

UNIVERSITY OF ICELAND

Microbes in Icelandic Marine Environments

Authors:

Mia CERFONTEYN
Clara JÉGOUSSE
Anouk LYVER

Supervisors:

Viggó Þór MARTEINSSON
René GROBEN
Pauline VANNIER

Last compiled: March 22, 2018 by Clara Jégousse

Contents

I 2018	2
1 March	3
1.1 March 1, 2018	3
1.1.1 Extraction with gentle bead beating and isolation of total DNA and RNA with AllPrep Mini Kit from micro-algae culture	3
1.1.2 DNA and RNA quantification with NanoDrop® ND-1000 Spectrophotometer	5
1.1.3 DNA quantification with Qubit™ DNA BR Assay Kit	7
1.1.4 RNA Quantification with Qubit™ RNA BR Assay	8
1.2 March 3, 2018	9
1.2.1 Macromolecular composition of cells with R	9
1.3 March 5, 2018	11
1.3.1 Pick up samples collected during winter survey	11
1.3.2 Cleaning teaching lab and setting up filtration system	11
1.4 March 6, 2018	11
1.4.1 Filtering seawater on Sterivex™ filters for metagenomic studies	12
1.4.2 Effect of Lugol Iodine	13
1.5 March 7, 2018	13
1.5.1 Filtering seawater from ST4	13
1.5.2 Filtering seawater from SB2	13
1.6 March 8, 2018	14
1.6.1 Sampling equipment inventory	14
1.6.2 Cleaning 10L bottles	15
1.6.3 Meeting with Hróar about the taxes	15
1.6.4 Cleaning teaching lab and putting away filtration system	15
1.7 March 9, 2018	15
1.7.1 Modeling diverse communities of marine microbes (?)	15
1.8 March 10, 2018	15
1.8.1 Inventory of samples collected during winter survey	16
1.9 March 11, 2018	16
1.9.1 MasterPure™ complete DNA and RNA purification	16
1.10 March 13, 2018	19
1.10.1 DNA and RNA quantification with NanoDrop® ND-1000 Spectrophotometer	19

1.10.2	DNA quantification with Qubit™ DNA BR Assay Kit	19
1.10.3	Quantification with Qubit™ RNA BR Assay	21
1.10.4	Finishing samples inventory	21
1.11	March 15, 2018	22
1.11.1	Inventory of nucleic acid samples	23
1.11.2	SYBR® Gold nucleic acid gel stain	23
1.11.3	Checking integrity of nucleic acids on agarose gel	24
1.12	March 16, 2018	26
1.12.1	Nucleic acid integrity assessment by electrophoresis	26
1.13	March 17, 2018	29
1.13.1	RNA integrity assessment by electrophoresis	29
1.14	March 18, 2018	32
1.14.1	Océans: une usine chimique qui se dérègle	32
1.15	March 20, 2018	32
1.15.1	Qiiime2 tutorials	33
1.15.2	Lab supervisors meeting	33
1.15.3	Extraction and purification of genomic DNA and total RNA from Sterivex™ filter with modified AllPrep® method	34
1.15.4	DNA and RNA quantification with NanoDrop® ND-1000 Spectrophotometer	36
1.15.5	DNA quantification with Qubit™ DNA BR Assay Kit	37
1.15.6	RNA quantification with Qubit™ DNA BR Assay Kit	37
1.16	March 21, 2018	37
1.16.1	Nucleic acid integrity assessment	38
1.17	March 22, 2018	40
1.17.1	Amplification by PCR with OneTaq® polymerase and Earth Microbiome Project (EMP) primers of DNA extracted with modified AllPrep® method	40
1.17.2	Migration of nucleic acids in agarose gel	43
1.17.3	Woman in science	44

People Index	45
---------------------	-----------

File Index	46
-------------------	-----------

Author Index	47
---------------------	-----------

Tag Index	48
------------------	-----------

Part I

2018

1 | March

1.1 March 1, 2018

1.1.1 Extraction with gentle bead beating and isolation of total DNA and RNA with AllPrep Mini Kit from micro-algae culture

Introduction

AllPrep® DNA/RNA Kits allows the simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample.

Two days ago, I performed this same experiment with a bead beating step as Viggó suggested. The experiment was a success as I obtained both DNA and RNA but surprisingly, I retrieved more DNA than RNA while I should be able to obtain more RNA than DNA.

There are two steps that could have affected my RNA yield:

- the bead beating step
- the fact that I did not totally dry the column before eluting the RNA

So this time, I will make sure I dry the column before eluting the RNA and I will use a more gently bead beating step: three times 10 seconds at 30 Hz instead of two times 30 seconds at 30 Hz. Also, Pauline Vannier recommended me to use icy water to cool down my tube instead of ice only.

This time, after the bead beating, I did not forget to take with me my ice bucket with the 70% Ethanol and the 10 mM Tris-HCl buffer to avoid running up and down the stairs.

Tag(s):
Laboratory (lab)
DNA (dna)
RNA (rna)
Extraction (extr)

Author(s):
Clara Jegousse (cj)

Icy water will cool down the tube efficiently because it increases the surface area of contact with the cold.

Sample disruption and homogenisation of cells

1. Harvest 2 mL of micro-algae cultures.

Maria came to talk to me before I had the time to place my tube in the centrifuge so it remained for about 15 min at room temperature.

2. Centrifuge for 20 min at maximum speed at 4°C.
3. Discard supernatent.
4. Add 600 µL of Buffer RLT.

5. Add approx. 0.2 mg of beads.
6. Disrupt MixerMill MM400 by Retsch using the program P9 (300 Hz) for 10 seconds three times and immerse tube icy water for 30 sec after each round.

Mia prepared them, I must check the diameters of the beads.

Next time, I will try to also cool down my tube before the bead beating step.

7. Centrifuge the lysate for 3 min at maximum speed (20 000 x g) at 4°C.
8. Transfer carefully the supernatant to an AllPrep DNA spin column placed in a 2 mL collection tube.
9. Close the lid.
10. Centrifuge for 30 sec. at 8000 x g.

I actually centrifuge for 1 min but I don't think it matters

11. Use the flow-through for RNA purification: proceed to Total RNA purification.
12. Place the AllPrep DNA spin column in a new 2 mL collection tube and keep at room temperature.

I leave my tube on ice.

Total RNA purification

1. Add 1 vol. of 70% ethanol to the flow-through collected previously.
Here, one volume is 600 µL
2. Mix well by pipetting.
3. Transfer up to 700 µL of the sample to an RNeasy spin column placed in a 2 mL collection tube.
4. Centrifuge for 15 sec. at 8000 x g at 4°C.
5. Discard the flow-through.
6. I repeat the three previous steps with the remaining volume of sample to make sure the column is saturated in RNA.
7. Add 700 µL of Buffer RW1 to the RNeasy spin column.
8. Close the lid.
9. Centrifuge for 15 sec. at 8000 x g at 4°C.
10. Discard the flow-thought.
11. Add 500 µL of Buffer RPE to the RNeasy spin column.
12. Close the lid.
13. Centrifuge for 15 sec. at 8000 x g at 4°C.
14. Discard the flow-thought.
15. Add 500 µL of Buffer RPE to the RNeasy spin column.

16. Close the lid.
17. Centrifuge for 2 min at maximum speed at 4°C.
18. Discard the flow-thought.

At this step, I could have performed the optional step to dry the column by centrifuging at maximum speed for one min.

19. Place the RNeasy spin column in a new 1.5 mL collection tube.
20. Add 50 µL of RNase-free water directly to the spin column membrane.
21. Close the lid gently.
22. Centrifuge for 1 min at 8000 x g to elute the RNA.

I repeated the elution of the RNA with an extra 30 µL of RNase-free water, which makes a final volume of 80 µL of RNase-free water containing the RNA.

Genomic DNA purification

1. Add 500 µL of Buffer AW1 to the AllPrep DNA spin column from the Lysis.
2. Close the lid.
3. Centrifuge for 15 sec. at 8000 x g at 4°C to wash the column membrane.
4. Discard the flow-thought.
5. Add 500 µL of Buffer AW2 to the AllPrep DNA spin column
6. Centrifuge for 2 min at maximum speed (20 000 x g) at 4°C to wash the column membrane.
7. Place the AllPrep DNA spin column in a new 1.5 mL collection tube.
8. Add 100 µL of Tris-HCl buffer (pH 8) to the DNA AllPrep spin column membrane and close the lid.
9. Incubate at room temperature for 1 min.
10. Centrifuge for 1 min at 8000 x g to elute the DNA.

I forgot to incubate for one minute the first time! lucky I actually repeat this step.

I added an extra 100 µL of Tris-HCl to the column, incubate 1 min and centrifuge at 8000 x g for 1 min to make sure all DNA was eluted.

Conclusion

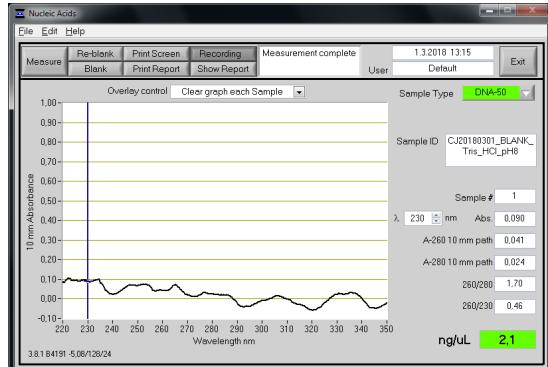
This procedure takes me 2 hours to complete (from a little after 10:00 AM to a little before 12:00 AM) even though I was interrupted few times.

1.1.2 DNA and RNA quantification with NanoDrop® ND-1000 Spectrophotometer

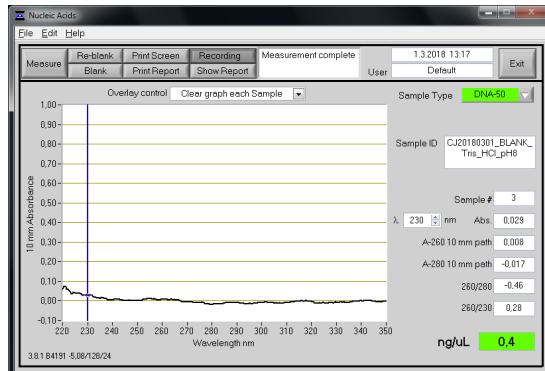
Tag(s):
Laboratory (lab)
DNA (dna)
RNA (rna)
Quantification (qnt)

Author(s):
Clara Jegousse (cj)

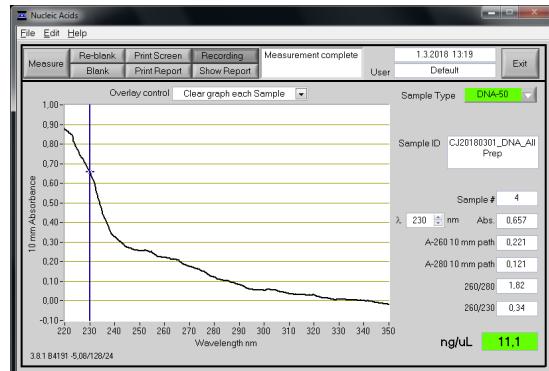
While I measure the absorbance of my samples with the NanoDrop® ND-1000, I leave the Qubit™ kits at room temperature so it will be ready once I am done.

Figure 1.1: NanoDrop spectra for DNA and RNA isolated with AllPrep Mini Kit

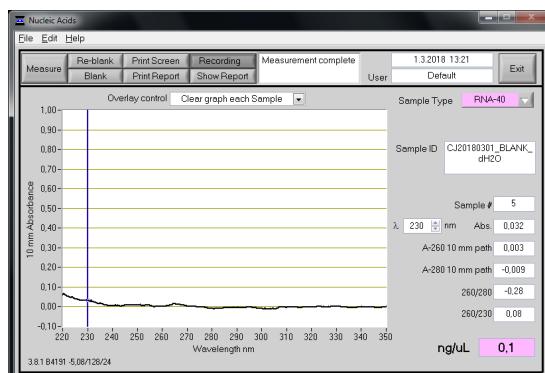
(a) Spectrum of the Tris-HCl buffer pH 8 used as Blank: this blank is not acceptable and would lead to inaccurate measurements.



