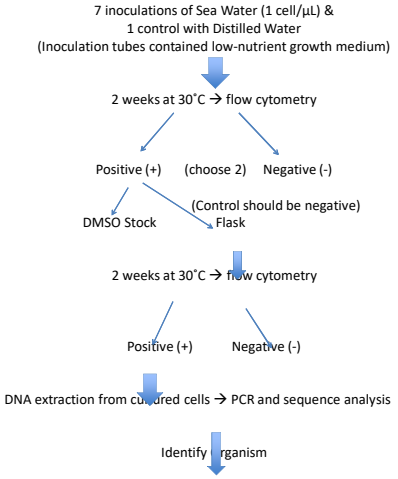


Figure 2. Procedure used to obtain a pure culture of a marine microorganism. The terms positive and negative refer to the culture results after being analyzed by flow cytometry.

Results and Discussion

Five out of our seven original inoculation tubes showed positive growth. The tubes that showed growth were numbers three (A6-3), seven (A6-11), four, six, and two (Table 1). Our control did not show any growth, so we believe that our tubes were not contaminated and the data can be trusted. Out of the five positive inoculation tubes, we chose numbers A6-3 and A6-11 to be transferred into larger flasks to further grow (Table 1). A6-11 showed positive growth, however, A6-3 was negative (Table 1). The DNA extracted from A6-11 yielded a concentration of 0.58 ng/µL (Table 1). The PCR we performed was successful and the negative control did not show a band upon gel electrophoresis. The gel image shows our PCR results (Fig. 1). From our BLAST results (Fig. 2), we discovered that we have possibly cultured a new microorganism that has never been grown *in vitro* before (Table 2). Our top two hits for our BLAST were Uncultured Bacterium Clone LGH02-C3-9-B-24 and *Limnobacter* sp. DG1290. Comparing the two hits, we gathered that the organism we cultured is possibly a new species or strain similar to *Limnobacter* sp. DG1290. *Limnobacter* sp. DG1290 is a b-proteobacteria that is commonly cultured from fresh water or volcanic deposits (3). Since our organism has a 95% identity similarity to *Limnobacter* sp. DG1290 and a 99% similarity to Uncultured Bacterium Clone LGH02-C3-9-B-24, we think that it is possibly a salt-water version of it, potentially a new species of *Limnobacter*. (Table 2). *Limnobacter* is a bacterial genus under the *Burkholderiaceae* family. The *Limnobacter* genus currently holds 99 different species (3). In general, a *Limnobacter* is a thiosulfate-oxidizing heterotrophic bacterium that is generally aerobic and grows on organic substrates, but not autotrophically, (3). In the environment, Thiosulfate is used in extracting silver from ores, manufacturing of paper, de-chlorinating water, and whitening cotton, fabrics, bone, straw, and ivory (4). The increase in industrialization could be causing an increase in the use of thiosulfate in the ways mentioned above. Increasing levels of thiosulfate lead to an increasing growth yield of *Limnobacters* able to grow chemolithoheterotropically by the oxidation of thiosulfate to sulfate (3). This is a probable explanation as to why we were able to culture a *Limnobacter* from a saltwater setting.

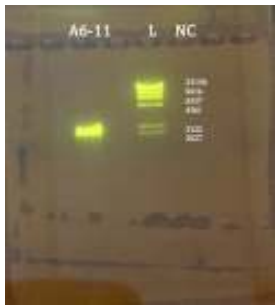


Figure 3. Gel Electrophoresis results from PCR amplification of the 16S rDNA gene in order to help identify the unknown microorganism being cultured. Letters and numbers at the top of each well indicate the sample loaded into the well. A6-11 indicates our culture sample, L indicates the ladder (number to the right are the sizes of bands in base pairs), and NC indicates our negative control.

Conclusions and Future Directions

With the use of HTC and DTE we were able to culture and grow one of our two isolates. These two methods helped up achieve our goal of isolating an organism through the decrease of competition and increase in probability of obtaining a pure culture. Our successful isolate is one that is very similar to *Limnobacter* sp. DG1290. Discovering that *Limnobacters* are mostly found in fresh water and volcanic deposits, future directions could include attempting to grow the *Limnobacter* we cultured, perhaps from the Gulf of Mexico or another setting. Also, one could take samples from locations with high usage of thiosulfate in attempt to discover if higher thiosulfate concentrations increase the amount of *Limnobacters* in that area. This could help determine if locations with usage of thiosulfate promote the growth of *Limnobacters* in that area.

