

# **RNA test 8/10/22**

T1-1 pasteroides

brought tube upstairs on ice

took piece out in hood in main CDE lab with flame sterilized forceps

replaced rest of sample in -80

put the chunk in a new 1.5 ml collection tube, but realized we should have put it directly in the provided 2 ml sample tube.

taking out 10 ul and putting the rest in the -80 on very top shelf

Nanodrop- 41.6 ng/ul 2.02 260/280

## **Log experiments repeat**

The following experiments were carried out on the following days, with the following parameters. Concentrations and results can be found in the DATA SHEET notation

### Microbial enrichment According to protocol

#### **6/1/2022**

T1-20 2019 MCAV (Healthy)

T2-16 2019 MCAV (Healthy)

T3-16 2019 MCAV (Healthy)

#### **6/15/2022**

T1-57 2022 MCAV (Healthy)

T3-1 2022 MCAV (Healthy)

T2-10 2022 MCAV (Healthy)

T3-13 2022 MCAV (Healthy)

T3-19 2022 MCAV (Healthy)

#### **6/22/2022**

T1-57 2022 MCAV (Healthy)

T3-1 2022 MCAV (Healthy)

T2-10 2022 MCAV (Healthy)

T3-13 2022 MCAV (Healthy)

T3-19 2022 MCAV (Healthy)

### **7/1/2022**

T1-16 2019 MCAV (Healthy)

T1-24 2019 MCAV (Healthy)

T3-9 2019 MCAV (Healthy)

T3-14 2019 MCAV (Healthy)

T3-15 2019 MCAV (Healthy)

T3-1 2019 MCAV (Healthy)

T3-8 2019 MCAV (Healthy)

### **7/6/2022**

T1-12 2022 MCAV (Margin)

T1-13 2022 MCAV (Diseased)

T1-40 2022 MCAV (Margin)

T1-70 2022 MCAV (Diseased)

T3-48 2022 MCAV (Margin)

T3-49 2022 MCAV (Diseased)

T3-51 2022 MCAV (Diseased)

T3-40 2022 MCAV (Margin)

T3-60 2022 MCAV (Diseased)

T1-1 2019 PAST (Healthy)

T1-8 2019 PAST (Healthy)

T2-21 2019 PAST (Healthy)

T3-31 2019 PAST (Healthy)

T3-7 2019 PAST (Healthy)

### **7/14/2022**

T1-1 2022 PAST (Healthy)

T1-52 2022 PAST (Diseased)

T2-11 2022 PAST (Healthy)

T3-25 2022 PAST (Healthy)

T3-8 2022 PAST (Healthy)

Library preparation - 1 ul of enzyme, 5 minutes fragmentation, cleanup according to >100ng protocol, largest insert size

**6/24/2022**

T1-57 2022 MCAV (Healthy)

**6/27/2022**

T3-1 2022 MCAV (Healthy)

T2-10 2022 MCAV (Healthy)

T3-13 2022 MCAV (Healthy)

T3-19 2022 MCAV (Healthy)

## **DATA SHEET SAMPLE PROCESSING**





22	M C A V	H ea lth y		6/1 5/2 02 2	37	6/ 22 /2 02 2	3.6	1.8	6/2 7/2 02 2	N O	N O				
	60	2	10	Ma y- 22	MC AV	H ea lth y		6/1 5/2 02 2	22. 4	6/ 22 /2 02 2	0. 3	0. 5	6/ 27 /2 02 2	N O	N O
	14	3	13	Ma y- 22	MC AV	H ea lth y		6/1 5/2 02 2	13. 2	6/ 22 /2 02 2	1	0. 5	6/ 27 /2 02 2	N O	N O
	21	3	19	Ma y- 22	MC AV	H ea lth y		6/1 5/2 02 2	38. 2	6/ 22 /2 02 2	6. 3	0	6/ 27 /2 02 2	N O	N O
	8	1	12	Ma y- 22	MC AV	M ar gi n		6/1 0/2 02 2	6.2	7/ 6/ 20 22	0. 1			N O	N O
	8	1	13	Ma y- 22	MC AV	D i se as ed	13 3	6/1 4/2 02 2	5.8	7/ 6/ 20 22	1.7			N O	N O
	15	1	40	Ma y- 22	MC AV	M ar gi n	70	6/1 4/2 02 2	18. 4	7/ 6/ 20 22	5. 2			N O	N O

	15	1	70	M ay - 22	M C A V	M se as ed	Di se as ed	117	6/1 4/2 02 2	95	7/ 6/ 20 22	5. 6			N O	N O
	15	3	48	M ay - 22	M C A V	M ar gi n	M ar gi n	155	6/1 4/2 02 2	38. 3	7/ 6/ 20 22	6. 4			N O	N O
	15	3	49	M ay - 22	M C A V	Di se as ed	Di se as ed	142	6/1 4/2 02 2	41. 9	7/ 6/ 20 22	5. 8			N O	N O
	17	3	50	M ay - 22	M C A V	M ar gi n	M ar gi n	56	6/1 4/2 02 2	0	RE TR Y				N O	N O
	17	3	51	M ay - 22	M C A V	Di se as ed	Di se as ed	104	6/1 4/2 02 2	30. 8	7/ 6/ 20 22	1. 5			N O	N O
	22	3	40	M ay - 22	M C A V	M ar gi n	M ar gi n	139	6/1 4/2 02 2	24. 7	7/ 6/ 20 22	7. 5			N O	N O
	22	3	60	M ay - 22	M C A V	Di se as ed	Di se as ed	187	6/1 4/2 02 2	4.4	7/ 6/ 20 22	(- 0. 35 )			N O	N O
	2	1	1	Ju n- 19	P A S T	H ea lth y	H ea lth y		6/1 7/2 02 2	2.9	7/ 6/ 20 22	14 .6			N O	N O

	19	1	8	Ju n- 19	P A S T	H ea lth y		6/1 7/2 02 2	13. 8	7/ 6/ 20 22	(- 0. 4)		N O	N O
	57	2	21	Ju n- 19	P A S T	H ea lth y		6/1 7/2 02 2	1.4	7/ 6/ 20 22	(- 0. 7)		N O	N O
	34	3	31	Ju n- 19	P A S T	H ea lth y		6/1 7/2 02 2	5.4	7/ 6/ 20 22	(- 0. 25 )		N O	N O
	10	3	7	Ju n- 19	P A S T	H ea lth y		6/1 7/2 02 2	13. 5	7/ 6/ 20 22	2. 3		N O	N O
	2	1	1	May - 22	P A S T	H ea lth y		6/1 7/2 02 2	72	7/1 4/ 20 22	0. 3		N O	N O
	19	1	52	May - 22	P A S T	Di se as ed		6/1 7/2 02 2	8.1	7/1 4/ 20 22	0. 6		N O	N O
	57	2	11	May - 22	P A S T	H ea lth y		6/1 7/2 02 2	141 .7	7/1 4/ 20 22	1. 8		N O	N O
	34	3	25	May - 22	P A S T	H ea lth y		6/1 7/2 02 2	14. 9	7/1 4/ 20 22	21 .2		N O	N O

	10	3	8	M ay - 22	P A S T	H ea lth y		6/1 7/2 02 2	15. 4	7/1 4/ 20 22	2.				N O	N O
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## 06/10/2022 Diseased coral test extraction

T1-17 SSID: Diseased tissue

T1-12 MCAV: Margin tissue

Omega Bio-tek soil kit

T1-12 MCAV margin: 6.7 ng, 2.08 (A260/A280)

T1-17 SSID diseased: 93.4 ng, 1.75 (A260/A280)

## Library preparation Illumina

### Preparation

1 Prepare the following consumables:

Item		
Storage		
Instructions		
ATM	-25°C to -15°C	Thaw on ice. Invert the thawed tubes 3–5 times, and then centrifuge briefly.
TD	-25°C to -15°C	Thaw on ice. Invert the thawed tubes 3–5 times, and then centrifuge briefly.
NT	15°C to 30°C	Check for precipitates. If present, vortex until all particulates are resuspended.