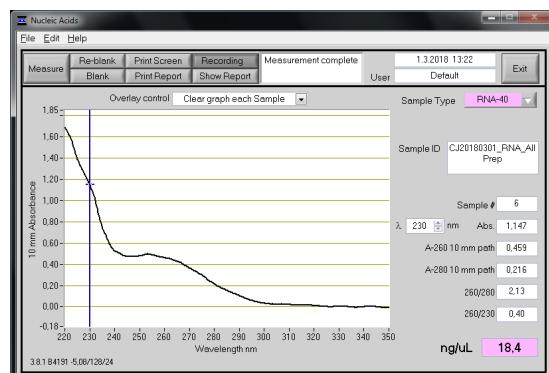
(b) Spectrum of the Tris-HCl buffer pH 8 used as Blank



(c) Spectrum of the DNA extracted with AllPrep



(d) Spectrum of the dH₂O used as Blank



(e) Spectrum of the RNA extracted with AllPrep

The spectra shown in figure 1.1 are not good spectra but they are consistent with previous spectra obtained for nucleic acids isolated with the AllPrep Mini Kit by QIAGEN.

Table 1.1: res/nanodrop/CJ20180301.txt

Sample ID	Time	ng/ µL	A260	A280	260/280	260/230
CJ20180301_BLANK_Tris_HCl_pH8	13:15	2,07	0,041	0,024	1,70	0,46
CJ20180301_BLANK_Tris_HCl_pH8	13:16	4,61	0,092	0,049	1,88	1,60
CJ20180301_BLANK_Tris_HCl_pH8	13:17	0,40	0,008	-0,017	-0,46	0,28
CJ20180301_DNA_AllPrep	13:19	11,05	0,221	0,121	1,82	0,34
CJ20180301_BLANK_dH2O	13:21	0,11	0,003	-0,009	-0,28	0,08
CJ20180301_RNA_AllPrep	13:22	18,36	0,459	0,216	2,13	0,40

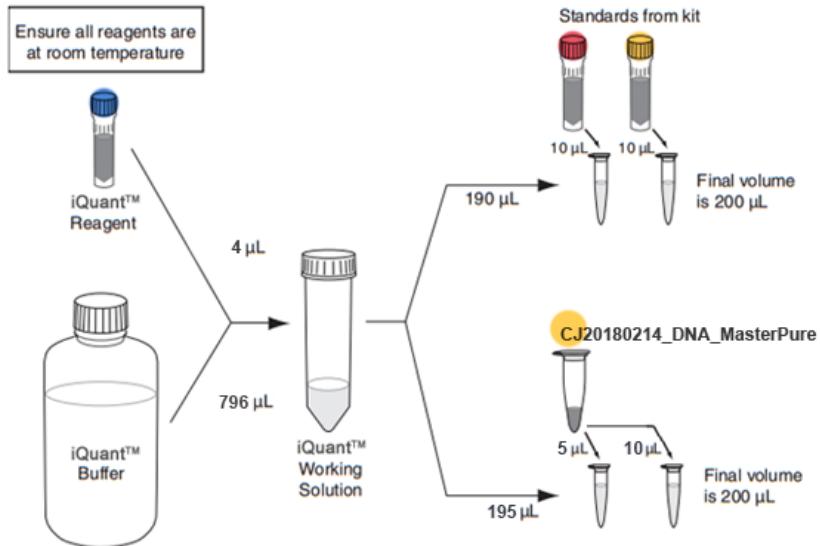
User: Default - Date: 1.3.2018 - Constant: 40,00 - Cursor position: 230

1.1.3 DNA quantification with Qubit™ DNA BR Assay Kit

Figure 1.2: Illustration for the Qubit™ DNA BR assay

Tag(s):
 Laboratory (lab)
 DNA (dna)
 Quantification (qnt)

Author(s):
 Clara Jegousse (cj)



It is exactly the same as what was done on the 20180215.

Qubit™DNA BR Assay Kit
 LOT:#1835789 opened by
 Elisabet on 20170815.

In table 1.2, the quantities of DNA are calculated based on the volume left: I know I eluted my DNA with 2 x 100 µL of Tris HCl buffer which makes it a total 200 µL, then I used 2 µL for the NanoDrop® measurements and finally I use 5 µL and 10 µL for the Qubit™ assay. Which means the volume left us 183 µL .

Table 1.2: Total DNA quantities in samples measured with Qubit™ DNA BR Assay Kit

Sample ID	$\mu\text{g/mL}$	V_f (mL)	m (μg)	m (ng)
CJ20180301_DNA_AllPrep_5	2.89	0.183	0.528	528.87
CJ20180301_DNA_AllPrep_10	2.93	0.183	0.536	536.19
CJ20180301_DNA_AllPrep_5	3.52	0.183	0.461	461.16
CJ20180301_DNA_AllPrep_10	3.46	0.183	0.450	450.18
CJ20180301_DNA_AllPrep_5	3.45	0.183	0.448	448.35
CJ20180301_DNA_AllPrep_10	3.44	0.183	0.446	446.52

In table 1.2, the two first rows shows measurements obtained with the previous calibration, while the four last rows were obtained with a new calibration. I was just curious to see how measurements would compare. I think the difference in the values measured can be explained by the pipetting errors when preparing the assay.

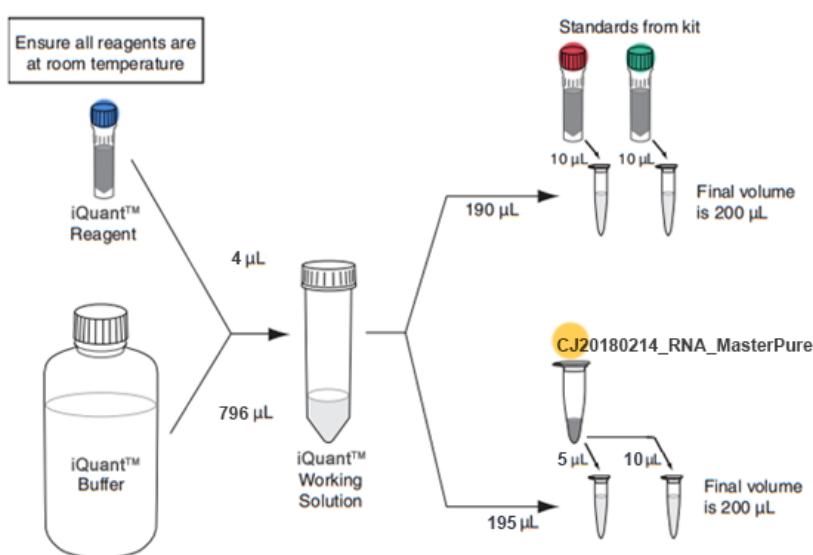
And since the volume used to elute the DNA was 200 μL and the concentration is at least 3.45 $\mu\text{g/mL}$, the DNA yield is 690 ng. This is not enough for a polymerase chain reaction (PCR)-free library preparation for shotgun metagenomics, but I must keep in mind that my starting material (2 mL of culture) is very likely smaller than the one I will get from my Sterivex™ filters.

1.1.4 RNA Quantification with Qubit™ RNA BR Assay

Tag(s):
Laboratory (lab)
RNA (rna)
Quantification (qnt)

Author(s):
Clara Jegousse (cj)

Qubit™RNA BR Assay kit;
LOT: 1924395 opened by
me on 20180210.

Figure 1.3: Illustration for the Qubit™ RNA BR assay

I repeated exactly the same
as I did on the 20180215
and on the 20180227.

Table 1.3: Total RNA quantities in samples measured with Qubit™ RNA BR Assay Kit

Sample ID	$\mu\text{g/mL}$	V_f (mL)	m (μg)	m (ng)
CJ20180301_RNA_AllPrep_5	14.4	0.080	1.152	1152.0
CJ20180301_RNA_AllPrep_10	14.0	0.080	1.120	1120.0
CJ20180301_RNA_AllPrep_5	14.3	0.080	1.144	1144.0
CJ20180301_RNA_AllPrep_10	14.4	0.080	1.152	1152.0
CJ20180301_RNA_AllPrep_5	14.0	0.080	1.120	1120.0
CJ20180301_RNA_AllPrep_10	14.4	0.080	1.144	1144.0

In table 1.3, the two first rows contain measures obtained with the last calibration while the four last rows contain values measured with a new calibration. I must say I am impressed by the consistency of these results (maybe because the kit is very new).

According to these measurements and knowing that the total volume of RNase-free water used to elute the RNA was 80 μL , my RNA yield is 1.120 μg , which is enough for metatranscriptomics.

And this time, the quantity of RNA isolated is higher than the quantity of DNA, which is what is expected. Therefore, when using the AllPrep Mini Kit for DNA and RNA isolation, I will use the gentle bead beating step for homogenisation.

1.2 March 3, 2018

1.2.1 Macromolecular composition of cells with R

Because I had surprising results regarding my yields of DNA and RNA, I decide to learn a little bit more about the proportions of macromolecules in cells and to try to represent it with diagrams.

?

After reading a webpage related to the article by ?,

Listing 1.1: Waffle plots to represent the macromolecular composition of different type of cell

```

1
2 img.path = "/Users/Clara/Projects/diary/graphics/plots/"
3 today <- "20180303"
4 # http://book.bionumbers.org/what-is-the-macromolecular-composition-of-the-cell/
5 ecoli <- c(
6   'protein'=55,
7   'other'=22,
8   'RNA'=20,
9   'DNA'=3
10
11   # 'lipid'=9,
12   # 'lipopolysaccharide'=3,
13   # 'peptidoglycan'=3,
14   # 'glycogen'=3,
15   # 'metabolites'=3,
16   # 'inorganic ions'=1
17 )

```

Tag(s):
DNA (dna)
RNA (rna)
R (r)

Author(s):
Clara Jegousse (cj)

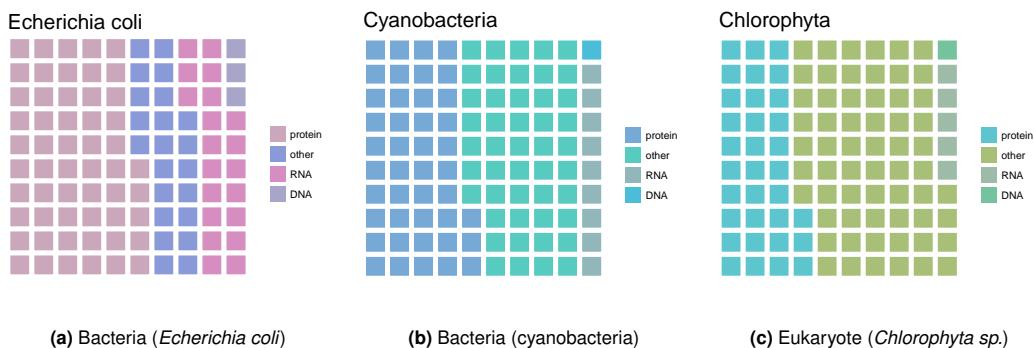
```

18
19 purple.palette <- c("#cba7bc",
20 "#8a99d8",
21 "#d68dc0",
22 "#a7a6c8")
23
24 pdf(file = paste(img.path, today, "_waffle_ecoli.pdf", sep="", collapse=NULL),
25       width=4, height=4)
25 waffle(ecoli,
26         title = "Echerichia coli",
27         colors=purple.palette)
28 dev.off()
29
30 # Phylogenetic Diversity in the Macromolecular Composition of Microalgae
31 cyanobacteria <- c(
32   'protein'=43,
33   'other' = 47,
34   'RNA'=9,
35   'DNA'=1
36   #'lipid'=12,
37   #'carbohydrates'=22,
38   #'ash'=8,
39   #'chlorophyll a'=1,
40   #'other'=4
41 )
42
43 cyano.palette <- c("#76a9d6",
44 "#58cbc0",
45 "#92b7bb",
46 "#4abdd8")
47
48 pdf(file = paste(img.path, today, "_waffle_cyano.pdf", sep="", collapse=NULL),
49       width=4, height=4)
49 waffle(cyanobacteria,
50         title = "Cyanobacteria",
51         colors=cyano.palette)
52 dev.off()
53
54 chlorophyta <- c(
55   'protein'=33,
56   'other' = 61,
57   'RNA'=5,
58   'DNA'=1
59 )
60
61 chlоро.palette <- c("#5dc5cd",
62 "#a7bf76",
63 "#9ebba8",
64 "#74c39e")
65
66 pdf(file = paste(img.path, today, "_waffle_chlorophyte.pdf", sep="", collapse=NULL
67   ), width=4, height=4)
67 waffle(chlorophyta,
68         title = "Chlorophyta",
69         colors=chlоро.palette)
70 dev.off()

```

The script in listing 1.1 generate waffle plots that are shown in figure 1.4.