Table 1. Summary of results from the cultured microorganisms. Results from the isolates that showed growth and the name of the isolate if applicable.

Isolate #	Did it transferto a larger volume?	DNA conc. ng/µL	PCR conc. ng/µL	Organism
1: A6-3	No	N/A	N/A	N/A
2: A6-11	Yes	0.58	17.5	*Limnobacter* sp. DG1290

Table 2. BLAST Results. 16S rDNA sequence from sample A6-11 aligned to previously cultured and uncultured microorganisms to help identify the cultured microorganism.

Similar Organism	% Identity	E Value	Query Coverage (%)
Uncultured Bacterium Clone LGH02-C3-9-B-24	99	0.0	100
Limnobacter sp. DG1290	95	0.0	100

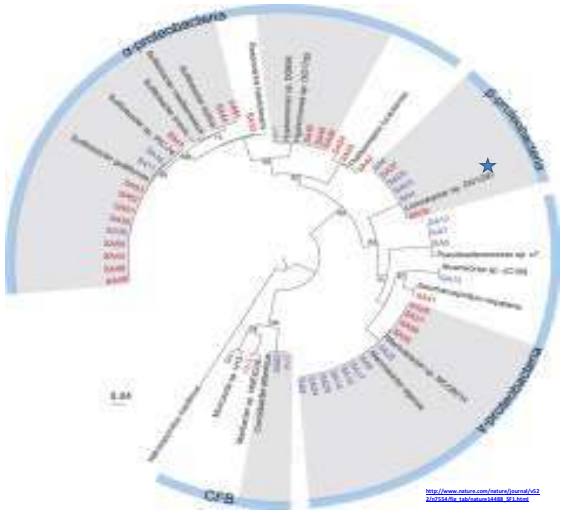


Figure 4. A Phylogenetic Tree of *P. multiseries*-associated Bacteria. *Limnobacter* sp. DG1290, marked with a blue star, was one of our top hits. We would assume the *Limnobacter* we cultured would be close to this species on the tree.

Author Contributions

Student 1: wrote all sections of the poster, formatted the poster, made the tables, did the follow up research about *Limnobacters*, found and cropped the photos, edited the poster, and cited all references.

Student 2: sent in research paper, sent in phylogenic tree photo, helped formulate formatting ideas, and edited the poster.

Acknowledgements

We want to thank our TA, for guiding us along this whole process. We couldn't have done any of this without her direction. Also, we would like to thank the lab for giving us this incredible opportunity of undergraduate research experience.

References

1. Staley JT, Konopka A. 1985. Measurement of in situ activities of non - photosynthetic microorganisms in aquatic and terrestrial habitats. Annual Review of Ecology and Systematics 39: 321–346.
2. Connon, S. A., and S. J. Giovannoni. 2002. "High-Throughput Methods for Culturing Microorganisms in Very-Low-Nutrient Media Yield Diverse New Marine Isolates." *Applied and Environmental Microbiology* 68.8: 3878-885.
3. Spring, S., Kampfer, P. and Schleifer, K.H. 2001. "Limnobacter thiooxidansgen. nov., sp. nov., a Novel Thiosulfate-oxidizing Bacterium Isolated From Freshwater Lake Sediment" *International Journal of Systematic and Evolutionary Microbiology* 51:1463–1470
4. "What Is Thiosulfate Ion, and What Is It Used For?" *General Chemistry Online: FAQ: Simple Compounds:*. Fred Senese, 1997-2010. Web. 27 Nov. 2015.

The Great Unculturable: Dilution to Extinction and High-Throughput Methods of Culturing Microbes Yield *Arthrobacter globiformis* Isolates from the Gulf of Mexico