**2**Save the following TAG program on the thermal cycler:

- Choose the preheat lid option and set to 100°C
- Set the reaction volume to 50 µl
- 55°C for 5 minutes
- Hold at 10°C

## Procedure

**1**Add the following volumes *in the order listed* to each well of a new 96-well PCR plate.

- TD (10 µl)

- 1 ng DNA (0.2 ng/μl per sample) (5 μl)

**2**Pipette to mix.

**3**Add 5 μl ATM to each well.

**4**Pipette 10 times to mix, and then seal the plate.

**5**Centrifuge at 280 × g at 20°C for 1 minute.

**6**Place on the preprogrammed thermal cycler and run the TAG program. When the program reaches 10°C, *immediately*proceed to step **7**because the transposome is still active.

**7**Add 5 μl NT to each well.

**8**Pipette 10 times to mix, and then seal the plate.

**9**Centrifuge at 280 × g at 20°C for 1 minute.

**10**Incubate at room temperature for 5 minutes.

## Amplify Libraries

### Preparation

**1**Prepare the following consumables:

Item		
Storage		
Instructions		
Index adapters	-25°C to -15°C	<p>Thaw at room temperature .</p> <p>[Tubes] Vortex to mix, and then centrifuge briefly.</p> <p>[Plates] Spin briefly before use.</p>
NPM	-25°C to -15°C	Thaw on ice for 20 minutes.

**2**Save the following NXT PCR program on a thermal cycler:

- Choose the preheat lid option and set to 100°C
- Set the reaction volume to 50 µl
- 72°C for 3 minutes
- 95°C for 30 seconds
- 12 cycles of:
  - 95°C for 10 seconds
  - 55°C for 30 seconds
  - 72°C for 30 seconds

- 72°C for 5 minutes

- Hold at 10°C

## Procedure

**1**Add the following index adapter volumes per sample according to your index adapter kit type.

Index Adapter Kit Type	Volume of Index Adapter per Sample
Index Adapter Tubes	5 µl i7 adapter 5 µl i5 adapter
Index Adapter Plate	10 µl pre-paired i7 and i5 index adapters

**2**Add 15 µl NPM to each well.

**3**Pipette 10 times to mix, and then seal the plate.

**4**Centrifuge at 280 × g at 20°C for 1 minute.

**5**Place on the preprogrammed thermal cycler and run the NXT PCR program.

## SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

# Clean Up Libraries

## Preparation

**1** Prepare the following consumables:

Item	Storage	Instructions
PB	2°C to 8°C	Let stand on the benchtop for 30 minutes to bring to room temperature. Vortex and invert to mix.
RSB	-25°C to -15°C	Thaw and bring to room temperature. Vortex to mix.  RSB can be stored at 2°C to 8°C after the initial thaw.

**2** Prepare fresh 80% EtOH from absolute ethanol.

## Procedure

**1** Centrifuge at 280 x g at 20°C for 1 minute to collect contents at the bottom of the well.

**2**Transfer 50 µl supernatant from each well of the PCR plate to corresponding wells of a new midi plate.

**3**If you are using standard DNA input, add 30 µl PBto each well containing supernatant.

**4**If you are using small PCR amplicon sample input, add the PBvolume according to your input size in the table below.

Input Size (bp)	AMPure XP Recommendation	AMPure XP Volume (µl)
300–500	1.8x AMPure XP	90
> 500	0.6x AMPure XP (0.5x AMPure XP for $\geq 2 \times 250$ cycles)	30 (25 µl for $\geq 2 \times 250$ cycles)

**5**Seal the plate, and then use a plate shaker at 1800 rpm for 2 minutes.

**6**Incubate at room temperature for 5 minutes.

**7**Place on the magnetic stand and wait until the liquid is clear (~2 minutes).

**8**Without disturbing the beads, remove and discard all supernatant.

**9**Wash two times as follows.

1. **a**With the plate on the magnetic stand, add 200 µl fresh 80% EtOH without mixing.
2. **b**Incubate for 30 seconds.
3. **c**Without disturbing the beads, remove and discard all supernatant.

**10**Use a 20 µl pipette to remove and discard residual EtOH.

**11**Air-dry on the magnetic stand for 15 minutes.

**12** Remove from the magnetic stand.

**13** Add 52.5 µl RSB to the beads.

**14** Seal the plate, and then use a plate shaker at 1800 rpm for 2 minutes.

**15** Incubate at room temperature for 2 minutes.

**16** Place on the magnetic stand and wait until the liquid is clear (~2 minutes).

**17** Transfer 50 µl supernatant to a new 96-well PCR plate.

### **SAFE STOPPING POINT**

If you are stopping, seal the plate with Microseal 'B' adhesive seal or Microseal 'F' foil seal and store at -25°C to -15°C for up to 7 days.

## **Check Library Quality**

**1** Run 1 µl undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA kit.

## **Normalize Libraries**

### **Warning**

**This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.** For additional

environmental, health, and safety information, see the SDS at [support.illumina.com/sds.html](http://support.illumina.com/sds.html).

## Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
LNA1	-25°C to -15°C	Prepare under a fume hood.  Bring to room temperature. Use a 20°C to 25°C water bath as needed.
LNB1	2°C to 8°C	Bring to room temperature. Use a 20°C to 25°C water bath as needed.
LNW1	2°C to 8°C	Bring to room temperature. Use a 20°C to 25°C water bath as needed.
LNS1	Room temperature	Bring to room temperature.

## Procedure

**1**Transfer 20  $\mu$ l supernatant from each well of the PCR plate to the corresponding well of a new midi plate.

**2**Combine the following volumes in a 15 mL conical tube to prepare the LN master mix. Multiply each volume by the number of samples being processed.

•LNA1 (46  $\mu$ l)

•LNA2 (8  $\mu$ l)

**3**Pipette 10 times to mix.

**4**Pour the LN master mix into a trough.

**5**Use a 200  $\mu$ l multichannel pipette to transfer 45  $\mu$ l LN master mix to each well.

**6**Seal the plate, and then use a plate shaker at 1800 rpm for 30 minutes.

**7**Place on the magnetic stand and wait until the liquid is clear (~2 minutes).

**8**Without disturbing the beads, remove and discard all supernatant.

**9**Wash two times as follows.

1. **a**Add 45  $\mu$ l LNW1 to each well.