Figure 1.4: Representation of the taxonomic differences of the median macromolecular composition as percent dry weight under nutrient-sufficient exponential growth conditions.



1.3 March 5, 2018

1.3.1 Pick up samples collected during winter survey

According to MarineTraffic the research vessel - Bjarni Sæmundsson - came back to Reykjavík on Sunday 4th March at 01:00 PM (cf. figure 1.5). After contacting Kristinn Guðmundsson to make sure the crew would be on board to help me with the crane, Tómas and I went to fetch the samples and Mia's personal belongings. We brought back the bottles of seawater with 1% lugol to Matís and then we had to go back to the harbour to fetch the two liquid nitrogen tanks and bring them back to Matís.

One of the two nitrogen tanks has no more cap. Mia warned me about this, apparently it was damaged during the survey.

Tag(s):
Samples (smp)

Author(s):
Clara Jegousse (cj)

Person(s):
Tómas Ármann Hafsteinsson
Kristinn Guðmundsson
Mia Cerfonteyn

www.marinetraffic.com

1.3.2 Cleaning teaching lab and setting up filtration system

Since I brought back the samples collected during the winter survey, I must filter the seawater that was collected. So the first step is to set up the filtration system in the teaching lab. Everything worked perfectly fine, and I just made sure to rinse the filtration system: in each 1L bottle of the filtration device, I rinse it once with tap water, and once with distilled MilliQ water (using a *dummy* 0.45µm Sterivex™filter). Doing this fake filtration with MilliQ water is time consuming but it allows me to check that the filtration is working fine and fix the pipe connections with parafilm.

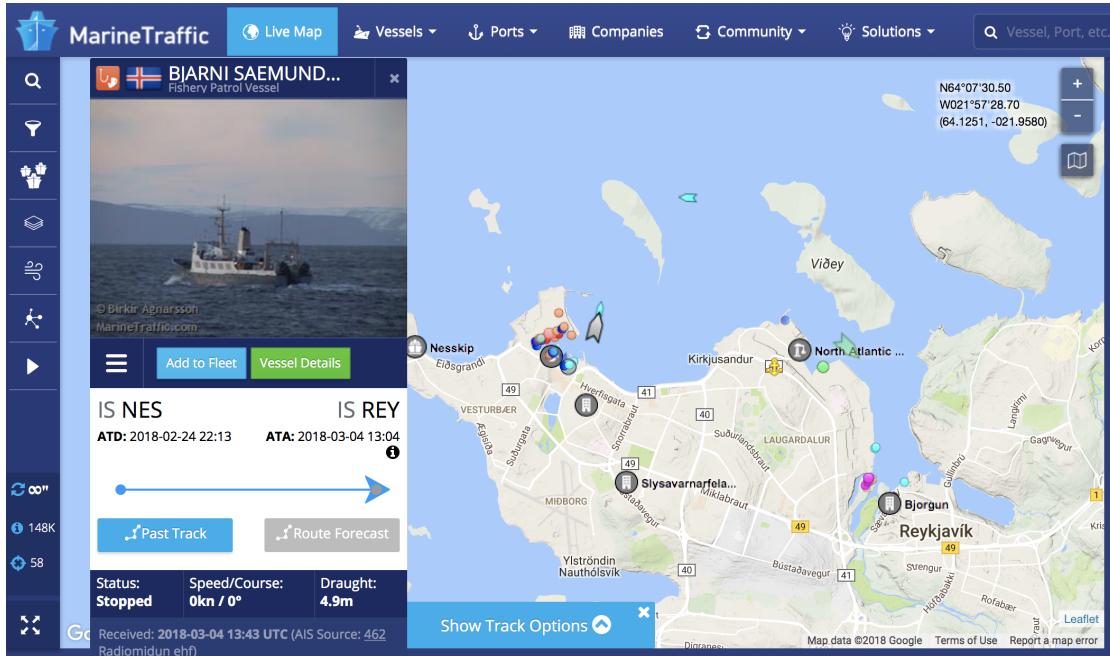
When everything is up and running, it is already 16:30 and I have to leave for my Icelandic course. So I decided to leave everything one more night so the seawater will be filtered consistently.

Tag(s):
Háskóli Íslands (hi)
Laboratory (lab)

Author(s):
Clara Jegousse (cj)

1.4 March 6, 2018

Figure 1.5: Screen capture showing the information about Bjarni Sædmundsson arrival and departure



1.4.1 Filtering seawater on Sterivex™ filters for metagenomic studies

I started the filtration of the metagenomic stations at 9:00 AM with SB5 0m (surface), SB5 bottom, and SB2 bottom and finally SB2 0m (surface).

I noticed that there was slightly less water for SB2 0m.

It was a little hard to work today because I was on my periods.

Anouk is busy with Alex and Sigurlaug organising the samples in the freezers.

I also took the time to partially record the filtering process with the GoPro so in case someone else has to go on the ship or do the filtration later, it will be a useful resource.

I talked to Biljana from Marinox about the new organisation of the teaching lab.

There is definitely one copepod in the Sterivex filter for station SB5 0m.

Tag(s):
Laboratory (lab)
Sterivex (svx)
Selvogsbanki (sb)

Author(s):
Clara Jegousse (cj)

Figure 1.6: Pictures of the filtration process

(a) Seawater samples (4.5L) with 1% lugol for metagenomic studies



(b) Homemade filtration device allowing simultaneous filtration on three Sterivex™ filters

1.4.2 Effect of Lugol Iodine

Many published sampling protocols make use of Lugol's solution. You add this preservative in amounts to achieve a 1% final concentration (1 part per 100). The iodine in Lugol's is effectively bacteriostatic, but it causes a number of changes in algal cells. For example, iodine will bind with starch to form a blue-black complex. This reaction is useful in identifying starch, which is present in some algal groups, but not in others. Moreover, Lugol's solution does not preserve cell structure well in many cases, making identification difficult. For later microscopic observation, it is preferable to preserve in glutaraldehyde (which we use for flow cytometry analysis).

Also, ? concluded that "*preserving the samples in acidic Lugol's solution resulted in equal DNA yields and PCR performance, but affected community profiles.*"

So in my opinion, this is a little concerning ... especially for the 16S and 18S rRNA studies, but also for the metagenomics.

In any case, we can't change it and I don't think we could have done better anyways since this survey was organised at the last minute.

I also read the paper by ?.

1.5 March 7, 2018

1.5.1 Filtering seawater from ST4

I start by rinsing the filtration system twice, once with tap water and once with dH₂O water. Then I start filtering ST4 bottom because it is likely that this water contains the least microorganisms.

1.5.2 Filtering seawater from SB2

For SB2, I only have depth 10m, 20m, 30m, 50m because the surface and the bottom were filtered for metagenomics. All these samples were collected plastic bottles and preserved with 1% lugol. I make sure I rinse the filtration device with MilliQ dH₂O water.

Tag(s):
Literature (lit)

Author(s):
Clara Jegousse (cj)

Binds to algal cells, but what about micro-algae?

Tag(s):
Laboratory (lab)
Stokksnes (st)

Author(s):
Clara Jegousse (cj)

Tag(s):
Laboratory (lab)
Selvogsbanki (sb)

Author(s):
Clara Jegousse (cj)

1.6 March 8, 2018

1.6.1 Sampling equipment inventory

Now that I am done filtering all the seawater, I decide to wash the boxes used to store and transport our sampling equipment. Once the boxes are nice and clean, I start reorganising all the items needed for sampling and I make a list that I print out and place in and on top of the box:

- 24 x cryotubes TM vials 5mL by Thermo Scientific
- 3 x 96-well plate Nucleon TM Delta Surface by Thermo Scientific for cell culture
- 2 x MULTIWELL TM 24 well plate for tissue Culture by Becton Dickinson
- 15 x disposable pasteur pipettes
- 50 x 2mL-Eppendorf tubes containing 1mL of 96% Ethanol with Yellow stickers on top
- 50 x 2mL-Eppendorf tubes, sterile with white stickers on top
- 17 x orange cap for Sterivex filter
- 45 x Sterivex filters (0.22 um)
- Few Sterivex filters (0.45 um)
- 1 x metal tube holder (for 40 tubes)
- 1 x box of burnt tips (96 tips prepared for winter survey)
- 2 x 20mL pipette (sterile plastic)
- 5 x 10mL pipette (sterile plastic)
- 1 x thermometer
- 1 x pipette bottle (for the sterile water)
- 1 x red cap for glass flask
- 1 x clip (presser)
- 3 x autoclave bags
- 1 x pair of thick plastic gloves
- 3 x elastic strap (with hooks)
- 3 x sterile 50mL srynge
- 3 x small cupboard boxes
- Transparent tape
- 1 x screw driver (USAG 326 PZ 3)
- 2 x small plastic box (Parafilm M; tissue paper, old 50mL srynges, Silicon High Temp tube; extra ziploc bags)
- 3 x pipes (non sterile)
- Black cable ties
- Stickers

Tag(s):
Laboratory (lab)

Author(s):
Clara Jegousse (cj)

- 2 x scalpel
- Ducked tape (black)
- Pump + cable

1.6.2 Cleaning 10L bottles

The 10L-Nalgene bottles used for metagenomic samples need to be cleaned. For each of the four bottles, I use soapy water and I shake the bottle, before rinsing it with tap water. Once there is no more bubbles, I rinse 3 times. Then I rinse three times with dH₂O (approx. 1L each rinse). Then I dry the bottles overnight at 40 °C.

After that, I will only need to autoclave the bottles, and leave them under UV for extra sterilisation (and removal of DNA).

Tag(s):
Laboratory (lab)

Author(s):
Clara Jegousse (cj)

1.6.3 Meeting with Hróar about the taxes

We must fill up some online form for the tax system here in Iceland, but the form is all in Icelandic, so Hróar spends one hour helping Anouk and I to do it.

Doing this, he realised that for some weird reasons my request for sport membership support was not recorded even though I remember doing it and I have proves with the emails and the comment left on the Matís Workplace social media. So Hróar said he will fix it next month which is very nice of him!

Tag(s):
Meeting (meet)

Author(s):
Clara Jegousse (cj)

Person(s):
Hróar Hugosson
Anouk Lyver

1.6.4 Cleaning teaching lab and putting away filtration system

I am now done filtering water and preparing our sampling equipment for long time storage (until May), so I can clean after myself, especially because I am now in charge of this lab (V14 Nemenda Lab) so I must be the role model!

