A.cer in nutrients and heat stress

Ana Palacio, Caroline Dennison, Celia Leto, Prati Rosen

Contents

Collection and maintenance

July 24, 2017

Adapting *Acropora cervicornis* to laboratory conditions

- Corals were obtained from MOTE laboratory from various locations in the Florida Keys
- MOTE provided 6 tagged genotype
  - Yellow (30)
  - Orange (31)
  - Green and Orange (31)
  - Red and Yellow (31)
  - Green (30)
  - Red and Orange (30)
- corals were added to a tank with other *A. cervicornis* kept at 26-27ºC with two pumps and build my LED light at 100% intensity

---

July 25, 2017

Epoxy

- Genotypes were epoxied together on clipboards and labelled to prevent the pumps from blowing the fragments into no man's land

---

July 26, 2017

Tagging fragments

- Plastic numbered tags + glue + previous epoxy

IPAM Time 0

- First IPAM data taken by CD and AP
  - The fu*(&^% IPAM is not working in 1/4 of the field
  - Average YII values ~ 0.6, one genotype is slightly lower than 0.6
- No pics taken

July 31, 2017

Death

- Corals 185, 200, 275, 279

Aug 1, 2017

New lights

- All the A.cer, including the old fragments were fed moved to tank 1A with the new lights.
- Same HOBO to follow temps

Aug 11 and 12, 2017

Death!

- Rapid tissue necrosis in many fragments

Genotype	Control	N	NP	Dead
Green	10	9	9	2
Green and Orange	10	10	9	2
Orange	6	6	6	13
Red and Orange	9	9	9	3
Red and Yellow	4	4	5	18
Yellow	3	3	3	21
Total	42	41	41	59

SAMPLING POINT TIME 1 (BASE LINE BEFORE EXPERIMENT STARTS)

Aug 30, 2017

IPAM Time 1

- Data IPAM taken by CD, AP and Becca using the lab (fixed) machine
- Pictures with color and size scale

Tissue samples Time 1

- Razor blade samples by PR

Buoyant Weight Time 1

- Data taken by CD and AP with CL scale
- YSI data taken every 10 fragments

Aug 31, 2017

Prok incubation of all samples T=1

- 100uL aliquotes + 6uL of prok / 3 hours

Sep 2-12, 2017

IRMA!!

- The corals were not cleaned or fed until 12th when Phil could get into RSMAS

Sep 18, 2017

- Cleaned and fed all the Acer, they look ok in general

Sep 19, 2017

- Samples 136 and 145 had mortality on the base, they were cut and re-glued
- Sample 192 fall and were re-glued

Oct 05, 2017

- Organic extraction and 1st EtOH from sample 101 to 236 by CD
- New 10 gallon aquaria and pumps set up

Oct 06, 2017

- 2nd EtOH from sample 101 to 236 by AP and PR
- Organic extraction and 1st EtOH from sample 236 to 300 by AP and PR

Oct 07, 2017

- 2nd EtOH from sample 237 to 300 by AP
- Make stock solutions and started the pumps.
- Took water samples for nutrient analysis day 1

Oct 08, 2017

- Took water samples for nutrient analysis day 2

Oct 09, 2017

- EtOH wash from sample 101 to 300 by AP and BG
  - 101 to 165 (BG)
  - 106 to 300 (AP)
- All the samples are in 100uL of 0.1X TE
- Took water samples for nutrient analysis day 3
- Cleaned all fragments

Oct 10, 2017

IPAM 2 for Acer and Pictures w color scale

* Values are lower vs Sep 1st (IPAM 0)

Oct 11, 2017

Preliminary qPCR (only symbionts)

- Clades A, B, C and D were tested for 2 samples of each genotype
  - F: 136, 139
  - Green: 176, 179
  - GO: 116, 119
  - Orange: 149, 1152
  - RO: 162, 166
  - RY: 204, 102
  - Yellow: 143, 231
- Results: Samples 162, 143, 139, 119 and 166 have one replicate with late D amplification, keep and eye on background, but all the corals are pretty much A