2. **b**Seal the plate, and then use a plate shaker at 1800 rpm for 5 minutes.

3. **c**Place on the magnetic stand and wait until the liquid is clear (~2 minutes).

4. **d**Without disturbing the beads, remove and discard all supernatant.

**10**Add 30  $\mu$ l 0.1 N NaOH to each well.

**11**Seal the plate, and then use a plate shaker at 1800 rpm for 5 minutes.

**12**Add 30  $\mu$ l LNS1 to each well of a new 96-well PCR plate labeled SGP.

**13**After the 5 minute elution completes, make sure that all samples in the midi plate are resuspended. If they are not, resuspend as follows.

1. **a**Pipette 10 times to mix or lightly tap the sample plate on the bench.
2. **b**Seal the plate, and then use a plate shaker at 1800 rpm for 5 minutes.

**14**Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

**15**Transfer 30 µl supernatant from each well of the midi plate to the corresponding well of the SGP plate.

**16**Seal the sample plate, and then centrifugeat 1000 × g for 1 minute.

## SAFE STOPPING POINT

If you are stopping, seal the plate with Microseal 'B' adhesive seal or Microseal 'F' foil seal and store at -25°C to -15°C for up to 7 days.

## Dilute Libraries to the Starting Concentration

Table 1: Recommended Read Length on IlluminaSystems

Sequencing System	Read Length

\*Assumes use of the 200 cycle kit.

**1**Calculate the molarity value of the library or pooled libraries using the following formula.

- For libraries qualified on a Bioanalyzer, use the average size obtained for the library.
- For all other qualification methods, use 600 bp as the average library size.

$$\frac{ng / \mu l \times 10^6}{660 \frac{g}{mol} \times average\ library\ size\ (bp)} = Molarity\ (nM)$$

**2**Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration for your system.

**3**Dilute libraries using RSB:

- **Libraries quantified as a multiplexed library pool**—Dilute the pool to the starting concentration for your system.
- **Libraries quantified individually**—Dilute each library to the starting concentration for your system. Add 10  $\mu$ l each diluted library to a tube to create a multiplexed library pool.

## Library preparation NEB

### Protocol for FS DNA Library Prep Kit with Inputs $\leq 100$

**Note:** Follow the protocol in this chapter for inputs  $\leq 100$  ng, as size selection is not recommended for this input range.

**Starting Material:** 100 pg–100 ng purified, genomic DNA. We recommend that the DNA be in 1X TE (10 mM Tris pH 8.0, 1 mM EDTA), however, 10 mM Tris pH 7.5–8, low EDTA TE or H<sub>2</sub>O are also acceptable. If the input DNA is less than 26  $\mu$ l, add TE (provided) to a final volume of 26  $\mu$ l.

#### 1.1. Fragmentation/End Prep

Fragmentation occurs during the 37°C incubation step. Use the chart below to determine the incubation time required to generate the desired fragment sizes. Incubation time may need to be optimized for individual samples. See Figure 1.1 for a typical fragmentation pattern.

FRAGMENTATION SIZE		
INCUBATION @ 37°C		
OPTIMIZATION		
100 bp-250 bp	30 min	30-40 min
150 bp-350 bp	20 min	20-30 min
200 bp-450 bp	15 min	15-20 min
<b>300 bp-700 bp</b>	<b>10 min</b>	<b>5-15 min</b>
500 bp-1 kb	5 min	5-10 min

1. Ensure that the Ultra II FS Reaction Buffer is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up, and quickly vortex to mix. Place on ice until use.

2. Vortex the Ultra II FS Enzyme Mix 5-8 seconds prior to use and place on ice.

**Note: It is important to vortex the enzyme mix prior to use for optimal performance.**

3. Add the following components to a 0.2 ml thin wall PCR tube on ice:

COMPONENT	
VOLUME PER ONE LIBRARY	
DNA	26 µl
NEBNext Ultra II FS Reaction Buffer	7 µl
NEBNext Ultra II FS Enzyme Mix	2 µl
<b>Total Volume</b>	<b>35 µl</b>

4. Vortex the reaction for 5 seconds and briefly spin in a microcentrifuge.

5. In a Thermocycler, with the heated lid set to 75°C, run the following program:

**5–30 min @ 37°C**

**30 min @ 65°C**

**Hold @ 4°C**

6. Check on the tape station! --> size distribution

### **Adaptor Ligation**

Determine whether adaptor dilution is necessary.

*If DNA input is < 100 ng, dilute the NEBNext Adaptor for Illumina in 10 mM Tris-HCl, pH 7.5–8.0 with 10 mM NaCl as indicated in Table 1.2.1.*

*Table 1.2.1: Adaptor Dilution*

INPUT		
ADAPTOR DILUTION (VOLUME OF ADAPTOR:TOTAL VOLUME)		
WORKING ADAPTOR CONCENTRATION		
100 ng-500 ng	No Dilution	15 µM
5 ng-99 ng	10-fold (1:10)	1.5 µM
less than 5 ng	25-fold (1:25)	0.6 µM

Check on tape station!

**Note: The appropriate adaptor dilution for your sample input and type may need to be optimized experimentally. The dilutions provided here are a general starting point.**

1. Add the following components directly to the FS Reaction Mixture:

COMPONENT	
VOLUME	
FS Reaction Mixture (Step 1.1.5)	35 µl
NEBNext Ultra II Ligation Master Mix*	30 µl
NEBNext Ligation Enhancer	1 µl
NEBNext Adaptor for Illumina**	2.5 µl
<b>Total Volume</b>	<b>68.5 µl</b>

\* Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.

\*\* The NEBNext adaptor is provided in NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E7600, #E7535 and #E6609) Oligos for Illumina.

**Note: The Ligation Master Mix and Ligation Enhancer can be mixed ahead of time and is stable for at least 8 hours @ 4°C. We do not recommend adding adaptor to a premix in the Adaptor Ligation Step.**

2. Set a 100 µl or 200 µl pipette to 50 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube. **(Caution: The NEBNext Ultra II Ligation Master Mix is very viscous.**

**Care should be taken to ensure adequate mixing of the ligation reaction, as incomplete mixing will result in reduced ligation efficiency. The presence of a small amount of bubbles will not interfere with performance).**

3. Incubate at 20°C for 15 minutes in a thermocycler with the heated lid off.

4. Add 3 µl of **USER® Enzyme** to the ligation mixture from Step 1.2.3.

**Note: Steps 1.2.4. and 1.2.5. are only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Singleplex (NEB #E7350) or Multiplex (NEB #E7335, #E7500, #E7710, #E7730, #E7600, #E7535and #E6609) Oligos for Illumina.**

1.2.5. Mix well and incubate at 37°C for 15 minutes with the heated lid set to ≥ 47°C.

**Samples can be stored overnight at -20°C.**

### **1.3. Size Selection or Cleanup of Adaptor-ligated DNA**

**Note: The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step (71.5 µl; Step 1.2.5.). AMPure XP Beads can be used as well. If using AMPure XP Beads, allow the beads to warm to room temperature for at least 30 minutes before use. These bead volumes may not work properly for a cleanup at a different step in the workflow,**

**or if this is a second cleanup at this step. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.**

1. Vortex AMPure XP Beads to resuspend.
2. Add 57 µl (0.8X) resuspended beads to the Adaptor Ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3–5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.
3. Incubate samples for at least 5 minutes at room temperature.
4. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.
5. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard the beads**).
6. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
7. Repeat Step 1.3.6. once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.
- 1.3.8. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

**Caution: Do not over-dry the beads. This may result in lower recovery of DNA. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.**

9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 17 µl 0.1X TE (dilute 1X TE Buffer 1:10 in water).

10. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 15 µl to a new PCR tube.

12. Proceed to PCR Enrichment of Adaptor-ligated DNA in Section 1.4.

Check tape station!

**Samples can be stored overnight at -20°C.**

#### **1.4. PCR Enrichment of Adaptor-ligated DNA**

1.4.1. Add the following components to a sterile strip tube:

**1.4.1B. Forward and Reverse Primers already combined**

Adaptor Ligated DNA Fragments (Step 1.3.12.)	15 µl
NEBNext Ultra II Q5 Master Mix	25 µl
Index/Universal Primer****	10 µl
<b>Total Volume</b>	<b>50 µl</b>

\* The primers are provided in NEBNext Singleplex ([NEB #E7350](#)) or Multiplex ([NEB #E7335](#), [#E7500](#), [#E7710](#), [#E7730](#), [#E7600](#)) Oligos for Illumina. For use with Dual Index Primers (NEB #E7600), look at the NEB #E7600 manual for valid barcode combinations and tips for setting up PCR reactions.

\*\* For use with NEBNext Multiplex Oligos (NEB #E7335, #E7500, #E7710 or #E7730) use only one index primer per PCR reaction. For use with Dual Index Primers (NEB #E7600) use

only one i7 primer per reaction.

\*\*\* For use with Dual Index Primers (NEB #E7600) use only one i5 Primer per reaction.

\*\*\*\* The primers are provided in NEBNext Multiplex Oligos for Illumina (NEB #E6609). Please refer to the NEB #E6609 manual for valid barcode combinations and tips for setting up PCR reactions.

1.4.2. Set a 100 µl or 200 µl pipette to 40 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

1.4.3. Place the tube on a thermocycler and perform PCR amplification using the following PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation Annealing/Extensio n	98°C 65°C	10 seconds 75 seconds	3-13*
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

\* The number of PCR cycles recommended in Table 1.4.1 are to be seen as a starting point to determine the number of PCR cycles best for standard library prep samples. Use Table 1.4.2 for applications requiring high library yields, such as target enrichment. The number of PCR cycles should be chosen based on input amount and sample type. Thus, samples

prepared with a different method prior to library prep may require re-optimization of the number of PCR cycles. The number of cycles should be high enough to provide sufficient library fragments for a successful sequencing run, but low enough to avoid PCR artifacts and over-cycling (high molecular weight fragments on Bioanalyzer).

Table 1.4.1.

<b>INPUT DNA IN THE FS REACTION</b>	<b># OF CYCLES REQUIRED FOR STANDARD LIBRARY</b>	<b>PREP: YIELD ~100 ng (5-35 nM)*</b>	
<b>INPUT DNA IN THE FS REACTION</b>	<b># OF CYCLES REQUIRED FOR TARGET ENRICHMENT LIBRARY PREP (YIELD ~750 ng-1 μg)*</b>		
100 ng	3-4	100 ng	4-5
50 ng	4-5	50 ng	5-6
10 ng	6-7	10 ng	8-9
5 ng	7-8	5 ng	9-10
1 ng	8-9	1 ng	11-12
0.5 ng	8-10	0.5 ng	12-13
0.1 ng	12-13	0.1 ng	N/A

Run tape station!

## 1.5. Cleanup of PCR Reaction

**Note:** The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. AMPure XP beads can be used as well. If using AMPure XP beads, allow the beads to warm to room temperature for at least 30 minutes before use. These volumes may not work properly for a cleanup at a different step in the workflow. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.

1.5.1. Vortex AmPure XP Purification Beads to resuspend.

1.5.2. Add 45 µl (0.9X) resuspended beads to the PCR reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

1.5.3. Incubate samples on bench top for at least 5 minutes at room temperature.

1.5.4. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

1.5.5. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard the beads**).

1.5.6. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

1.5.7. Repeat Step 1.5.6. once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

1.5.8. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

**Caution: Do not over-dry the beads. This may result in lower recovery of DNA. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.**

1.5.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 33 µl of 0.1X TE (dilute 1X TE Buffer 1:10 in water).

1.5.10. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

1.5.11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 30 µl to a new PCR tube and store at -20°C.

## **1.6. Assess Library Quality on a Bioanalyzer**

1.6.1. Dilute library (from Step 1.5.11.) 5-fold in 0.1X TE Buffer (inputs ≤ 1 ng may not require dilution to run on a Bioanalyzer).

1.6.2. Run 1 µl on a DNA High Sensitivity Chip.

1.6.3. Check that the library size shows a narrow distribution with an expected peak size based on fragmentation time (Figure 1.2).

**Note: If a peak ~80 bp (primers) or 128 bp (adaptor-dimer) is visible in the Bioanalyzer trace, bring up the sample volume (from Step 1.5.11.) to 50 µl with 0.1X TE Buffer and repeat the Cleanup of PCR Reaction in Section 1.5. You may see adaptor-dimer when starting with inputs ≤ 1 ng**

# **NEBNext library prep with enriched dna**

 If DNA input is < 100 ng, dilute the ● (red) NEBNext Adaptor for Illumina in 10 mM Tris-HCl, pH 7.5-8.0 with 10 mM NaCl as indicated in Table 1.2.1.

Table 1.2.1: Adaptor Dilution

INPUT	ADAPTOR DILUTION (VOLUME OF ADAPTOR:TOTAL VOLUME)	WORKING ADAPTOR CONCENTRATION
100 ng-500 ng	No Dilution	15 µM
5 ng-99 ng	10-fold (1:10)	1.5 µM
less than 5 ng	25-fold (1:25)	0.6 µM

## Library prep test illuminate 6/2

Sample name	Concentrat ion 1	Concentration final	Volume DNA	Volume water
T1-20	4.3	0.2	4.7	95.3
T2-16	2.9	0.2	6.9	93.1
T2-21	3.6	0.2	5.6	94.4
T3-16	4.4	0.2	4.5	95.5

Trying to get 1 ng of dna in 5 ul

		i7 -
		02
i5 -	17	1
i5 -	02	2
i5 -	03	3- i703
i5 -	04	4

## Enrichment test

Taking 2 low and 2 high concentrations of past and Mcav

1	sample	ng/ul	volume	ng	Volume beads	Volume protein	final volume	Bead volume
2	T1-20	20.8	20	416	66.56	6.656	91.56	164.808
3	T2-16	6.1	20	122	19.52	1.952	44.52	80.136
4	T2-21	7.5	20	150	24	2.4	49	88.2
5	T3-16	30.1	20	602	96.32	9.632	121.32	218.376
6					206.4			
7								

Have to make up the beads before you add the DNA! Made up one stock then divided into four tubs based on above volumes

Eluted bother enriched microbial dna and host dna. (Put in separate boxes)

Cleaned enrichment with ampure beads, eluded into 40 ul

Post cleaning concentrations:

T1-20 4.3 ng/ul

T2-16 2.9 ng/ul

T2-21 3.6 ng/ul

T3-16 4.4 ng/ul

## **05/24 DNA extraction**

Extraction of *Porites asteorides* (healthy) from Belize

Sample 1: T1-1 - 125 mg

Sample 2: T1-8 - 265 mg

Sample 3: T2-21 - 195 mg

Sample 4: T2-29 - 122 mg

Sample 5: T3-10 - 164 mg

Sample 6: T3-23 - 142 mg

Extract according to Omega Bio-tek Soil protocol

## **5/20 Extraction MCAV t3-14**

P2 buffer was added to the disruptor tube before centrifuging. Observation: foaming, supernatant becomes more whitish (reaction with the skeleton?)