Tag(s):
Laboratory (lab)

Author(s):
Clara Jegousse (cj)

1.7 March 9, 2018

1.7.1 Modeling diverse communities of marine microbes (?)

Today I read this very interesting review. I am clearly not at this stage of the research because I do not even have my data yet but it is good to think a step ahead.

Today, I also updated my diary ... because when I was busy in the lab, I did not update it for the entire week, so I spend a fair amount of time writing today.

Tag(s):
Literature (lit)

Author(s):
Clara Jegousse (cj)

1.8 March 10, 2018

1.8.1 Inventory of samples collected during winter survey

I arrived a little after 10:00 AM and I was showing Helene how to switch off the alarm system. Ali is also here today so we plan to have lunch together.

I prepared boxes, and I start transferring samples from the liquid nitrogen to the -80°C freezer, and each time, I make sure I log the sample information into an excel spreadsheet.

I took me the whole day and I am not even done, but I just can't continue because I can feel that I will start making mistakes now.

Tag(s):
Laboratory (lab)
Samples (smp)
Excel (xls)

Author(s):
Clara Jegousse (cj)

Person(s):
Alexandra Leeper

1.9 March 11, 2018

1.9.1 MasterPure™ complete DNA and RNA purification

Today is Sunday, I forgot that the first bus was at 10:00 AM this morning, so I arrived at 10:50 AM, and since Ali was here also, we decided to start our day by having porridge together.

Tag(s):
Laboratory (lab)
DNA (dna)
RNA (rna)
Extraction (extr)

Author(s):
Clara Jegousse (cj)

Person(s):
Alexandra Leeper

Introduction

Today I just want to perform the isolation of DNA and RNA from micro-algae cultured cells using a modified version MasterPure™ Complete DNA and RNA Purification kit. The modification consists of an addition step of bead beating during the cell lysis and softer DNase treatment. Last time I extracted DNA and RNA with this method, my RNA yield was a lot lower than expected, and I suspect it could be explained by the DNase treatment for 30 min at 37°C. Therefore, I will try a DNase treatment at room temperature for 1 hour.

Also, when I added a bead beating step to the AllPrep® DNA/RNA mini kit, I obtained more DNA than RNA which is not the expected results but then I repeated this experiment with a gentle bead beating step which lead to the expected results.

So this time, I just wanna make sure that I can obtain consistent results with the Master-Pure™ complete DNA and RNA purification method.

Lysis of cells

1. Transfer 2 mL of micro-algae cultures into a new Eppendorf tube.
2. Pellet cells by centrifugation at maximum speed (21460 g; 15300 rpm) for 20 min at 4°C.
3. Discard supernatant, leaving approximately 25 µL of liquid.

Using the centrifuge for 20 min at full speed really allows me to obtain a good pellet that does not move around.

4. Add 600 µL of Tissue and Cell Lysis solution.
5. Dilute 2 µL of proteinase K into the lysate.

There was no more proteinase K from the kit, so I used some of the proteinase K prepared by Solveig.

6. Vortex for 10 seconds to resuspend the cell pellet.

7. Incubate at 65°C for 15 min, vortex every 5 min.
8. Cool down samples in icy water.
9. Add 0.2 g of beads.
10. Shake at 30 Hz for 10 seconds and cool down in icy water and repeat this three times.
11. Place the sample on ice for 3-5 min before proceeding with the total nucleic acid precipitation.

Precipitation of total nucleic acids

1. Add 175 µL of MPC Protein Precipitation Reagent to the 600 µL of lysed sample.

It is hard to see the effect of the MPC reagent because of the bubbles resulting from the bead beating step.

2. Pellet the debris by centrifugation at 4 °C for 10 min at 20000g in a microcentrifuge.

The resulting pellet (beads and precipitate) is too loose so I decide to add an extra 200 µL of MPC precipitation reagent and repeat the centrifugation step.

3. Transfer the supernatant to 2 clean microcentrifuge tubes.

Tubes are labeled: one for DNA and one for RNA. I manage to transfer 400 µL in each tube.

I transfer the supernatant to the RNA-labeled tube last because it is the most likely to contain contaminants (close to the pellet) but there will be another precipitation step for the RNA later.

4. Discard the pellet.

5. Add 800 µL of isopropanol to the recovered supernatant.

I keep my isopropanol and ethanol on ice.

I increase the volume of isopropanol used so that it is twice the volume of liquid containing nucleic acids

6. Invert the tube 30-40 times.

7. Pellet the total nucleic acids by centrifugation at 4 °C for 10 min at maximum speed (21460 x g).

8. Proceed to either DNA or RNA isolation.

DNA isolation

1. Carefully pour off the isopropanol without dislodging the pellet.
2. Rinse twice with 70% ethanol, being careful to not dislodge the pellet.

I use 500 µL of 70% ethanol.

3. Centrifuge briefly if the pellet is dislodged.

I centrifuge at maximum speed for 2 min at 4 °C.

4. Remove all residual ethanol with a pipette.

The pellet is easy to see and therefore it is easy to remove all the ethanol.

5. Resuspend the total nucleic acids in 35 μL of TE buffer.

I DO NOT RESUSPEND THE PELLET IN TE BUFFER: it inhibits downstream PCR. I use 50 μL of 10 mM Tris HCl buffer pH 8.

Resuspending the pellet in more volume than recommended decreases the final concentration of DNA, but it also decrease the concentration of eventual contaminants and will allow me to work with bigger volumes when trying to amplify the DNA: it is always easier to work with slightly bigger volumes than 0.5 μL !

RNA isolation

1. Remove all residual isopropanol with a pipette.
2. Add 200 μL of DNase I solution.
3. Add 10 μL of RNase-free DNase I to the sample containing the DNase I solution and the nucleic acids pellet.

Here I did not have a 10 μL pipette, so I used $5 \times 2 \mu\text{L}$.

4. Resuspend the total nucleic acids pellet in the DNase I solution.
5. Incubate at room temperature (on ice) for 1H.
6. Instead of 30 min at 37°C so hopefully it does not damage the RNA too much.
7. Add 200 μL of 2X Tissue and Cell Lysis solution.
8. Vortex for 5 seconds.
9. Add 200 μL of MPC Protein Reagent.
10. Vortex for 10 seconds.
11. Place on ice for 3-5 min.
12. Pellet the debris by centrifugation for 10 min at maximum speed (21460 g) at 4°C.
13. Transfer the supernatant containing the RNA into a clean eppendorf tube and discard the pellet.

This time, the pellet is very white.

14. Add 500 μL of isopropanol to the supernatant.
15. Mix by inversion 30-40 times.
16. Pellet the purified RNA by centrifugation at 4°C for 10 min at maximum speed.
17. Remove carefully all isopropanol without dislodging the pellet.
18. Rinse twice with 70% ethanol, being careful to not dislodge the pellet.

I use 500 μL of 70% ethanol.

19. Centrifuge briefly if the pellet is dislodge.

I actually dislodge the pellet and centrifuge again at maximum speed for 1 min at 4°C.

20. Remove all residual ethanol with a pipette.

The pellet is easy to see and therefore it is easy to remove all the ethanol.

21. Resuspend the total nucleic acids in 35 µL of TE buffer.

I DO NOT RESUSPEND THE PELLET IN TE BUFFER: it inhibits downstream PCR. I use 50 µL of 10 mM Tris HCl buffer pH 8.

22. Add 1 µL of RiboGard RNase Inhibitor.

Once I am done, I place all reagents where they belong and place my DNA and RNA samples in the freezer at -20 °C.

1.10 March 13, 2018

1.10.1 DNA and RNA quantification with NanoDrop® ND-1000 Spectrophotometer

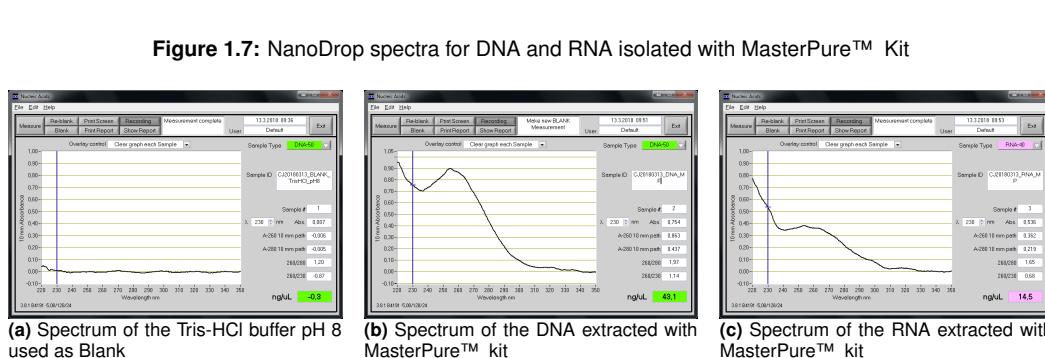


Figure 1.7: NanoDrop spectra for DNA and RNA isolated with MasterPure™ Kit

Tag(s):
Laboratory (lab)
Quantification (qnt)
DNA (dna)
RNA (rna)

Author(s):
Clara Jegousse (cj)

File(s):
ndv_to_latex_tab.py

Sample ID	Time	ng/ul	A260	A280	260/280	260/230
CJ20180313_BLANK_TrisHCl_pH8	09:35	-0,32	-0,006	-0,005	1,20	-0,87
CJ20180313_DNA_MP	09:37	43,13	0,863	0,437	1,97	1,14
CJ20180313_RNA_MP	09:53	14,49	0,362	0,219	1,65	0,68

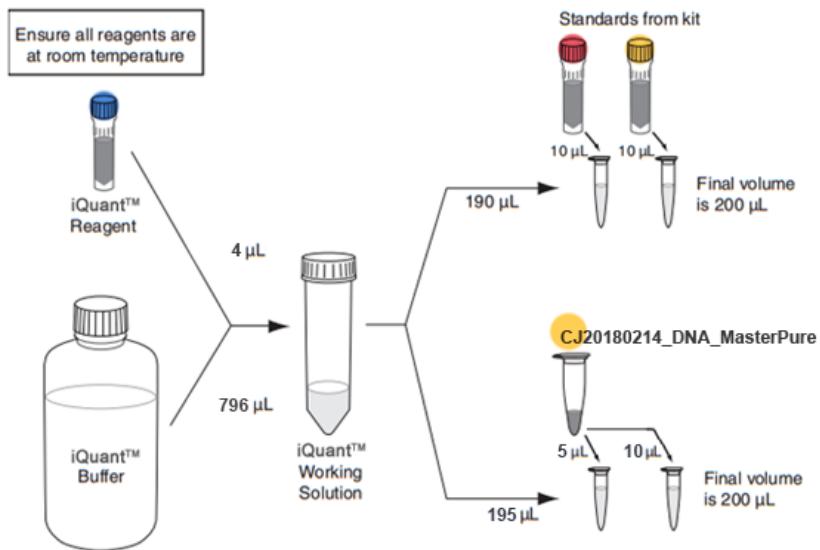
User: Default - Date: 13.3.2018 - Constant: 40,00 - Cursor position: 230

1.10.2 DNA quantification with Qubit™ DNA BR Assay Kit

Tag(s):
Laboratory (lab)
Quantification (qnt)
DNA (dna)

Author(s):
Clara Jegousse (cj)

Qubit™ DNA BR Assay Kit
LOT:#1835789 opened by
Elisabet on 20170815.

Figure 1.8: Illustration for the Qubit™ DNA BR assay

It is exactly the same as what was done on the 20180215.

For the Qubit™ DNA BR Assay, I consider that I have 4 samples: the two standards and one DNA sample that I will measure twice, once using 5 µL and once using 10 µL.

Also, I repeat the measure three times. The first time, I use the old calibration. The second time, I re-calibrate the spectrophotometer with the standards prepared by myself.