Oct 15, 2017

New Nutrient stocks

- Made more Stock 1 and Stock 2 Nutrients
  - Stock 1 N: 4.011825g of NH4Cl + 250mL of RO water
  - Stock 1 P: 1.034925g of NaH2PO4.H2O + 250mL of RO water
  - Stock 2 N: 21mL of Stock 1 + 987mL of RO water
  - Stock 2 NP: 21mL of N_Stock 1 + 42mL of P_Stock 1 + 945mL of RO water
- New Stock 2 solutions were attached to the pumps at 4:06PM to follow volume delivery for 24hours

---

Oct 18, 2017

Tissue samples Time 3 for Acer

- Samples taken by PR
-
- SDS incubation

Buoyant weight 3 for Acer

- Data taken by CD, PR and AP

Oct 19, 2017

IPAM 3 for Acer

- Values are higher than time 2, but still lower than time 0

ProK incubation samples Time 3

- Prok by CD

2017-10-27 ## Oct 23, 2017

Corals moved inside aquaria, not nutrients added yet

Oct 25, 2017

Organic Extraction of 77 samples by CD and PR

- R1 and some from R2

Oct 26, 2017

2nd EtOH for 77 samples

- R1 and some from R2
- AP and AG

Oct 27, 2017

EtOH wash 77 samples

- R1 and some from R2
- Tested some samples for other clades (contamination) with ER plate 2 SSID. Samples are contaminated with CD

Organic Extraction

- Last 57 Samples with new Prok, new CTAB and New NAOH. If there is contamination it is in the SDS...

Oct 28, 2017

EtOH wash 57 last samples

- Samples labeled with red sharpie

qPCR

- Acer 181, 232 checked in qPCR for clade C and D contamination

---

Nov 6, 2017

IPAM 4 for Acer

- Values are higher than 0.6!! and than time 0!
- The corals look great! Ready to start the experiment!

Nov 10, 2017

Buoyant weight before sampling

- Data taken by CD and BG

Nov 15, 2017

Tissue samples T=5

- Acer by AP

Pictures T=5

Nov 16, 2017

IPAM T=5

- Values are lower than time 4 :/ May be because they were sampled one day before?

Buoyant weight after sampling

- Data taken by CD and AP

STARTED NUTRIENT ADDITION!!!

Nov 17, 2017

- To start concentrations:
  - N: 1mL of NH4Cl [Stock 1 = 300mM] in 30L filtered sea water
  - NP:1mL of NH4Cl [Stock 1 = 300mM] + 1mL NaH2PO4 [Stock 1 = 30mM] in 30L filtered sea water
- To keep concentrations
  - N: 24mL of NH4Cl / day [Stock 2 = 6.25uM]
  - NP: 24mL of NH4Cl + NaH2PO4 / day [Stock 2 = 6.25uM + 1.25uM]

Nov 19, 2017

Fed Acer

- Inside the aquariums with the larvae food

Nov 20, 2017

ProK incubation

- All Acer samples by CD

Nov 22, 2017

Fed Acer

- Inside the aquariums with the larvae food

Nov 23, 2017

IPAM time = 6

- AP and CD
- Small increase in general

Water change and horizontal rotation

Nov 27, 2017

Mesenterial filaments out in NP

- Mesenterial filaments were out only in the NP treatment
- The water was murky
- Opened the new water income and let it run for a long time. Then water was removed and added more stock 1 nutrients

---

Nov 28, 2017

Fed Acer

- Inside the aquariums with the larvae food

Nov 29, 2017

IPAM time = 7

- AP and BG
- Small increase in general

Water change and vertical rotation

Water sample for nutrient analysis

```
* ~ 50 mL from each aquarium
```

Dec 06, 2017

IPAM time = 8

- PR and CD
- Constant values

Water change and vertical rotation

Pictures with color scale

- One coral was found broken

Dec 13, 2017

IPAM time = 8

- CD and AP
- Values look stable, control R2 is a bit lower

Water change and horizontal rotation

- Celia

Buoyant weight before samples

- BG and PR

Dec 14, 2017

Tissue samples + Picture with color scale

- Taken by AP
- Three polyps per coral

Dec 17, 2017

ProK incubation Acer R1 and begging R2

- 100uL sample + 10uL Prok
- Control, N and NP - R1
- Control-R2, + samples N R2 (236-278)