Concentration might not be correct anymore

16.1 ng/ul

A260/A280: 1.98 (slightly too high)

## **05/18 DNA extraction**

Samples:

1: T1-4 *M. cavernosa* DEPLETED - 157 mg

2: T1-20 *M. cavernosa* - 237 mg (unable to separate a piece of coral)

3: T2-16 *M. cavernosa* DEPLETED - 188 mg

4: T2-28 *M. cavernosa* - 108 mg

5: T3-8 *M. cavernosa* - 167 mg

6: T3-16 M. cavernosa - 111 mg (ACCIDENTAL DOUBLE, MISREAD THE TABLE)

Extract using Omega Bioteck Soil kit

Step 3: due to a small oversight, centrifuge + RNase step were skipped on accident

Final concentrations:

Sample	Name	Concentration (ng/ul)	A260/280 ration
1	T1-4	11.9	1.94
2	T1-20	20.8	1.81
3	T2-16	6.1	1.92
4	T2-28	4.7	1.81
5	T3-8	7.8	1.82
6	T3-16	21.8	1.94

# 05/13/2022

PCR with brute force

Repeat the Kapa mix, this time add 5 uL of FULLY THAWED DNA

# 05/12/2022

2 negative kapa, 2 with DNA previously used, 2 with the DNA Sarah used:

1- negative 1

2 - SSID-2

### 3- SSID-1 (?)

4- T3-28 SSID

5 - T1-15 SSID

6 - negative 2

## Primers: our own primers

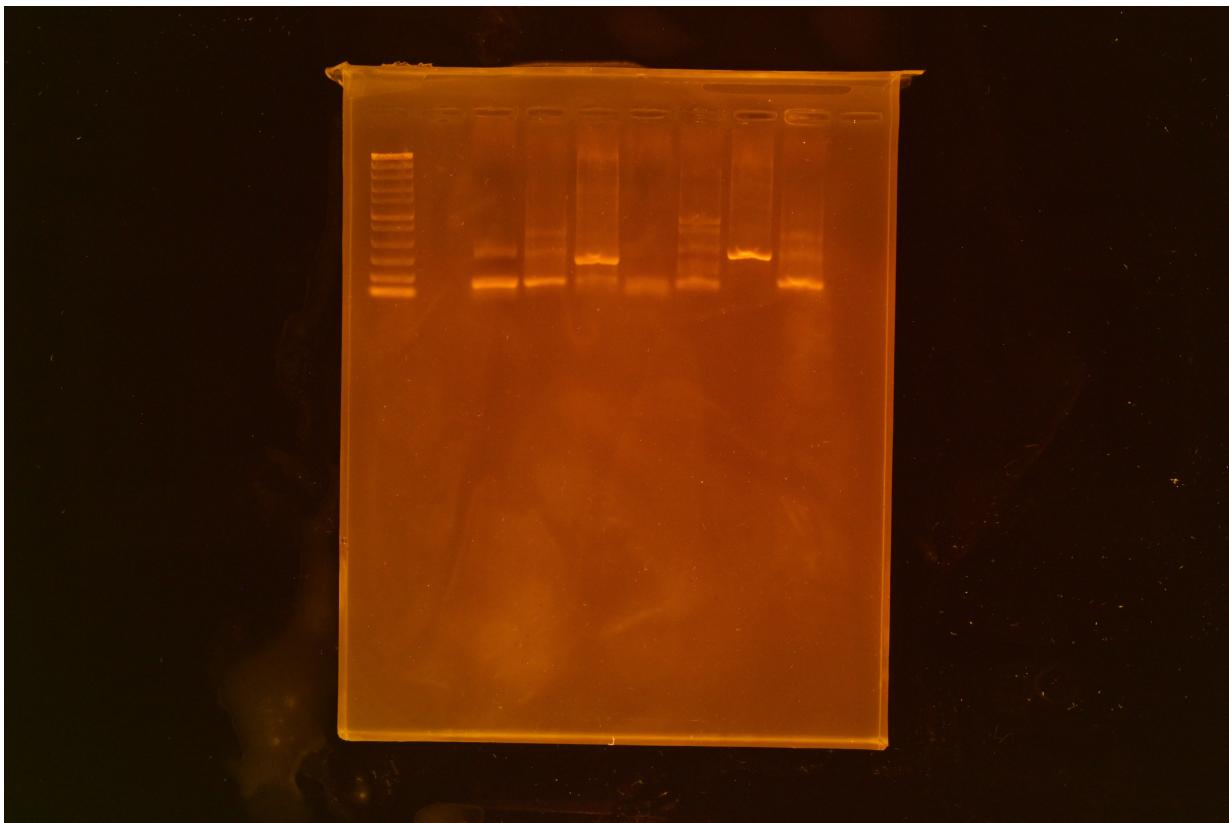
1uL of DNA

Volume 1st pcr to add	volume water to add						
1	9.5						
<b>Initial MM</b>							
#samples	water	divided by 2 (needed when kapa)		divided by 2	Check volume is correct		
6	59.85	29.925	78.75	39.375		25.1	
#rows	#columns	RowMM kapa (vertical)	RowMM N50xx index (vertical)	ColumnMMKapa (horizontal)	ColumnMM N7xx index n(horizontal)		
8	12	138.6	12.72			92.4	8.48
<b>Volume of each mm to add</b>							
12							