Because I received a new Qubit™ DNA BR assay kit last week, I want to make sure the measurements are consistent. So today, I will quantify the DNA with the old kit (LOT:#1835789) and then with the new one (LOT:#1927400). Measurements obtained with the old kit are presented in table 1.5 and Measurements obtained with the new kit are presented in table 1.6. It seems that the results are fairly consistent and therefore I can *trust* the new kit.

Table 1.5: Total DNA quantities in samples measured with Qubit™ DNA BR Assay Kit

Sample ID	$\mu\text{g/mL}$	$V_f (\text{mL})$	$m (\mu\text{g})$	$m (\text{ng})$
CJ20180313_DNA_MP_5	20.0	0.018	0.360	360.0
CJ20180313_DNA_MP_10	22.7	0.018	0.408	408.6
CJ20180313_DNA_MP_5	14.8	0.018	0.266	266.4
CJ20180313_DNA_MP_10	16.1	0.018	0.289	289.8
CJ20180313_DNA_MP_5	15.3	0.018	0.275	275.4
CJ20180313_DNA_MP_10	16.2	0.018	0.291	291.6

Qubit™ DNA BR Assay Kit
LOT:#1927400 opened by
myself today.

Table 1.6: Total DNA quantities in samples measured with Qubit™ DNA BR Assay Kit recently received

Sample ID	$\mu\text{g/mL}$	V_f (mL)	m (μg)	m (ng)
CJ20180313_DNA_MP_5	8.15	0.018	0.146	146.7
CJ20180313_DNA_MP_10	8.94	0.018	0.160	160.9
CJ20180313_DNA_MP_5	17.5	0.018	0.315	315.0
CJ20180313_DNA_MP_10	18.4	0.018	0.331	331.2
CJ20180313_DNA_MP_5	17.0	0.018	0.306	306.0
CJ20180313_DNA_MP_10	18.3	0.018	0.329	329.4

In table 1.2, the quantities of DNA are calculted based on the volume left: I know resuspended the DNA 50 μL of Tris HCl buffer, which means I was able to obtain at least 850 ng of DNA. Then I used 2 μL for the NanoDrop® measurements and finally I use 5 μL and 10 μL twice for the Qubit™ assays. Which means the volume left us 18 μL in which I have - at least - 300 ng of DNA left.

1.10.3 Quantification with Qubit™ RNA BR Assay

Table 1.7: Total RNA quantities in samples measured with Qubit™ RNA BR Assay Kit

Tag(s):
Laboratory (lab)
Quantification (qnt)
RNA (rna)

Author(s):
Clara Jegousse (cj)

RNA BR Assay Kit
LOT:1924395

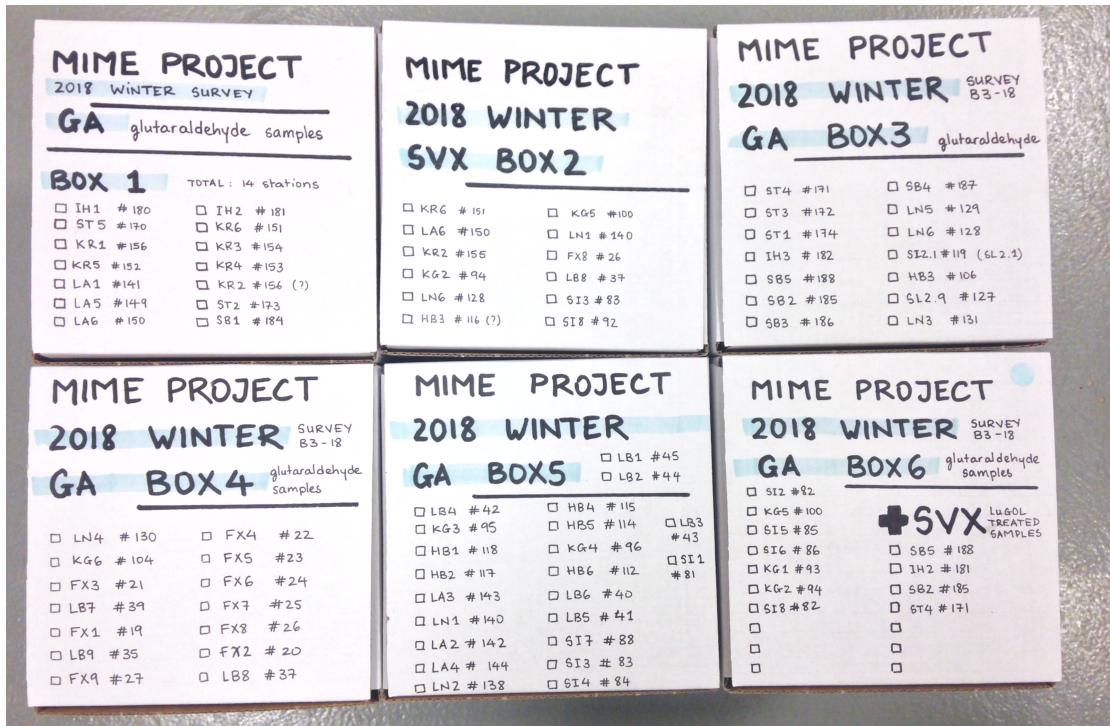
Sample ID	$\mu\text{g/mL}$	V_f (mL)	m (μg)	m (ng)
CJ20180313_RNA_MP_5	7.54	0.050		
CJ20180313_RNA_MP_10	21.2	0.050		
CJ20180313_RNA_MP_5	7.19	0.050	0.359	359.5
CJ20180313_RNA_MP_10	20.6	0.050		
CJ20180313_RNA_MP_5	7.56	0.050		
CJ20180313_RNA_MP_10	21.0	0.050		

1.10.4 Finishing samples inventory

I finished the samples inventory today and sent the results to the team.

Tag(s):
Laboratory (lab)
Samples (smp)

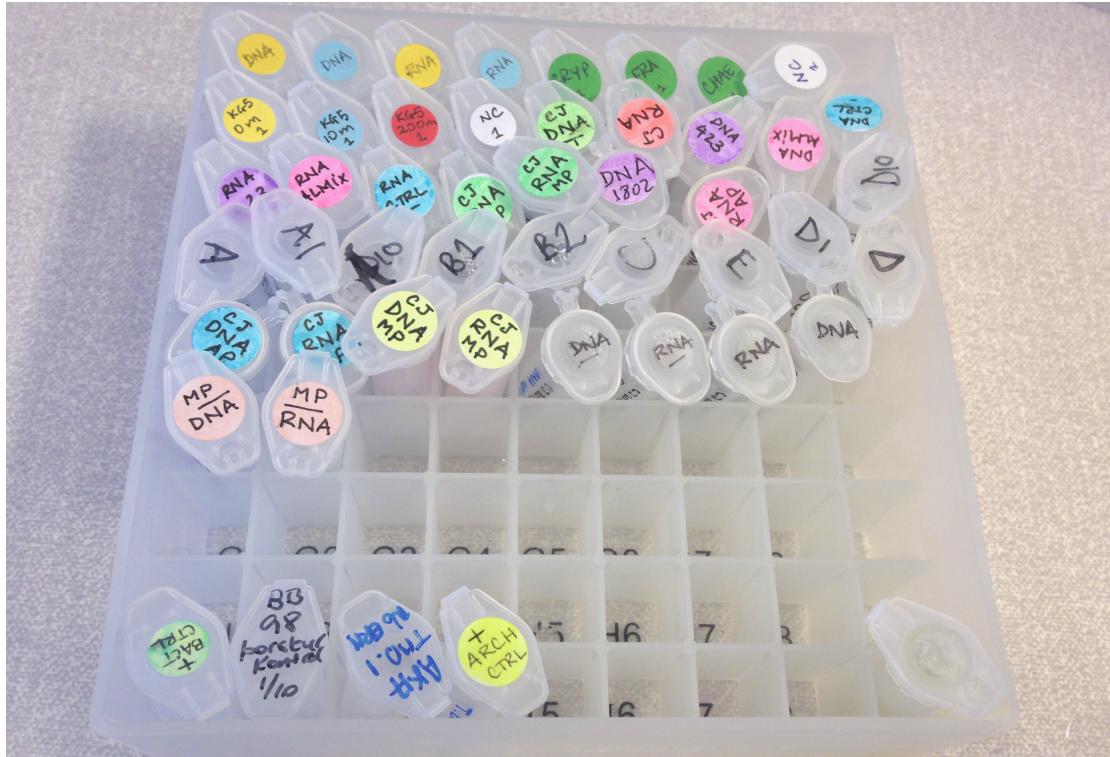
Author(s):
Clara Jegousse (cj)

Figure 1.9: Picture of the labels on storage boxes containing winter survey samples in -80°freezer

1.11 March 15, 2018

1.11.1 Inventory of nucleic acid samples

Figure 1.10: Picture of my nucleic acid samples in the storage box kept in freezer at -20°C



Tag(s):
Laboratory (lab)
Samples (smp)
DNA (dna)
RNA (rna)

Author(s):
Clara Jegousse (cj)

USER	LABEL	LABEL COLOR	BOX POSITION	EXTRACTION METHOD	STARTING MATERIAL	CONCENTRATION QBIT
CJ	DNA MP	GREEN	C4	MasterPure	Micro-algae culture	3,11
CJ	RNA MP	GREEN	C5	MasterPure	Micro-algae culture	7,20
CJ	DNA 1802	PURPLE	C6	AllPrep	Micro-algae culture	2,44
CJ	RNA AP	PINK	C7	AllPrep	Micro-algae culture	8,77
CJ	DNA AP	BLUE	E1	AllPrep	Micro-algae culture	5,86
CJ	RNA AP	BLUE	E2	AllPrep	Micro-algae culture	31,50
CJ	DNA MP	YELLOW	E3	MasterPure	Micro-algae culture	18,20
CJ	RNA MP	YELLOW	E4	MasterPure	Micro-algae culture	32,20
CJ	DNA	NONE	E5	AllPrep	Micro-algae culture	7,71
CJ	RNA	NONE	E6	AllPrep	Micro-algae culture	3,94
CJ	DNA	NONE	E7	AllPrep	Micro-algae culture	3,45
CJ	RNA	NONE	E8	AllPrep	Micro-algae culture	14,00
CJ	MP DNA	PEACH	F1	MasterPure	Micro-algae culture	17,00
CJ	MP RNA	PEACH	F2	MasterPure	Micro-algae culture	7,19

1.11.2 SYBR® Gold nucleic acid gel stain

SYBR® Gold is a nucleic acid gel stain. It is very sensitive and allows detection of double- or single-stranded DNA and RNA in electrophoresis gels while our usual stain (SYBR® Safe) does not allow detection of RNA and is less sensitive.

Tag(s):
Literature (lit)
RNA (rna)
DNA (dna)

Author(s):
Clara Jegousse (cj)

However, because SYBR® Gold is different from SYBR® Safe, I must adapt my protocol.

Considerations:

- It seems that it is preferable to not cast SYBR stains into the gels (which is what we usually do!). Here is what the documentation manual says:

Smearing and Distorted Bands: If the dye is cast into the gel, or the nucleic acid prestained during loading, you may see some smearing or distortion of the bands. SYBR Green stains are very sensitive to nucleic acid overloading. We recommend that each lane contain 1–5 ng of nucleic acid per band to avoid this problem. We do not recommend including the dye in the running buffer as it will disrupt the migration of the nucleic acids and cause smearing of the bands.

- allow SYBR® Gold to warm up at room temperature before preparing the working solution.

https://www.researchgate.net/post/Why_does_SYBR_Gold_interfere_with_1Kb_DNA_ladder2

<https://tools.thermofisher.com/content/sfs/manuals/td004.pdf>

1.11.3 Checking integrity of nucleic acids on agarose gel

Introduction

The first DNA and RNA quality parameters assessed earlier were based on DNA 260/280 and 260/230 spectrophotometric ratios measured by NanoDrop® ND-1000 and detectability by PCR. Another important quality parameter is the size range and integrity. Integrity does not mean purity. It means intactness or state of degradation of nucleic acids. DNA and RNA integrity and size range can be assessed by agarose gel electrophoresis.