Water change and vertical rotation

- AP

New Stock 2 of nutrients

-

AP

Dec 18, 2017

Death

- Fragment 237 in NP R2

}

Dec 19, 2017

ProK incubation Acer R2

- from 285 to 235 (38 samples) with R2 Ssid

Dec 20, 2017

Organic extraction last Acer R2 from Dec 14

- from 285 to 235 (38 samples) with R2 Ssid
- CTAB AP- 1st EtOH PR

Dec 21, 2017

Organic extraction Acer R1 and begging of R2 from Dec 14

- from 137 to 154 (96 samples)
- AP

Second EtOH last Acer R2 from Dec 14

- from 285 to 235 (38 samples) with R2 Ssid
- AP

Cleaned tanks

- AP, corals left without nutrients

Dec 22, 2017

Second EtOH Acer R1 and begging of R2 from Dec 14

- from 137 to 154 (96 samples)
- AP

EtOH wash last Acer R2 from Dec 14

- from 285 to 235 (38 samples) with R2 Ssid
- AP

Cleaned corals

- AP, horizontal rotation

Dec 27, 2017

Fed corals + vertical rotation

Jan 03, 2018

Fed corals + horizontal rotation

- With S.D

IPAM time = 10

- SD and AP
- Values look stable, but control R2 declined more

Jan 05, 2018

New nutrient stock (1) and (2)

- Replaced stock 2 in pumps

Jan 08, 2018

Water change with non filtered water

- Added more stock 1

Organic Extraction samples from 11-15

- All R1 and begging of R2 (Control and few N)
- Samples 137-187
- With S.D

Jan 09, 2018

Acer (Dec) qPCR with all 4 clades

- CD
- Samples in the left rows did not amplified ok
- B positive did not amplified

Jan 10, 2018

2nd ETOH from 11-15

- All R1 and begging of R2 (Control and few N)
- Samples 137-187
- With S.D

Acer (Dec) qPCR with B, C and D clades

- CD
- We figured that the MM was not mixed, maybe that was the problem?
- B did not amplified in any positive control

Fed corals and stayed in extra tank for cleaning next day

- CD and SD

Jan 11, 2018

ETOH wash from 11-15

- All R1 and begging of R2 (Control and few N)
- Samples 137-187
- With S.D

Acer (Dec) qPCR with A and Acer

- CD
-

Cleaned corals + vertical rotation

- CD and SD

Jan 16, 2018

Cleaned tanks + horizontal rotation

- CD and SD

Mesenterial filaments were out, specially in N and NP

- We figured that evaporation may increase salinity??

Acer buoyant weight

- CD and SD
- We did not take BW data after sampling last time, so this will only be useful as initial values for the next time point

Organic Extraction last batch of samples from 11-15

- Most of N and all NP from R2
- 39 Samples from 278 to 235 plus - control
- S.D

Jan 17, 2018

Second EtOH, last batch of samples from 11-15

- Most of N and all NP from R2 (S.D)
- 39 Samples from 278 to 235 plus - control Se ### Second Pictures of N weird tissue lost in N
- CD

Jan 18, 2018

EtOH wash last batch of samples from 11-15

- Most of N and all NP from R2 (S.D)
- 39 Samples from 278 to 235 plus - control

Water change

- CD, the corals were left in holding tank to IPAM next day
- Corals were fed

Jan 19, 2018

ITS-2 PCR last batch of samples from 11-15

- Most of N and all NP from R2 (S.D)
- 39 Samples from 278 to 235 plus - control

IPAM T=11

- CD and AP
- Values look stable, but control R2 does not recovered

Corals back to treatments

- Corals moved inside the tanks and nutrients added
- Vertical rotation
- We bleached the whole recirculationg system