# 05/03 PCR Kapa

40.95 ul of Water

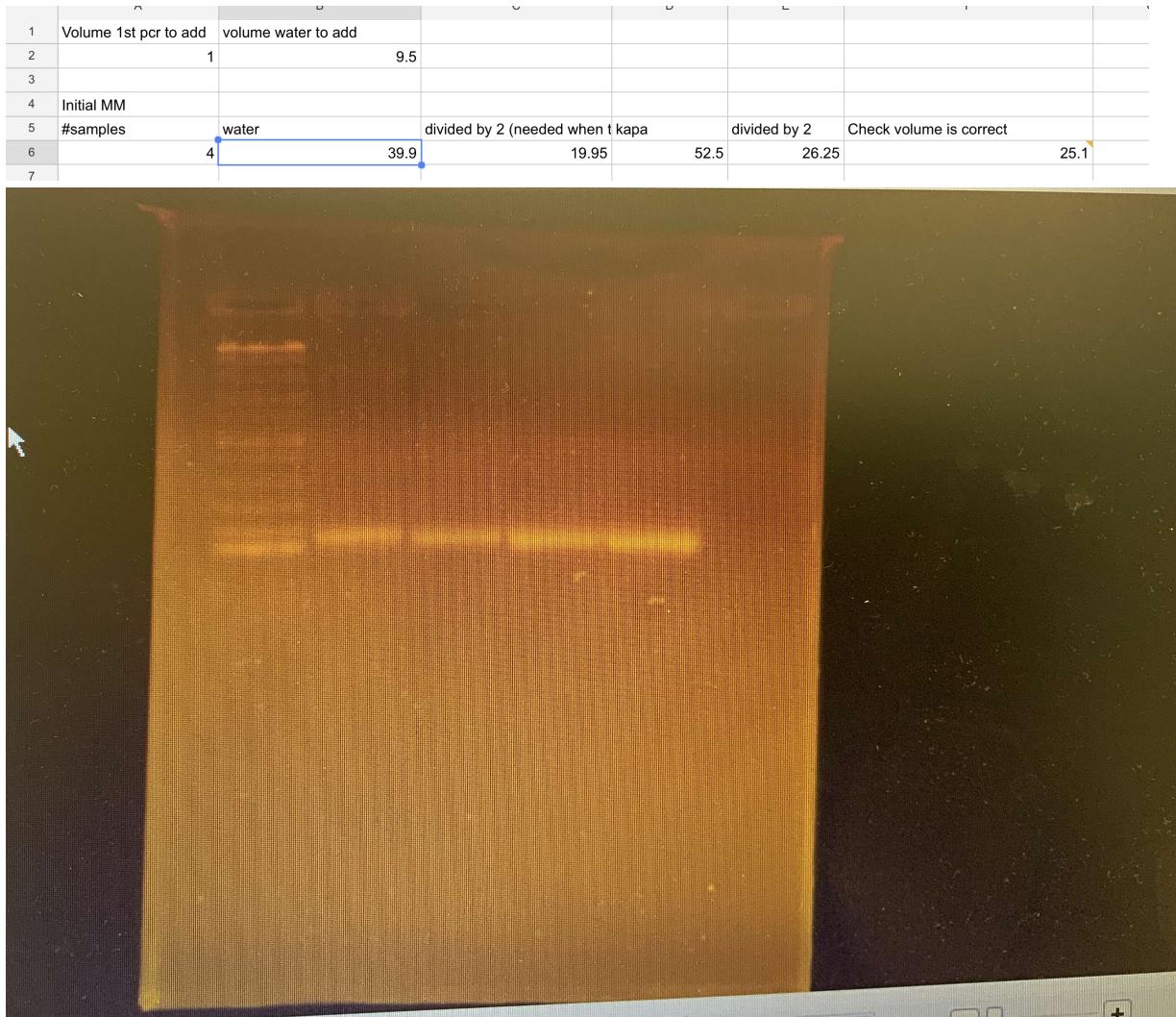
58.95 ul kapa



## 5/10/22 PCR

SGW trying pcr with kappa master mix

2 DNA samples, 2 negative controls



## 04/28 PCR check

Run PCR on 2 Siderea samples with NEB primers

Also run 1 with our normal primers

Run 2 blanks: 1 with NEB and 1 with our primers

**Dilute NEB primers first!!! --> 2x**

Reagent	Amount	MM amount	#Samples
Buffer	2.5	13.75	5
dNTP (10 mM)	0.5	2.75	
MgCl2	1.5	8.25	
F primer (10 uM)	1	5.5	
R primer (10 uM)	1	5.5	
DNA	1		
Polymerase	0.125	0.6875	
Water	17.125	94.1875	
BSA	0.25	1.375	
Total MM:		132	24
		25	

Primer amounts NEB: 3.5 (only once, diluted!)

Primer amounts our own: 2.5

Sample 1 - NEB blank

Sample 2 - NEB SSID-2

Sample 3 NEB T3-28 S.Sid

Sample 4 Own blank

Sample 5 pos. control T3-28 S.sid

## PCR contamination check

04/26/2022

	A	B	C	D	E
1	Reagent	Amount	MM amount	#Samples	triplicate
2	Buffer	2.5	28.75	11	
3	dNTP (10 mM)	0.5	5.75		
4	MgCl <sub>2</sub> <i>thijs steg</i>	1.5	17.25		
5	F primer (10 uM)	1	11.5		
6	R primer (10 uM)	1	11.5		
7	DNA	1			
8	Polymerase	0.125	1.4375		
9	Water	17.125	196.9375		
10	BSA	0.25	2.875		
11		Total MM:	276	24	
12			25		
13					
14					

In which the reaction will not run without Taq and primers, and dNTPS

Reagent	Amount	MM amount
Buffer	2.5	21.25
dNTP (10 mM)	0.5	4.25
MgCl <sub>2</sub>	1.5	12.75
F primer (10 uM)	1	8.5
R primer (10 uM)	1	8.5
DNA	1	
Polymerase	0.125	1.0625
Water	17.125	145.5625
BSA	0.25	2.125
	Total MM:	204
		25

Make initial Master Mix with: water, buffer, mgCl, and Taq and DNTPs

Split into 4 equal parts of 15 ul:

Group 1: Add BSA and our primers

Group 2: Add 2.6 ul of water and our primers

Group 3: Add NEB primers and 2.6 ul of water

Group 4: our primers and 2.6 ul of water

Replicate 2

## 4/21 PCR amplification test

	A	B	C	D	E
1	Reagent	Amount	MM amount	#Samples	triplicate
2	Buffer	2.5	28.75	11	
3	dNTP (10 mM)	0.5	5.75		
4	MgCl <sub>2</sub> <i>this steg</i>	1.5	17.25		
5	F primer (10 uM)	1	11.5		
6	R primer (10 uM)	1	11.5		
7	DNA	1			
8	Polymerase	0.125	1.4375		
9	Water	17.125	196.9375		
10	BSA	0.25	2.875		
11		Total MM:	276	24	
12		25			
13					
14					

1. P.ast T3-12
2. Ssid T3-28
3. Dstr T1-29
4. SSid-1 DR
5. PStr 7 DR
6. Past T2-25
7. Ssid T1-15
8. DSTR-T1-30
9. SSID-2 DR
10. PSTR-4 DR
11. Negative control

# Gel check + first data samples

## 04/20/2022

First data samples - ladder - samples 04/19

B	E	F	G	H	I	J	M
1	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
2		55.5 ng/ $\mu$ l		1.11	1.894	0.59	0.13
3	sample2	48.7 ng/ $\mu$ l		0.974	1.65	0.59	0.12
4	sample4	51 ng/ $\mu$ l		1.02	1.72	0.59	0.12
5	sample5	68.4 ng/ $\mu$ l		1.368	2.004	0.68	0.15
6	sampleT3-4	60.1 ng/ $\mu$ l		1.203	2.03	0.59	0.13
7	sampleT3-21	79.9 ng/ $\mu$ l		1.597	2.498	0.64	0.15
8							
9							
10							
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29							
30							
31							
32							
33							

# DNA extraction 4/19/2022

**Sample 1** - Porites asteroides - Belize - T2-25 - 142.8 mg

**Sample 2** - Siderastrea siderea - Belize - T1-15 - 150.8 mg

**Sample 3** - Diploria strigosa - Belize - T1-30 - 146.1 mg

**Sample 4** - S. siderea DR - SSID-2 - 143.7 mg

**Sample 5** - P. strigosa DR - PSTR-4 -

Sample 4 remains fairly cloudy, even after spinning down with cHTR reagent and P2 buffer. This resulted in a clogged filter downstream, so therefore the centrifuge time was increased with an additional 2 minutes for step 7.