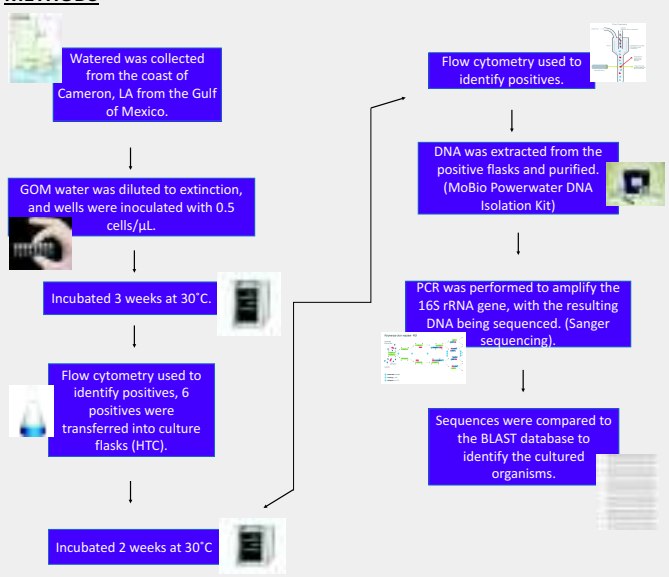
ABSTRACT

Studies show that only a marginal fraction of microorganisms are currently isolated in scientific culture collections. Only about one percent of marine microorganisms have been viable in culture labs, an anomaly referred to as "The Great Plate Count Anomaly"[8]. A solution for solving a microorganism's viability lies with the process of High Throughput and Dilution to Extinction methods of culturing. The purpose of this experiment is to use the natural medium befitting most sea-water microbial samples in order to isolate and discover new strains of DNA. Through this experimentation, we can determine certain strains that can heavily influence immunity to prevalent diseases and can also be used to determine similar life processes shared between microbes and other organisms, including the nitrogen cycle[3].

INTRODUCTION

- "The Great Plate Count Anomaly" refers to the the lack of viability or cultivability amongst collected microbes[8].
- The samples' low magnitude of growth could result from:[1,2]
  1) lack of proper nutrients or conditions in the media
  2) natural processes of the microbes, such as dormancy
  3) improper preparation by the microbiologists
     - contaminating the samples
- Solutions for this phenomenon are:[1]
  1) Dilution to Extinction Culturing (DTE)- diluting a natural sample of media to the smallest possible concentration.
  2) High Throughput Culturing (HTC)- allows a larger number of cultures to be identified so findings or success can be measured through a larger sample of isolates.
- The motivation for this experiment is to discover more information about the vital role microbes play in life-sustaining functions, including the nitrogen cycle.
- The goal of this study is to use Dilution to Extinction and High Throughput methods of culturing to yield undiscovered microbes from the Gulf of Mexico.

METHODS



RESULTS

- Six of our original fourteen inoculation tubes were positive.
- Four tubes of six that were transferred into flasks yielded positives.

* One specific culture lost to experimental error.

- The results of sequence analysis yielded two of the three* samples that were sent off with sequential DNA.
- The DNA extraction from our positive flask yielded a DNA concentration of 2.35 ng/µL and
- 17.2 ng/ µL.
- BLAST sequence analysis of LSUCC3243 and LSUCC3247 PCR products resulted in identification of the samples as two strains of *Arthrobacter*: *globiformis* and *sp.AGF35.*

Table 1: Graph of positive cultures from initial tubes and then culture flasks, including organism found and PCR outcome.

LSU CC #	Did it transfer into a larger volume?	MEDIA	PCR	PCR con. (ug/µL)	Organism	BLAST Identification
3215	YES	MWH3	YES	n/a	n/a	n/a
3236	YES	MWH3	n/a	n/a	n/a	n/a
3243	YES	MWH3	YES	2.35	*Arthrobacter globiformis*	93%
3247	YES	MWH3	YES	17.2	*Arthrobacter sp. AGF35*	99%
3260	YES	MWH2	YES	n/a	n/a	n/a
3262	YES	MWH2	n/a	n/a	n/a	n/a

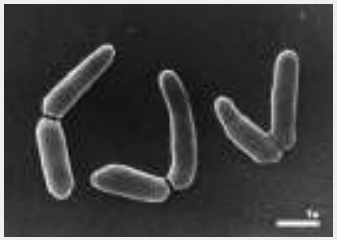


Figure 1 (5): View of *Arthrobacter globiformis* under microscope.*Arthrobacter are* single celled and young cultures are shaped as rods, and yield to more coccoid forms as they mature and as their growth stagnates This view of *A. globoformis,* depicts the generalities in shape of *A. globiformis* as well it's early stage in development.