Jan 20, 2018

3 plates of Acer to test Pax-C and CAM primers

- See R project

Recirculation drained and added fresh water

Jan 22, 2018

last BW NP R2 data is bullshit

Jan 23, 2018

Water change by CD + horizontal rotation

- Corals in control(both replicates) had mesenterial filaments out
- Another coral in Nitrogen R2 is showing signs of tissue loss

· Temperature and Salinity measurements taken using YSI from Langdon Lab

- Control ** R1: 26.1, 37.2 R2: 26.3, 36.9
- Nitrogen ** R1: 26.1, 36.9 R2: 26.2, 36.9
- NP ** R1: 26.2, 37.0 R2: 26.2, 36.8

Jan 25, 2018

IPAM Time=12

- AP and CD

Jan 26, 2018

Nutrient Stock Replenished/HOBOs checked

- Changed the bottles, but used stock made before.

Jan 28, 2018

Water change + vertical rotation

- Fed corals
- Corals (both replicates) had mesenterial filaments out
- AP and CL

Jan 29, 2018

BW Before Samples->Sample-> BW after samples (Time = 13)

- BW Before Samples by CD and AP
- 3 polyps samples by AP
- BW After Samples by CD and SD

ProK incubation samples Time = 13

- By SD and CD

Jan 30, 2018

IPAM T=13

- CD and AP

Feb 01, 2018

Organic extraction of Acer replicates 1 and 2 from Jan 29 sampling

- CD

RAMPING UP

Feb 02, 2018

Second EtOH precipitation of Acer (both replicates) from Jan 29

- SD

Thermal stress begins (26 –> 27)

- AP

Feb 03, 2018

EtOH wash and resuspension of Jan 29 samples

- 1st 16 re-suspended in 0.1xTE (made by AP) and the 2nd 16 re-suspended in 1xTE (made by SD)
- SD

Feb 05, 2018

Took samples for blasting and put them in -80

- chose 1 sample per genotype (where available)

Water changes and horizontal AND vertical rotation

- done by AP and PR

Took water samples after nutrients were added for analysis

- done by PR

Temperature increase from 27 to 28

- corals were kept at 27 over the weekend

Feb 06, 2018

Acer corals found to be in wrong treatments for ~24 hours

- Control (R2) was in NP (R1)
- Control (R1) was in Control (R2)
- N (R1) was in Control (R1)
- NP (R1) was in N (R1)

Temperature increase 29

Feb 07, 2018

IPAM Time=14

- IPAM done by CD and AP
- Corals were at 29 degrees and temperature increased to 30 at the end of the day
- So far no decline in YII values for Acer corals in any treatment

Water samples taken

- CD

Feb 08, 2018

Water samples taken

- SD

qPCR from Dec samples

- Plates 12 and 13 by CD
- A-actin and Acer CaM SYBER assays

Feb 09, 2018

qPCR from Dec samples

- Plates 14 to 17 by CD
- A-actin and Acer CaM SYBER assays

Feb 11, 2016

Temperature set to 31

Feb 12, 2016

IPAM + Pictures Time=15

- IPAM done by CD and AP
- Corals in N and NP keep losing tissue. Two corals completly dead (164 and 135)

Salinity and Temperature

- Control 1: 40 - 30.6
- Control 2: 39.9 - 30.6
- N1: 40.3 - 30.7
- N2: 39.8 - 30.6
- NP1: 39.9 - 30.7
- NP2: 35.5 - 30.5

Water change

- Vertical and horizontal rotation

Feeding + Cleaning

- PR, SD, AP

Feb 13, 2018

Salinity and Temperature

- Control 1: 34.4 - 30.6
- Control 2: 34.5 - 30.7
- N1: 34.6 - 30.6
- N2: 34.4 - 30.7
- NP1: 34.5 - 30.6
- NP2: 34.6 - 30.8

Feb 14, 2018

Salinity and Temperature

- Control 1: 34.8 - 31.4

- Control 2: 35.4 - 31.7
- N1: 35.3 - 31.4
- N2: 35.2 - 31.6
- NP1: 35.2 - 31.5
- NP2: 35.3 - 31.7