Sample 3 is of lower concentration due to a pipetting error: it has an end volume of 125  $\mu$ l instead of 75  $\mu$ l

The screenshot shows a Microsoft Excel spreadsheet titled "Omega Biotek 04-19.xml - Microsoft Excel". The table contains data for 11 samples, with columns for Sample ID, Nucleic Acid Conc. (Unit), A260, A280, 260/280, 260/230, Sample Factor, and DNA concentration. The DNA concentration column is highlighted in blue.

	B	E	F	G	H	I	J	K	L	M	N	O	P
1	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Factor					
2	Biotek2_sample1		7.9 ng/ $\mu$ l	0.159	0.072	2.19	0.13	DNA	50				
3	Biotek2_sample2		11.5 ng/ $\mu$ l	0.23	0.11	2.09	0.9	DNA	50				
4	Biotek2_sample3		35.6 ng/ $\mu$ l	0.712	0.362	1.97	1.89	DNA	50				
5	Biotek2_sample4		93.2 ng/ $\mu$ l	1.864	0.997	1.87	2.06	DNA	50				
6	Biotek2_sample5		42.6 ng/ $\mu$ l	0.853	0.446	1.91	2.11	DNA	50				
7	Biotek2_sample1		9.5 ng/ $\mu$ l	0.189	0.076	2.5	0.13	DNA	50				
8	Biotek2_sample2		11.1 ng/ $\mu$ l	0.222	0.102	2.19	0.89	DNA	50				
9	Biotek2_sample3		39.4 ng/ $\mu$ l	0.788	0.415	1.9	1.68	DNA	50				
10	Biotek2_sample4		94.4 ng/ $\mu$ l	1.888	1.019	1.85	2.08	DNA	50				
11	Biotek2_sample5		44 ng/ $\mu$ l	0.88	0.465	1.89	1.96	DNA	50				
12													
13													
14													
15													
16													
17													
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21													
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35													
36													
37													

## Notes on DNA extraction protocol

1. Add 100-250 mg soil sample to a Disruptor Tube.

2. Add 725 µL SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse samples.

3. Centrifuge at 500g for 5 seconds to remove drops of liquid from the lid.

4. Add 72 µL DS Buffer. Vortex to mix thoroughly.

*Supposedly, this lyses bacteria and fungi for further treatment. Wonder how much DNA is extracted from animal cells and viruses. It is the detergent that, together with the mechanical lysis, disrupts the membranes completely. I would add RNase here*

5. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.

*I think this is thermo-inactivation of DNases and other disruptive proteins.*

6. Centrifuge at 10,000g for 5 minutes at room temperature.

*Removal of cellular debris*

7. Transfer 400 µL supernatant into a new 1.5 mL microcentrifuge tube (not provided).

8. Add 135 µL chilled P2 Buffer and 200 µL cHTR Reagent. Vortex to mix thoroughly. Note: P2 Buffer must be chilled on ice before use. Completely resuspend cHTR Reagent by shaking the bottle before use. It may be necessary to cut the end of the pipette tip to aspirate and dispense cHTR Reagent.

*Removal of humic acid, metals, etc. Or I would add RNase here*

9. Centrifuge at maximum speed ( $\geq 13,000\text{g}$ ) for 1 minute.

10. Transfer cleared supernatant (~500 µL) to a new 1.5 mL microcentrifuge tube. Note: If supernatant still has a dark color from the soil, perform the following steps for a second cHTR Reagent step. This will require additional cHTR Reagent that can be purchased separately. • Add 200 µL cHTR Reagent. Vortex to mix thoroughly. • Centrifuge at maximum speed ( $\geq 13,000\text{g}$ ) for 1 minute. • Transfer cleared supernatant to a new 1.5 mL microcentrifuge tube. • Continue with Step 11.

11. Add an equal volume XP1 Buffer. Vortex to mix thoroughly.

*Stabilisation of the mixture for DNA binding to column*

### **Rest of the protocol is washing and eluting, so no real improvement to be made**

Notes from Weber, DeForce, & Apprill, 2017:

*Optimization of DNA extraction for advancing coral microbiota investigations*

Species: *Porites lobata* (collected in Micronesia), *Pocillopora verrucosa* (Micronesia), *Acropora humilis* (Australia), *Orbicella faveolata*, *Montastraea cavernosa*, *Orbicella annularis*, and *Diploria strigosa* (Florida Keys)

Kits used: Powersoil, PowerBiofilm, Plant Tissue, Ultraclean Blood & Tissue

PowerBiofilm and Ultraclean Blood &Tissue were best. UC doesn't exist any longer. Overall, they have lower yields than we do??? Highest concentration is 18.5 ng/ul

#### On viruses:

Most of the things I found claim that virus coats will disintegrate upon major acidity changes in the environment they find themselves in. Analysis of the data that we have will have to prove this. Lysis of viruses is a challenge but may be achieved with proteinases.

## To do

### Hydra/BioIT - Metagenomes

- Install DAS\_tools
- Install BBTools
- Fix Kaiju installation-jq dependancy
- CHECK WHETHER READS NEED QUALITY FILTERING AND CRY IF THEY DO
- Run extra quality control
  - Fix script
- Run assembly for extra quality control
- Write conditional MegaHIT script
- Run MetaBAT for filtered Cavernosa
- Run MetaBAT for unfiltered Cavernosa
- Run CONCOCT for non-complex Cavernosa
- Run MetaBAT for non-complex Cavernosa
- Run CheckM for filtered Cavernosa MetaBAT
- Run CheckM for unfiltered Cavernosa MetaBAT
- Run CheckM for non-complex Cavernosa CONCOCT
- Run CheckM for non-complex Cavernosa MEtaBAT
- Fix Kaiju
  - Install Kaiju Mar database
- Normalize reads using BBNorm
  - Fix metaspace issue
- Run assembly with normalized reads
- Run checkM on DAS\_tools
- integrate collection DAS\_tools into Anvi'O profile
- Add taxonomy to Anvi'O profile
- Run DAS\_tools on filtered samples
- Run best binning practice MetaBAT to decide best approach

- Move Anvi'0 taxonomy database to overarching database
- FastQC screen to remove host reads
- Figure out how to pull the 16s gene from the metagenome assembly
- Write down all possible bugs and errors you might get from this pipeline