I have previously tried to test the integrity of nucleic acid with SYBR® Safe stain, but unfortunately SYBR® Safe did not stain my RNA samples. Therefore, I want to repeat this experiment with SYBR® Gold. SYBR® Gold is a very sensitive stain for detecting double- and single-stranded DNA or RNA in electrophoretic gels.

Tag(s):
Laboratory (lab)
DNA (dna)
RNA (rna)

Author(s):
Clara Jegousse (cj)

The size of my expected DNA would determine what percentage agarose gel should be used. Usually 0.8% to 1% will be good enough to see how degraded is the DNA or RNA.

1% Agarose gel preparation

1. Measure with erlenmeyer 50 mL of 1% agarose gel.
2. Warm up the erlenmeyer for 15s in microwave: agarose will be more fluid and it will avoid bubbles when casting the gel
3. Cast the gel with the comb (for 15 wells)
4. Let the gel set for 20 min

The 1% agarose gel is kept at 60°C in the gel room.

Nucleic acid samples preparation

- 5 µL of each sample of nucleic acid sample
- 5 µL of blue dye (bromothymol blue)
- Tap gently tubes to mix up everything
- Centrifuge briefly to bring all the liquid to the bottom

Electrophoresis

For this electrophoresis migration, we run:

- 9 µL of each sample
- 5 µL of the 1 kb ladder
- 5 µL of the 2-log ladder

I leave empty lanes between ladders and samples.

1. Place the agarose gel into the gel box (electrophoresis unit) containing TAE buffer
2. Load 5 µL of molecular weight ladder into the first and last lane of the gel
3. Load 9 µL of each sample into the additional wells of the gel
4. Run for 65 min with the electrophoresis power supply (EPS) 301 at 80 V, 400 mA

I change the usual settings for a slightly longer run at a lowest voltage (the usual settings I use for electrophoresis are 50 min at 100 V), so that the separation of nucleic acid fragments will be slower but clearer.

5. Remove gel from the migration tank and place in the staining container (a recycled tip box).

SYBR® Gold Staining

1. Ensure SYBR® gold is properly thawed.
2. Ensure the pH of TAE buffer is between 7.5 and 8 (cf. figure 1.11a)

I made a new batch of 10L of TAE buffer in the gel room thanks to Águsta.

3. Prepare the staining solution at 1X SYBR® Gold in TAE buffer.

For 100 mL of staining solution, I mixed 10 µL of SYBR® stain with 100 mL of TAE buffer.

4. Transfer 100 mL of staining solution in the staining container.

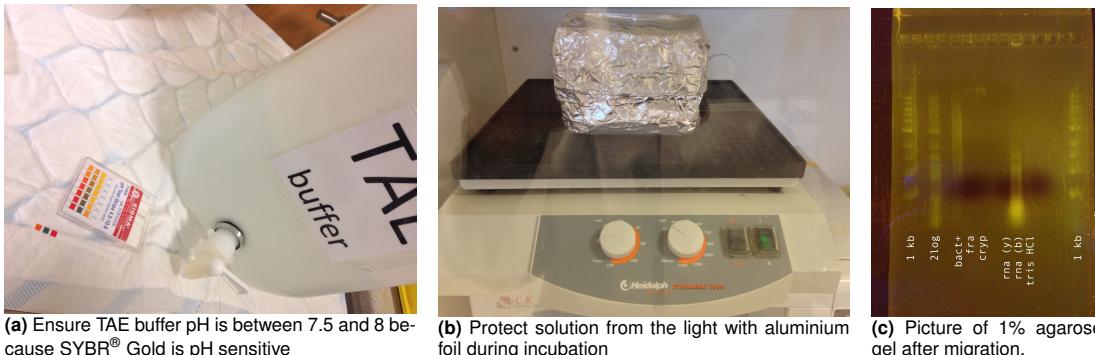
I recycle a broken box of tips.

5. Incubate under agitation for 10 to 40 min.

I incubated 20 min under 150 rpm at room temperature on the TITRAMAX 1000 by Heidolph (cf. figure 1.11b)

6. Visualize your nucleic acid fragments with ultra violet (UV) lights.

Our usual CCD camera by BioRad does not work anymore. In the meanwhile, we use transilluminator and take the picture with my cell phone.

Figure 1.11: SYBR® Gold Staining considerations and results

Results

Figure 1.11c shows a picture of the gel under UV lights after 65 min migration. The ladders are slightly smudged which could be due to the SYBR® Gold stain. The DNA extracted from AK17 (labelled bact+) is smeared which indicates degradation of the genomic DNA. Since the 1 kb ladder not is sharp and fine, the degradation could have happened during electrophoresis but also during the extraction process or because of repeated freeze-thaw (I use this DNA as positive control for my PCR so it has been frozen and thawed a lot of times). But on the other hand, the genomic DNA extracted from micro-algae cultures (labeled fra and cryp) show sharp band which indicates a rather good integrity of the genomic DNA. Regarding RNA samples, RNA (y) was below the detection level for the Qubit™ so this is no surprise that there is nothing to see. RNA (b) is smeared indicating a poor intergrity of the RNA, but we can still distinguish two bands: the heavier one is approximately at 1.5 kb while the lightest one is at 1 kb. According to Thermo Fisher documentation, for *E. coli* 16S rRNA is 1.5 kb and the 23s rRNA is 2.9 kb. Therefore the two bands observed for the RNA (b) sample could be slightly degraded 16S and 23S rRNA. Finally, the Tris-HCl does not show any band which is a good thing as I just wanted to make sure there was no nucleic acid in the buffer because I had been using it to resuspend my nucleic acids at the end of all my extractions.

Pauline Vannier suggests that I just make a test with same migration settings for two ladders with SYBR® Safe and Gold to see the difference.

If genomic DNA is smeared, using DNase inhibitors while extracting the DNA can prevent degradation of the genomic DNA.

<https://www.thermofisher.com/is/en/home/references/ambion-tech-support/rna-isolation/general-articles/ribosomal-rna-sizes.html>

Conclusion

SYBR® Gold successfully stains deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) using the procedure performed today so I can now perform this experiment confidently with my RNA and RNA samples extracted from micro-algae culture with AllPrep® and MasterPure™ kits.

1.12 March 16, 2018

1.12.1 Nucleic acid integrity assessment by electrophoresis

1% Agarose gel preparation

1. Measure with erlenmeyer 50 mL of 1% agarose gel.

Tag(s):
Laboratory (lab)
DNA (dna)
RNA (rna)

Author(s):
Clara Jegousse (cj)

The 1% agarose gel is kept at 60°C in the gel room.

Table 1.8: Volumes needed for nucleic acid migration on agarose gel so that all samples contain 20 ng of nucleic acid

Usr	Label	Label colour	Box pos.	Extr. method	Starting material	Qubit™ ($\mu\text{g/mL}$)	vol. to transfer	vol. dye
CJ	DNA MP	GREEN	C4	MasterPure	Micro-algae culture	3,11	6,43	3,57
CJ	RNA MP	GREEN	C5	MasterPure	Micro-algae culture	7,20	2,78	7,22
CJ	DNA 1802	PURPLE	C6	AllPrep	Micro-algae culture	2,44	8,20	1,80
CJ	RNA AP	PINK	C7	AllPrep	Micro-algae culture	8,77	2,28	7,72
CJ	DNA AP	BLUE	E1	AllPrep	Micro-algae culture	5,86	3,41	6,59
CJ	RNA AP	BLUE	E2	AllPrep	Micro-algae culture	31,50	0,63	9,37
CJ	DNA MP	YELLOW	E3	MasterPure	Micro-algae culture	18,20	1,10	8,90
CJ	RNA MP	YELLOW	E4	MasterPure	Micro-algae culture	32,20	0,62	9,38
CJ	DNA	NONE	E5	AllPrep	Micro-algae culture	7,71	2,59	7,41
CJ	RNA	NONE	E6	AllPrep	Micro-algae culture	3,94	5,08	4,92
CJ	DNA	NONE	E7	AllPrep	Micro-algae culture	3,45	5,80	4,20
CJ	RNA	NONE	E8	AllPrep	Micro-algae culture	14,00	1,43	8,57
CJ	MP DNA	PEACH	F1	MasterPure	Micro-algae culture	17,00	1,18	8,82
CJ	MP RNA	PEACH	F2	MasterPure	Micro-algae culture	7,19	2,78	7,22

2. Warm up the erlenmeyer for 15s in microwave: agarose will be more fluid and it will avoid bubbles when casting the gel
3. Cast the gel with the comb (for 15 wells)
4. Let the gel set for 20 min

I casted my gels 3 times because the agarose was way to soft and the gel would break when removing the comb. I suspect a new intern prepared the stock solution of 1% but diluted it too much ... this delayed my progress by almost 2 hours ... In the end, Elísabet prepared a new stock solution that I was able to use.

Sample preparation

In a PCR 8-well rack mix:

- sample of nucleic acid sample according to volumes shown in table 1.8
- blue dye (bromothymol blue) according to volumes shown in table 1.8

For each sample, the final quantity of nucleic acids is 20 ng.

- Tap gently tubes to mix up everything
- Centrifuge briefly to bring all the liquid to the bottom

Electrophoresis

For this electrophoresis migration, we run:

- 10 μL of each sample
- 5 μL of the 1 kb ladder
- 5 μL of the 2-log ladder

I leave empty lanes between ladders and samples.

1. Place the agarose gel into the gel box (electrophoresis unit) containing TAE buffer
2. Load 5 μL of molecular weight ladder into the first and last lane of the gel

3. Load 9 µL of each sample into the additional wells of the gel
4. Run for 65 min with the EPS 301 at 80 V, 400 mA

I change the usual settings for a slightly longer run at a lowest voltage (the usual settings I use for electrophoresis are 50 min at 100 V), so that the separation of nucleic acid fragments will be slower but clearer.

5. Remove gel from the migration tank and place in the staining container (a recycled tip box).

SYBR® Gold Staining

1. Ensure SYBR® gold is properly thawed.
2. Ensure the pH of TAE buffer is between 7.5 and 8
3. Prepare the staining solution at 1X SYBR® Gold in TAE buffer.

I actually use the staining solution solution I made yesterday and kept in aluminium foil at room temperature.

4. Transfer 100 mL of staining solution in the staining container.
5. Incubate under agitation for 10 to 40 min.

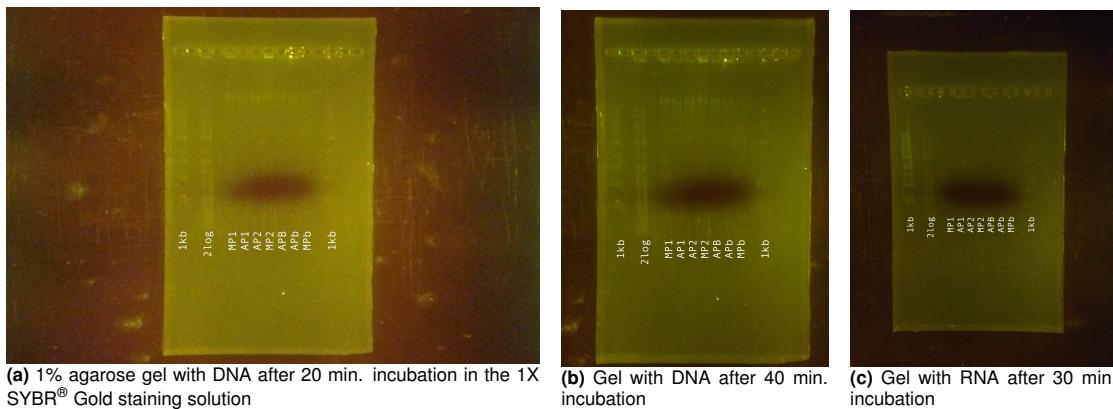
Gel with DNA was incubated 20 min but since it was hard to take a good picture, I decided to incubate the gel another 20 min to see the difference and it did not change much. Therefore for the gel with RNA, the incubation lasted 30 min.