BW corals at 31 degrees

- weighted by CD and Celia

Feb 15, 2018

Salinity and Temperature

- Control 1: 36.6 - 30.6
- Control 2: 36.7 - 30.6
- N1: 36.4 - 30.9
- N2: 36.2 - 30.6
- NP1: 36.3 - 30.9
- NP2: 36.5 - 30.7

IPAM and pictures with color scale time=16

- IPAM done by CD and AP

Water changes

- Vertical and horizontal shift of aquaria
- Done by AP and CD

Stopped nutrient addition

Corals with tissue loss were flash frozen for future gene expression work

---

Feb 18, 2018

- Added fresh water to aquaria

~ 100mL of RO water was added to each one

Feb 19, 2018

- Blasted 13 Acer fragments (CD, CL and AP)

Salinity and Temperature before WC

- Control 1: 37.4 - 31.1
- Control 2: 38.1 - 31.5
- N1: 37.9 - 31.2
- N2: 38.2 - 31.5
- NP1: 38.2 - 31.2
- NP2: 38.0 - 31.5

IPAM and pictures with color scale time=17

- IPAM done by CD and AP

Water changes + Feeding

- Vertical and horizontal shift of aquaria
- Done by AP and PR

Feb 20, 2018

Salinity and Temperature

- Control 1: 35.1 - 31.5
- Control 2: 35.5 - 31.8
- N1: 35.3 - 31.6
- N2: 35.4 - 31.8
- NP1: 35.5 - 31.6
- NP2: 35.4 - 31.7

Feb 21, 2018

Salinity and Temperature

- Control 1: 36.1 - 31.5
- Control 2: 36.0 - 31.7
- N1: 36.0 - 31.7
- N2: 36.1 - 31.8
- NP1: 36.2 - 31.6
- NP2: 36.1 - 31.7
- RO water added to aquaria to reach target salinity (34.5-35.0)

Feb 22, 2018

IPAM and pictures with color scale time=18

- IPAM done by CD and AP

Water changes and feeding

- Vertical and horizontal rotation

Got HOBOs data from Jan-Feb

Feb 23, 2018

Buoyant weight

- Done by CD and AP

Razor blade samples taken

- Done by AP

Salinity and Temperature

- Control 1: 35.4 - 31.7
- Control 2: 35.7 - 31.6
- N1: 35.6 - 31.8
- N2: 35.8 - 31.6
- NP1: 35.9 - 31.8
- NP2: 35.8 - 31.6
- RO water added to aquaria to reach target salinity (34.0-34.3)

Feb 25, 2018

- Added ~ 1 cup of RO water

Feb 26, 2018

Salinity and Temperature

- Control 1: 35.8 - 31.7
- Control 2: 35.8 - 31.6
- N1: 36.0 - 31.7
- N2: 36.2 - 31.6
- NP1: 36.4 - 31.7
- NP2: 36.3 - 31.6

Blasted 15 Acer fragments

- done by CD, AP, SD and CL

IPAM and pictures with color scale time=19

- done by CD, AP, and SD

Water changes and feeding

- Vertical and horizontal rotation

Feb 27, 2018

Salinity and Temperature

- Control 1: 34.4 - 31.5
- Control 2: 34.6 - 31.6
- N1: 34.9 - 31.6
- N2: 34.8 - 31.7
- NP1: 35.0 - 31.6
- NP2: 34.8 - 31.7

Chlorophyll for 15 blasted samples on Feb 26, 2018

- Done by CD and AP

Feb 28, 2018

Salinity and Temperature

- Control 1 35.5 - 31.6
- Control 2: 35.6 - 31.6
- N1: 35.8 - 31.6
- N2: 35.7 - 31.6

- NP1: 35.8 - 31.6
- NP2: 35.8 - 31.6

CTAB incubation, organic extraction, and 1st EtOH precipitation of Acer samples taken on Feb 23, 2018

- Done by CD and AP

Mar 01, 2018

Water changes and feeding

- Vertical and horizontal rotation

IPAM and Pics T=20

- Done by CD and AP

2nd EtOH precipitation and wash of Acer samples taken on Feb 23, 2018

- Done by CD and SD

Mar 02, 2018

Sampled dying corals

- Acer 282, 288, 221, 177, 123, and 178 were sampled and frozen

---

Mar 03, 2018

Salinity and Temperature (adjusted with ~ 2 cups of RO)