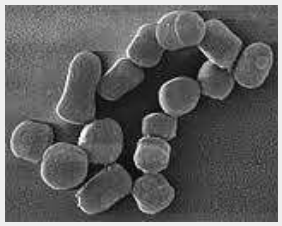


Figure 2 (5): View of adult *Arthrobacter globiformis* under microscope.

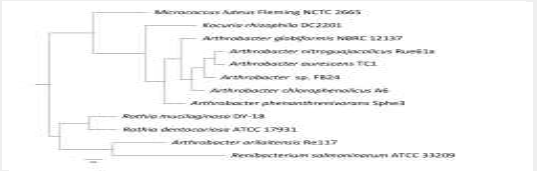


Figure 2 (9): Phylogenetic tree of closest relatives of *Arthrobacter globiformis. A. globiformis* is most closely related to varying strands of *Arthrobacter: nitroguajacolicus, aurescens, chlorophenolicus, and phenanthrenivorans.*

DISCUSSION

Arthrobacter globiformis can be found in key components of sandy, clay, or grassy soil and has the ability to readily reduce any compound found in nature.

APPLICATIONS:

Industry:[5]

1) Crop preservation:
-Ex: sugar cane
-can produce artificial sweeteners
2) Bioremediation:
= biodegradables
-Ex: solving chemical contaminations (i.e, oil spills)
3) Manufacturing:
- effective oil remover (can be used for dish detergents)

Study:

1) Environmentalism:
- part of nitrogen cycle (can help us understand natural cycles)
-Oil consumption can lead to healthier environment
2) Medicine:[3]
- metabolically and adaptably resilient (can help with developing resistance to diseases)
3) Science:
- genetic makeup allows for compatibility with lab equipment and other artificially grown media.

CONCLUSIONS

Dilution to extinction and high-throughput culturing techniques proved successful in the isolation of the novel microbe *Arthrobacter globiformis* from the Gulf of Mexico

•*Arthrobacter*'s genetic resilience allowed for easy culturing by HTC:

- Resilience allows them to be grown on any media, including those that are both natural and artificially produced.

•Some cultures were lost due to experimental error: LSUCC 3215 was lost during DNA extraction, when it was knocked over and sample contaminated. LSUCC 3260 may have been lost due to error during DNA extraction: losing DNA's location in vital step during the extraction process.

FUTURE DIRECTIONS

Continue research in the role of *A. globiformis* in degradation of agricultural pesticides and oil.

Find the evolutionary trait that make *A. globiformis* able to contribute so exponentially to the environment.

ACKNOWLEDGMENTS

We would like to thank our instructor, Austen Webber, for the wonderful guidance throughout this experiment and the abundance of successful handlings.

We would like to thank Dr. Thrash for welcoming us in taking part in his experiment.

We would like to thank Michigan State University for sequencing our DNA strands.

(Author contributions: Liza contributed most of the information and Hannah contributed information as well as inputting everything into PowerPoint format).

REFERENCES

1) **Connon SA, Giovannoi SJ.** 2002. High-Throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl Environ Microbiol **68**:3878-3885.
2) **Deming, J. W., and J. A. Baross.** 2000. Survival, dormancy, and nonculturable cells in extreme deep-sea environments, p. 147-197. *In* R. R. Colwell and D. J. Grimes (ed.), Nonculturable microorganisms in the environment. ASM Press, Washington, D.C.
3) **Guttman, Jared.** 2011. Investigations into the Current Usage of Microorganisms in Medicine. Worchester Polytechnic Institute IQP Report.
4) **Jannasch, H.W., and G.E. Jones.** 1959. Bacterial populations in seawater as determined by different methods of enumeration. Limnol. Oceanogr. **4:** 128-139.
5) **Megharaj, M. et al.** 2003. Toxicity of Hexavalent Chromium and Its Reduction by Bacteria Isolated from Soil Contaminates with Tannery Waste. Current Microbiology, **47**: 51-54
6) **Robinson, Richard**. 2014. *Encyclopedia of Food Microbiology*. Academic Press.
7) **Sperling, Bert.** "Best Places to Live in Cameron, Louisiana." *Best Places to Live in Cameron, Louisiana*. Web. 18 Nov. 2016.
8) **Staley, J.T., and A. Konopka.** 1985. Measurements of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. **39**:321-346.
9) **T. Tamura, T. Nishii & K. Hatano.** Arthrobacter globiformis IFO 12137. "Micrococcus, Microbacterium and related genera including the Nocardioides cluster"