6. Visualize your nucleic acid fragments with UV lights.

The CCD camera by BioRad does not work anymore. In the meanwhile, we use transilluminator and take the picture with my cell phone.

Results

Figure 1.12: Pictures of the gels after migration



In figures ??, we can see the ladders and for all samples except the last one (MPb), the bands are rather sharp and clear which indicated a good integrity of DNA. However, for the RNA, there

is nothing to see. I suspect that 20 ng is enough to see the genomic DNA since there is one big molecule of genomic DNA while there are multiple type of RNAs. Therefore the 20 ng of RNA end up being splitted into many tiny bands that are therefore too light to be seen. So I must try again with a higher quantity of RNA.

Conclusion

The integrity of the DNA extracted with AllPrep® and with MasterPure™ is rather good except for the modified MasterPure™ protocol (involving a gentle bead beating). When working with good quality genomic DNA, 20 ng is enough to visualise the DNA on the gel with SYBR® Gold stain.

Regarding the RNA, I must repeat the experiment with more RNA. I think 100 ng should be good knowing that previously I got really bright results with 300 ng of RNA.

1.13 March 17, 2018

1.13.1 RNA integrity assessment by electrophoresis

It's saturday, so I arrive rather late around 10:00 AM, and I start right away by casting the gels and defrosting my RNA samples as well as the SYBR® Gold.

Tag(s):
Laboratory (lab)
RNA (rna)

Author(s):
Clara Jegousse (cj)

Introduction

Because I failed to see the RNA on the gel yesterday, I must repeat the experiment but this time, I will use a lot more RNA than previously.

1% Agarose gel preparation

1. Measure with erlenmeyer 50 mL of 1% agarose gel.
2. Warm up the erlenmeyer for 15s in microwave: agarose will be more fluid and it will avoid bubbles when casting the gel
3. Cast the gel with the comb (for 15 wells)
4. Let the gel set for 20 min

The 1% agarose gel is kept at 60°C in the gel room.

Sample preparation

In a PCR 8-well rack mix:

- sample of nucleic acid sample according to volumes shown in table ??
- blue dye (bromothymol blue) according to volumes shown in table 1.9

For each sample, the final quantity of nucleic acids is 20 ng.

- Tap gently tubes to mix up everything

- Centrifuge briefly to bring all the liquid to the bottom

Table 1.9: nucleicacidssamples.csv

User	Label	Label colour	Box pos.	Extr. method	Start mat.	Qubit™ (ug/mL)	vol. a prelever	vol. loading buffer
CJ	DNA MP	GREEN	C4	MasterPure	Micro-algae culture	3,11	32,15	-17,15
CJ	RNA MP	GREEN	C5	MasterPure	Micro-algae culture	7,20	13,89	1,11
CJ	DNA 1802	PURPLE	C6	AllPrep	Micro-algae culture	2,44	40,98	-25,98
CJ	RNA AP	PINK	C7	AllPrep	Micro-algae culture	8,77	11,40	3,60
CJ	DNA AP	BLUE	E1	AllPrep	Micro-algae culture	5,86	17,06	-2,06
CJ	RNA AP	BLUE	E2	AllPrep	Micro-algae culture	31,50	3,17	11,83
CJ	DNA MP	YELLOW	E3	MasterPure	Micro-algae culture	18,20	5,49	9,51
CJ	RNA MP	YELLOW	E4	MasterPure	Micro-algae culture	32,20	3,11	11,89
CJ	DNA	NONE	E5	AllPrep	Micro-algae culture	7,71	12,97	2,03
CJ	RNA	NONE	E6	AllPrep	Micro-algae culture	3,94	25,38	-10,38
CJ	DNA	NONE	E7	AllPrep	Micro-algae culture	3,45	28,99	-13,99
CJ	RNA	NONE	E8	AllPrep	Micro-algae culture	14,00	7,14	7,86
CJ	MP DNA	PEACH	F1	MasterPure	Micro-algae culture	17,00	5,88	9,12
CJ	MP RNA	PEACH	F2	MasterPure	Micro-algae culture	7,19	13,91	1,09

Electrophoresis

For this electrophoresis migration, we run:

- 10 µL of each sample
- 5 µL of the 1 kb ladder
- 5 µL of the 2-log ladder

I leave empty lanes between ladders and samples.

1. Place the agarose gel into the gel box (electrophoresis unit) containing TAE buffer
2. Load 5 µL of molecular weight ladder into the first and last lane of the gel
3. Load 9 µL of each sample into the additional wells of the gel
4. Run for 60 min with the EPS 301 at 80 V, 400 mA
5. Remove gel from the migration tank and place in the staining container (a recycled tip box).
1. Ensure SYBR® gold is properly thawed.
2. Ensure the pH of TAE buffer is between 7.5 and 8
3. Prepare the staining solution at 1X SYBR® Gold in TAE buffer.

I actually use the staining solution solution I made yesterday and kept in aluminium foild at room tempature.

4. Transfer 100 mL of staining solution in the staining container.
5. Incubate under agitation for 10 to 40 min.

Gel with DNA was incubated 20 min but since it was hard to take a good picture, I decided to incubate the gel another 20 min to see the difference and it did not change much. Therefore for the gel with RNA, the incubation lasted 30 min.

6. Visualize your nucleic acid fragments with UV lights.

The CCD camera by BioRad does not work anymore. In the meanwhile, we use transilluminator and take the picture with my cell phone.

Results

Figure 1.13: 1% agarose gel with RNA after 30 min. incubation in the 1X SYBR® Gold.

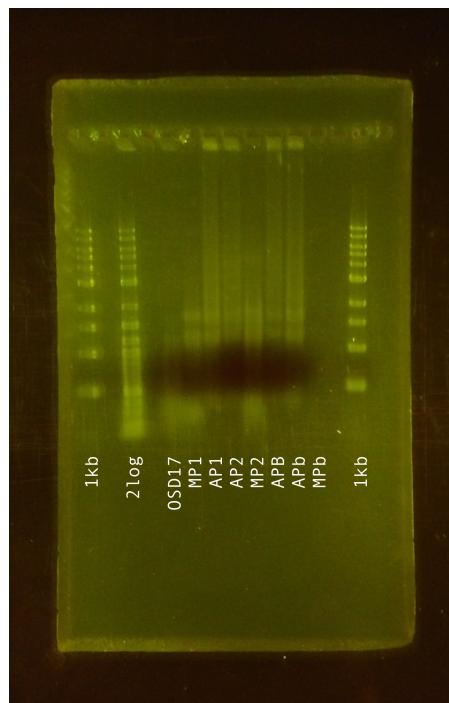


Figure 1.13 shows the gel with RNA samples after 60 min. migration and 30 min. staining in SYBR® Gold. It is possible to see the RNA samples except for sample labeled MPb: because the RNA concentration was very low, the volume I transferred to load 100 ng of RNA did not let me use a loading buffer, and when loading the sample in the gel, it did not fall at the bottom of the well and I lost the sample in the buffer ... As expected, sample OSD is the most degraded because it is the oldest sample. For all other samples, it is possible to see the bands corresponding to rRNAs (likely 16S and 23S). It seems that RNA extracted with MasterPure™ (MP) because it is possible to see smears at low molecular weight: small broken RNA fragment and nucleotides. Samples extracted with the AllPrep® mini kit seem to have better integrity with higher molecular fragments and no smears at the low molecular weight.

Conclusion

I was able to assess the integrity of RNA samples by electrophoresis with SYBR® Gold stain. While it was not possible to determine which kit was the best when looking at DNA integrity, it is clear that AllPrep® kit allows to retrieve RNA with high integrity compared to the Master-Pure™ kit.

This is consistent with the quantity of nucleic acid extracted with these two kits, and it confirms that the RNA molecules very likely get damaged during the MasterPure™ protocol because it is longer and it involves a DNase treatment while AllPrep® is really quick and only relies on columns to separate DNA from RNA.

These results reinforce my decision of using the AllPrep® kit.

1.14 March 18, 2018

1.14.1 Océans: une usine chimique qui se dérègle

Comme souvent dans le bus, j'écoute des podcast et j'ai trouvé l'émission de La Méthode Scientifique du 13 mars 2018 très intéressante. L'émission s'intitule *Océans: une usine chimique qui se dérègle*

Tag(s):
Literature (lit)

Author(s):
Clara Jegousse (cj)

Person(s):
Nicolas Martin
Marilaure Grégoire
Laurent Bopp
Pascal-Jean Lopez

<https://www.franceculture.fr/emissions/la-methode-scientifique/la-methode-scientifique-du-mars-2018-100344>

Quelle est la chimie propre aux océans et quel rôle joue-t-elle ? Pour quelles raisons cette chimie est-elle perturbée et quelles sont les conséquences de ces perturbations ? Quelles solutions sont aujourd'hui envisagées pour enrayer le phénomène ?

Saviez-vous que l'on trouve dans les océans terrestres tous les éléments chimiques connus, ne serait-ce qu'à l'état de traces parce qu'avant d'être en endroit où faire tremper pour se rafraîchir l'été, l'Océan Mondial est avant tout une incroyable, immense usine physico-chimique, qui brasse les éléments terrestres et atmosphériques dans des processus de conversion qui sont encore pour partie mal compris. Ce que l'on comprend bien en revanche, c'est que l'activité humaine, depuis près de deux siècles, est en train de bouleverser cet équilibre. Résultat : l'océan se réchauffe, s'étouffe, s'acidifie. La dernière fois que cela s'est produit, 90% des espèces vivantes ont disparu.

Océans : une usine physico-chimique qui se dérègle : c'est le problème qui va nous occuper pour l'heure qui vient.

Et pour en parler, nous avons le plaisir de recevoir aujourd'hui Marilaure Grégoire, directrice de recherche à l'Université de Liège, vice-président du groupe international de recherche Global Ocean Oxygen Network, son équipe vient de publier début janvier une étude dans la revue Science qui décrit l'ampleur planétaire des processus de désoxygénéation des océans et Laurent Bopp, océanographe et climatologue, directeur de recherche CNRS au Laboratoire des Sciences du Climat et de l'Environnement de l'Institut Pierre Simon Laplace.

Le reportage du jour

Rencontre avec Pascal-Jean Lopez, chargé de recherche CNRS au sein de l'unité de recherche "Biologie des organismes et des écosystèmes aquatiques" au Muséum National d'Histoire Naturelle. Comment les diatomées, ces microalgues qui jouent un rôle prépondérant dans la photosynthèse océanique peuvent-elles réagir à l'acidification des océans ?

1.15 March 20, 2018

1.15.1 Qiime2 tutorials

Introduction

Tag(s):
Meeting (meet)

Connecting to ISCaR

Author(s):
Clara Jegousse (cj)

Preparing your data

Person(s):
Gregory Farrant
Sigurlaug Skirnisdóttir
Elisabet Eik Guðmundsdóttir
Snaðís Björnsdóttir
Jérémie Courtin
Pauline Bergsten
Anouk Lyver
Mia Cerdoncyn

Running Qiime2

The meeting was actually one hour longer than expected ... so we did not really cover the end of the program.

1.15.2 Lab supervisors meeting

It was a very interesting meeting for me just to get an idea of what are global issues in the different laboratories.

Tag(s):
Meeting (meet)

Needed input

Author(s):
Clara Jegousse (cj)

- Teaching lab
 - Install pumps + sign
- Chemical room new organisation
 - Storage room for waste and hazardous chemicals.
 - Split the room in two in order to have a working space.
- Daily routine checklist
 - As part of documents given during induction day
 - For new students working in the lab: always make them perform the risk assessment of their experiments/equipment so they are aware of the risks.
- Freezers
 - Monitoring temperature and samples
 - Responsibility in case of emergency? (especially after hours)
 - Accessibility of keys to freezers
 - Frystilager on SharePoint
- Ventilation incident

It is now recommended to drain the pipes with few litres of water every week.