- Control 1 36.2 - 31.3 (35.3)
- Control 2: 34.4 - 31.6 (35.4)
- N1: 35.8 - 31.7 (35.4)
- N2: 36.2 - 31.6 (35.4)
- NP1: 36.4 - 31.7 (35.3)
- NP2: 36.3 - 31.6 (35.4)

Sampled dying corals

- Acer 117, 288, 221 were sampled and frozen

Mar 05, 2018

Water changes and feeding

- Vertical and horizontal rotation

IPAM and Pics T=21

- Done by CD and AP

ProK removed fragments

- 117, 288 and 221

Mar 06, 2018

Tissue samples

- Taken by AP, only controls and some NP are left
- SDS incubation

Organic Extraction (Initial blastate samples)

- SD and CD

Mar 07, 2018

Acer initial cell counts diluted

- 1st batch was already diluted in ~ 1/2 by CD
- 1st and 2nd batch were further diluted in 1/4
- Dilutions by CL

Salinity and Temperature

- Control 1: 37.0 - 31.5
- Control 2: 37.0 - 31.7
- N1: 37.0 - 31.5
- N2: 37.2 - 31.7
- NP1: 37.1 - 31.6
- NP2: 37.1 - 31.8

ProK incubation 3/6/18 samples

2nd EtOH (Initial blastate samples)

- SD

Mar 08, 2018

Female corals strike

Salinity and Temperature

- Control 1: 35.9 - 31.4
- Control 2: 35.4 - 31.6
- N1: 35.6 - 31.3
- N2: 35.7 - 31.6
- NP1: 35.5 - 31.4
- NP2: 35.7 - 31.7

Bouyant W before recovery

- Controls R1 and R2, few NP2
- Corals have been sampled 2x since last BW, so we need to correct for this loss

IPAM and pictures with color scale T=22

- AP and CD

Took fragments for blasting and put in -80

- AP and CD

Mar 12, 2018

qPCR for Acer samples taken on 2/23/2018

- Acer - SYBR, A - SYBR
- Quant Studio 3 machine
- Run by CD

Mar 13, 2018

qPCR for Acer samples taken on 2/23/18 and blastate samples

- Acer - SYBR, A - SYBR
- Quant Studio 3 machine
- Run by CD

CTAB incubation, Organic extraction, and 1st EtOH for Acer samples taken on 3/6/18

- Done by CD

Mar 14, 2018

2nd ETOH for Acer samples taken on 3/6/18

- Done by SD and CD

Mar 15, 2018

Corals in recovery moved outside the aquaria and put on recirculating system

- Ssid and Ofav dip in insecticide

Lights with calibrated lightmeter

- Control 1: Right top: 321
- Control 2: Right bottom: 420
- N1: Left, top: 350
- N2: Left, bottom: 365
- NP1: Center, top: 345
- NP2: Center, bottom: 435

Mar 16, 2018

qPCR run for Acer blastate and sick samples

- Run by CD

Mar 19, 2018

EtOH wash and resuspension of DNA samples taken on 3/6/18

- Done by CD

Mar 20, 2018

Blasted all Acer fragments collected on Mar 08

- done by CD, AP, SD

Blasted all Ofav fragments collected on Mar 08 (bleaching)

- done by CD, AP, SD
- Samples taken from the -80
- Took alliquots for:
  - Cell counts (0.5mL + 50uL lugols)
  - DNA (0.5mL + 1/2(500DNAB + 83uL of 10% SDS)) SDS incubation for 1:30 at 65C
  - Chlorophyll A (~ 3-4mL filtered + 4mL of Methanol)
  - Lipids (~ 3-4mL filtered + moved back to -80), some samples did not have enough blastate for lipids