### BioIT - 16S 2017

- Set up DADA2 on Hydra
- Read DADA2 documentation
- Demultiplex data
- Read Sarah GitHUB link
- Download NCBI sequences + upload to HYDRA
- Figure out if the option --split-3 works better for gathering read data
- Run quality trimming
- Write Cutadapt script
- Make Cutadapt job file
- Write DADA2 script
- Check paths in scripts
- Annotate code
- Discuss with Sarah about pooling samples

### Lab work

- Make safety label Qiagen Powersoil
- Make safety label Monarch
- Find Sarah's notes
- Purify ethanol leftovers from samples
- Look into clean-up procedures for post-purified DNA
- Make new labels for coral samples
- Sort coral boxes
- Select samples to be extracted
- Look at different library kits
- Find extra boxes for diseased coral samples

### Literature

- Lewis 2018
- Thornhill 2006
- Endozooicomonas reading
- Difference pangenomics and metagenomics
- Link between Endozocomonas and sulfur metabolites

### WUR report

- Introduction

- Material & Methods
- Results
- Discussion
- Abstract

# DNA extraction 04/11/2022 -04/13/2022

DNA extraction from the following species:

Porites asteorides - Belize **Sample 1**

Siderastrea siderea - Belize **Sample 2**

Diploria strigosa - Belize **Sample 3**

Sidestrea siderea - DR **Sample 4**

Diploria Strigosa - DR **Sample 5**

Using the following kits:

E.Z.N.A Soil (Omega Biotek) **EZNA Soil** (<https://www.omegabiotek.com/product/soil-dna-extraction-kit-e-z-n-a-soil-dna-kit/?cn-reloaded=1> )

Monarch Genomic DNA Purification Kit (New England Biolabs) **Monach**

(<https://www.neb.com/products/t3010-monarch-genomic-dna-purification-kit#Product%20Information> )

DNeasy Blood & Tissue (Qiagen) **Blood & Tissue**

([https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dnearly-blood-and-tissue-kit/](https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit/) )

DNeasy Powersoil Kit (Qiagen) **Powersoil** (<https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dnearly-powersoil-pro-kit/> )

	<b>EZNA Soil</b>	<b>Powersoil</b>	<b>Monarch</b>	<b>Blood &amp; Tissue</b>
<b>Sample 1</b>	170 mg	180 mg	30 mg	27 mg
<b>Sample 2</b>	82 mg	108 mg	31 mg	21 mg
<b>Sample 3</b>	229 mg	237 mg	29 mg	29 mg
<b>Sample 4</b>	184 mg	188 mg	20 mg	30 mg
<b>Sample 5</b>	129 mg	100 mg	26 mg	29 mg

Note EZNA: Disruption of coral fragments takes far longer than 5 minutes. Manual vortexing was performed until no large fragments were left

Lystate was cloudy. Some fragments seem to contain skeleton particles. Lysate of Sample 2 seemingly lighter than the rest (seems correct with lower amount)

Note Monarch: Sample 1 has less volume due to a high amount of debris that could not be removed using a pipette

Note Blood and Tissue: After adding ethanol and vortexing, samples were spun down and supernatant transferred to a new clean microcentrifuge tube, to avoid membrane clogging downstream (3 minutes at maximum rpm). IMPORT: Step 2 and 3 were switched by accident. Might affect downstream steps

Note Powersoil: step 8, no pellet forms, but small flocculation occurs, which will not settle, even at higher speeds. Continued regardless. C3 solution does the same thing, so assuming that samples are relatively devoid of C2 contaminants

Gel:

Top row: Biotek soil, power soil 1-5

Bottom row: blood and tissue, monarch 1-5

**Note:** Would it matter that Qiagen Powersoil samples spent overnight in the fridge?

B	E	F	G	H	I	J	K
Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type
2 Bioteck1	7 ng/μl		0.139	0.07	2	0.37	DNA
3 Bioteck2	15.6 ng/μl		0.312	0.149	2.09	0.83	DNA
4 Bioteck3	60.4 ng/μl		1.208	0.648	1.86	0.88	DNA
5 Bioteck4	60.7 ng/μl		1.214	0.636	1.91	1.19	DNA
6 Bioteck5	145.6 ng/μl		2.913	1.591	1.83	1.53	DNA
7 Power1	3.3 ng/μl		0.065	0.014	4.59	0.76	DNA
8 Power2	11.4 ng/μl		0.228	0.099	2.3	1.12	DNA
9 Power3	16.4 ng/μl		0.327	0.162	2.02	1.29	DNA
10 Power4	5.9 ng/μl		0.118	0.044	2.69	1.03	DNA
11 Power5	27.1 ng/μl		0.542	0.282	1.93	1.47	DNA
12 Blood1	28.2 ng/μl		0.564	0.288	1.96	0.36	DNA
13 Blood2	11 ng/μl		0.22	0.081	2.71	0.15	DNA
14 Blood3	14.3 ng/μl		0.285	0.122	2.35	0.23	DNA
15 Blood4	10.7 ng/μl		0.214	0.091	2.37	0.17	DNA
16 Blood5	8.7 ng/μl		0.173	0.055	3.13	0.15	DNA
17 Monarch1	36.7 ng/μl		0.734	0.4	1.83	1.64	DNA
18 Monarch2	134.5 ng/μl		2.69	1.46	1.84	2.12	DNA
19 Monarch3	2 ng/μl		0.041	0.018	2.24	0.07	DNA
20 Monarch4	1.7 ng/μl		0.034	0.018	1.89	0.25	DNA
21 Monarch5	4.2 ng/μl		0.084	0.05	1.69	0.69	DNA
22							
23							
24							
25							
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Samples with a 260/280 close to 2 might be RNA contaminated. Generally, Biotek values seem fine, but might be worth it to add a RNA lysis step.

260/230: Anything between 1.8 and 2.5 is considered clean (so only Monarch 2 would be considered clean). Minor protein contamination lowers the value a little. Guadanine thiocyanate may lower the 260/230 ratio significantly, as do non-ionic detergents.

DNA extraction experiment						
EZNA Soil Kit	ng/ul	Total V	ng DNA	mg tissue	ng/mg tissue	
	1	7	50	350	170	2.1
	2	15.6	50	780	82	9.5
	3	60.4	50	3020	229	13.2
	4	60.7	50	3035	184	16.5
	5	145.6	50	7280	129	56.4
<b>Powersoil</b>						
	1	3.3	75	247.5	180	1.4
	2	11.4	75	855	108	7.9
	3	16.4	75	1230	237	5.2
	4	5.9	75	442.5	188	2.4
	5	27.1	75	2032.5	100	20.3
<b>Blood &amp; Tissue</b>						
	1	28.2	75	2115	27	78.3
	2	11	75	825	21	39.3
	3	14.3	75	1072.5	29	37.0
	4	10.7	75	802.5	30	26.8
	5	8.7	75	652.5	29	22.5
<b>Monarch</b>						
	1	36.7	75	2752.5	30	91.8
	2	134.5	75	10087.5	31	325.4
	3	2	75	150	29	5.2
	4	1.7	75	127.5	20	6.4
	5	4.2	75	315	26	12.1

Look at metagenomics protocols for degraded dna do we want to clean up?