SDS incubation of blastate samples

Mar 21, 2018

Chl-a for Acer fragments collected on Mar 08

- done by CD, AP

PCR ITS-2 selected samples to sequence symbionts
*** 2 fragments per genotype**

- Done by SD

Mar 22, 2018

qPCR for Acer Nutrients Plates 23 and 24

ProK incubation of Acer blastate taken on Mar 08, 2018 (bleaching)

- Done by SD

Mar 23, 2018

qPCR for Acer Nutrients Plates 25 and 26

Mar 25, 2018

Last IPAm before babaies come back to the ocean (T=23)

- CD and AP

Mar 26, 2018

They took them back to the nursery :/

- Good bye babies... Giraffe will be watching you!
- Make your mom's proud!!!!

:'(

Mar 28, 2018

qPCR plates 27 and 28 for Acer Nutrients

- Sybr Green for Acer/A

Apr 11, 2018

1:8 dilutions for Acer cell count aliquots BEFORE BLEACHING

- Old dilutions were thrown out
- 20uL of cells, 130uL DNAB, 10uL of lugols
- done by CD

Cell counts for Acer before bleaching

- CL

Apr 12, 2018

Water samples taken from experiment tank and cleaning tank (5B) to measure DIC and TA for insight to what CO2 levels are in system

- Samples taken from incoming water, recirculating system, aquarium with pumps, and aquarium with pump and bubbler
- 8 samples taken total (1 replication)
- Done by Emma Pontes and new lab manager in Langdon Lab

Apr 22, 2018

1:5 dilutions for Acer cell count aliquots AFTER BLEACHING

- 20 uL of cell, 75 uL DNAB, 5 uL of lugols
- Done by CD

Apr 26, 2018

Copy number estimation for Symbiodinium type A using 2 strategies:

- PCR A, B, C and D standards at CR2-60 (PR) using actin primers... was supposed to use the M13 primers to amplify the plasmids :(

1. Using DNA already extracted and estimating the number of cells/ul

Spp	Colony	Treatment	Replicate	Fragment	CELLS/ul archive	CELLS/ul DNA	uL for 8000 cells/10uL	Take 10uL DNA and add water
A. cer	Green	NP	R2	181	1734.56	1656.51	20.71	10.71
A. cer	Green	Control	R2	290	1432.25	1367.80	17.10	7.10
A. cer	Orange	N	R1	158	1513.72	1445.60	18.07	8.07
A. cer	Green and Orange	Control	R1	129	969.13	925.51	11.57	1.57
A. cer	Green	Control	R1	189	896.23	855.90	10.70	0.70
A. cer	Red and Yellow	NP	R2	110	866.21	827.23	10.34	0.34

- Found the DNA samples and caculated the amount of DNA to be diluted with water to get a target of 800 cells/uL using a DNA extraction efficiency of 95%

2. Extracting DNA from balstate of known of cells/ul

Spp	Colony	Treatmen	Replicate	Fragment	CELLS/ul archive	Aliquote for 100.000 cells	Add SDS	Volumen of extraction
A. cer	Green	NP	R2	181	1734.56	57.65	142.35	100000 cells in 100TE
A. cer	Green	Control	R2	290	1432.25	69.82	130.18	100000 cells in 100TE
A. cer	Orange	N	R1	158	1513.72	66.06	133.94	100000 cells in 100TE
A. cer	Green and Orange	Control	R1	129	969.13	103.19	96.81	100000 cells in 100TE
A. cer	Green	Control	R1	189	896.23	111.58	88.42	100000 cells in 100TE
A. cer	Red and Yellow	NP	R2	110	866.21	115.45	84.55	100000 cells in 100TE

- Got 5 replicate aliquots (equivalent to 100.000 cells) of each blastate and ProK

Apr 27, 2018

Copy number estimation for Symbiodinium type A using 2 strategies

- Standars (actin) gel check (A, B, D worked, rePCR C)
- PCR A, B, C and D standards at CR2-57 with M13 primers (SD)

1. Using DNA already extracted and estimating the number of cells/ul

- Dilute DNA to get target concentration (SD)

2. Extracting DNA from balstate of known of cells/ul

- Organic extraction adding SDS to get a 200uL archive volume (SD)

Apr 28, 2018

Copy number estimation for Symbiodinium type A using 2 strategies

- Standars (actin) gel check (A, B, D worked, rePCR C)
  - Nanodrop and calculate initial dilution for D, but do not have A, B amplicon lenght or C PCR product :/
- Standars (M13) gel check (A, B, C, D worked, for almost all the samples)
  - Samples 5, 6 7,8 worked better in general, however, A2 did not worked at all
  - Samples A1, B1 and B2, C1-4 abd D1, D3, D4 had very weak amplification.

2. Extracting DNA from balstate of known of cells/ul

- 2nd EtOh and EtOh wash (AP)

Apr 29, 2018

Copy number estimation for Symbiodinium type A strategy #2

- PCR clean up all samples (SD)
- Nanodrop (3 times) the A standards (SD)
  - Sample A4 had a very variable concentration, discarded

Standard Sample	ng/uL	Vector+Amplicon (Da/copy]	Da/copy	ng/copy	Copy/uL	Copy/uL *10^10	TE Added	Final Volume of 10^10/uL
A1 (22-3)Big	43.63	860	567600	9.43E-10	46294238598.8363	4.63	36.29	46.29
A2 (22-4)Big	48.00	860	567600	9.43E-10	50927199069.7679	5.09	40.93	50.93
A3 (22-3)Sm	45.97	860	567600	9.43E-10	48769866331.3958	4.88	38.77	48.77
A4 (22-4)Sm	Fucked	860	567600	9.43E-10	??	#VALUE!	#VALUE!	#VALUE!

- Bring the samples to 10^10 concentration and make serial dulitions
  - 10^8: 5uL of 10^10 + 495 0.1XTE
  - 10^6: 5uL of 10^8 + 495 0.1XTE
  - 10^4: 5uL of 10^6 + 495 0.1XTE
  - 10^2: 5uL of 10^4 + 495 0.1XTE
  - 10^1: 5uL of 10^2 + 45 0.1XTE
- SYBER plate with the Standars and samples extracted by duplicate
- Copy number estimation ~ 9, but high SE

Apr 30 - May 2, 2018

Recount for high SE Acer cell counts at all timepoints

- Done by CD

qPCR for Ssid Nutrients Plates 32-35

- done by CD

May 03, 2018

qPCR of multiple times to compare flourescence

*Fragments 184, 152, 160 abd 265

May 16, 2018

New positive (+) control made for qPCR with A,B,C,D symbionts

- 20 uL of OfavSC (Ofav Plate 16 columns 7-12) qPCR product
- 10 uL of A1 standard actin cleaned PCR product
- 10 uL of A2 standard actin cleaned PCR product
- 10 uL of B actin standard #3 PCR product
- 10 uL of B actin standard #4 PCR product
- 10 uL of D actin standard #3 PCR product
- 10 uL of D actin standard #4 PCR product
- 20 uL of Acer CaM (Acer Plate 31 rows BCD column 1)
- 10 uL of C standard #3 M13 PCR product
- 10 uL of C standard #4 M13 PCR product
- Total volume of 100 uL then cleaned using Wizard SV Gel and Clean-up System protocols
- Done be CD and AP

May 17, 2018

qPCR for Acer Nutrients from 03/06/18

- Check for A,B,C,D symbionts post bleachin and to rule out contamination
- Master mix: Actin primers an probes and EMM

May 21, 2018

PCR stock made

- Done by CD

1:10 dilutions of the super positive made on 05/16/18

- 10 uL of (+) and 90 uL of UP water

May 22, 2018

PCR for Ssid, Ofav, and Acer Nutrient using Ssid PaxC, OfacSC and AcerCaM primers respectively

- this will be used for copy #, fluormetry corrections, and specialized/species super positive qPCR sample

- Done by CD

Jul 2, 2018

PCR a subset of samples to look at the bacterial community

- PCR + gel. All the samples worked, there is not - control Samples: 284, 217, 102, 231, 187, 120, 232, 234, 188, 128, 104, 233

Jul 3, 2018

PCR cleanup and qubit

-