Person(s):
Beata Wawiernia
Natasa Desnica
Hildur Inga Sveinsdóttir
Magrétt Geirsdóttir
Halldóra Viðarsdóttir

1.15.3 Extraction and purification of genomic DNA and total RNA from Sterivex™ filter with modified AllPrep® method

Introduction

AllPrep® DNA/RNA Kits allows the simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample.

This is the final test for me before I extract my samples for stable-isotope probing (SIP) or metagenomics/metatranscriptomics.

I found in the -80 °freezer, I found a lost Sterivex™ filter with no label whatsoever. So I decided to use this Sterivex™ to make sure I can perform the extraction starting with a filter.

Tag(s):
 Laboratory (lab)
 Extraction (extr)
 DNA (dna)
 RNA (rna)
 Sterivex (svx)

Author(s):
 Clara Jegousse (cj)

Sample disruption and homogenisation of cells

1. Add 600 µL of Buffer RLT in the Sterivex™ filter.
2. Vortex for 10 seconds.

Lysate is green-brown, and filter looks clean and white.

3. Withdraw lysate using a 2mL serynge and transfer into a clean 2mL-Eppendorf tube.
4. Add approx. 0.2 mg of beads.
5. Disrupt MixerMill MM400 by Retsch using the program P9 (300 Hz) for 10 seconds three times and immerse tube icy water for 30 sec after each round.
6. Centrifuge the lysate for 3 min at maximum speed (20 000 x g) at 4 °C.
7. Transfer carefully the supernatent to an AllPrep® DNA spin column placed in a 2 mL collection tube.
8. Close the lid.
9. Centrifuge for 30 sec. at 8000 x g.
10. Use the flow-through for RNA purification: proceed to Total RNA purification.
11. Place the AllPrep® DNA spin column in a new 2 mL collection tube and keep at room temperature.

Mia prepared them, I must check the diameters of the beads.

Icy water allows more surface to be in contact and therefore a more efficient cooling effect compared to ice.

1. Add 1 vol. of 70% ethanol to the flow-through collected previously.

Here, one volume is 600 µL

2. Mix well by pipetting.
3. Transfer up to 700 uL of the sample to an RNeasy spin column placed in a 2 mL collection tube.
4. Centrifuge for 15 sec. at 8000 x g at 4 °C.
5. Discard the flow-through.
6. I repeat the three previous steps with the remaining volume of sample to make sure the column is saturated in RNA.

7. Add 700 μL of Buffer RW1 to the RNeasy spin column.
8. Close the lid.
9. Centrifuge for 15 sec. at 8000 $\times g$ at 4°C.
10. Discard the flow-thought.
11. Add 500 μL of Buffer RPE to the RNeasy spin column.
12. Close the lid.
13. Centrifuge for 15 sec. at 8000 $\times g$ at 4°C.
14. Discard the flow-thought.
15. Add 500 μL of Buffer RPE to the RNeasy spin column.
16. Close the lid.
17. Centrifuge for 2 min at maximum speed at 4°C.
18. Discard the flow-thought.

At this step, I could have performed the optional step to dry the column by centrifuging at maximum speed for one min.

19. Place the RNeasy spin column in a new 1.5 mL collection tube.
20. Add 50 μL of RNase-free water directly to the spin column membrane.
21. Close the lid gently.
22. Centrifuge for 1 min at 8000 $\times g$ to elute the RNA.

I repeated the elution of the RNA with an extra 30 μL of RNase-free water, which makes a final volume of 80 μL of RNase-free water containing the RNA.

Genomic DNA purification

1. Add 500 μL of Buffer AW1 to the AllPrep® DNA spin column from the Lysis.
- I accidentally added the AW2 buffer instead of AW1, so I just centrifuged for 15 sec., discarded flow-through, and then added the 500 μL of Buffer AW1 in the AllPrep® DNA spin column and I proceeded with the following steps as described.
2. Close the lid.
 3. Centrifuge for 15 sec. at 8000 $\times g$ at 4°C to wash the column membrane.
 4. Discard the flow-thought.
 5. Add 500 μL of Buffer AW2 to the AllPrep® DNA spin column
 6. Centrifuge for 2 min at maximum speed (20 000 $\times g$) at 4°C to wash the column membrane.
 7. Place the AllPrep® DNA spin column in a new 1.5 mL collection tube.
 8. Add 100 μL of Tris-HCl buffer (pH 8) to the DNA AllPrep® spin column membrane and close the lid.
 9. Incubate at room temperature for 1 min.

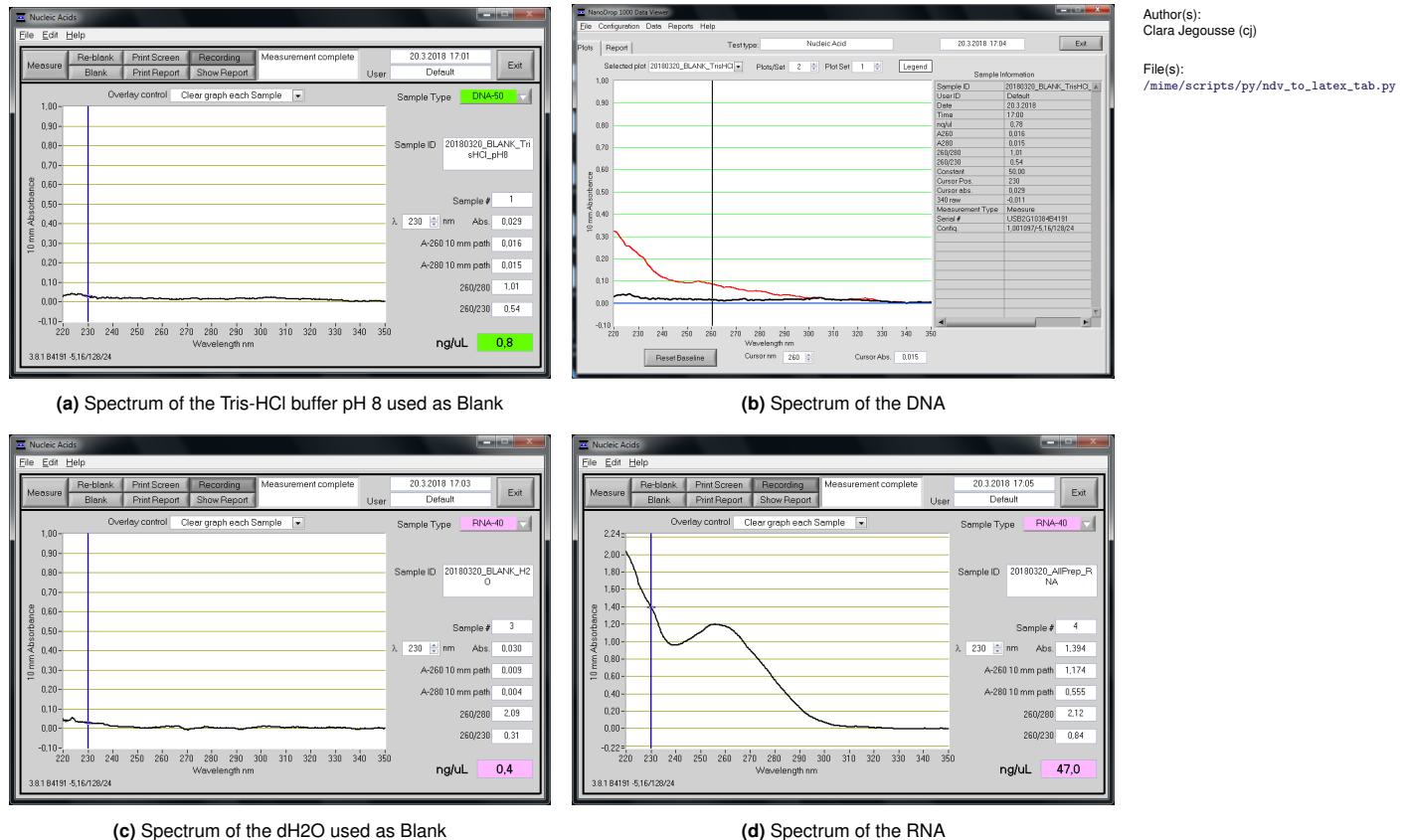
10. Centrifuge for 1 min at 8000 x g to elute the DNA.

I forgot to incubate for one minute the first time! lucky I actually repeat this step.

I did not repeat the elution with an extra 100 μL , so my final volume is 100 μL .

1.15.4 DNA and RNA quantification with NanoDrop® ND-1000 Spectrophotometer

Figure 1.14: NanoDrop spectra for DNA and RNA isolated with AllPrep® Mini Kit



I find that the spectrum obtained for the RNA is not too bad (cf. figure 1.14d).

Table 1.10: CJ20180320.txt

Sample ID	Time	ng/ul	A260	A280	260/280	260/230
20180320_BLANK_TrisHCl_pH8	17:00	0,78	0,016	0,015	1,01	0,54
20180320_A11Prep_DNA	17:02	4,28	0,086	0,054	1,59	0,39
20180320_BLANK_H2O	17:03	0,37	0,009	0,004	2,09	0,31
20180320_A11Prep_RNA	17:05	46,95	1,174	0,555	2,12	0,84

User: Default - Date: 20.3.2018 - Constant: 40,00 - Cursor position: 230

1.15.5 DNA quantification with Qubit™ DNA BR Assay Kit

I made 3 measurements: the first one with the previous calibration, and then two measurements with a new calibration.

Tag(s):
 Laboratory (lab)
 DNA (dna)
 RNA (rna)
 Quantification (qnt)

Table 1.11: Total DNA quantities in samples measured with Qubit™ DNA BR Assay Kit

Author(s):
 Clara Jegousse (cj)

Sample ID	μg/mL	V _f (mL)	m (μg)	m (ng)
CJ20180320_DNA_AP_5	1.37	0.083		
CJ20180320_DNA_AP_10	1.16	0.083		
CJ20180320_DNA_AP_5	1.37	0.083		
CJ20180320_DNA_AP_10	1.12	0.083	0.092	92.9
CJ20180320_DNA_AP_5	3.09	0.083		
CJ20180320_DNA_AP_10	2.34	0.083		

Qubit™ DNA BR Assay Kit
 LOT:#1927400 opened by myself today.

According to the measurements shown in table 1.11, I can say that I have about 100 ng of DNA left and that I was able to retrieve a little more than 100 ng of DNA.

1.15.6 RNA quantification with Qubit™ DNA BR Assay Kit

Table 1.12: Total DNA quantities in samples measured with Qubit™ DNA BR Assay Kit

Sample ID	μg/mL	V _f (mL)	m (μg)	m (ng)
CJ20180320_RNA_AP_5	45.0	0.063		
CJ20180320_RNA_AP_10	42.1	0.063		
CJ20180320_RNA_AP_5	43.2	0.063		
CJ20180320_RNA_AP_10	39.9	0.063		
CJ20180320_RNA_AP_5	44.7	0.063		
CJ20180320_RNA_AP_10	41.4	0.063		

1.16 March 21, 2018

1.16.1 Nucleic acid integrity assessment

1% Agarose gel preparation

1. Measure with erlenmeyer 50 mL of 1% agarose gel.
2. Warm up the erlenmeyer for 15s in microwave: agarose will be more fluid and it will avoid bubbles when casting the gel
3. Cast the gel with the comb (for 15 wells)
4. Let the gel set for 20 min

I cast my gel just after I arrived at work.

Tag(s):
Laboratory (lab)
DNA (dna)
RNA (rna)

Author(s):
Clara Jegousse (cj)

The 1% agarose gel is kept at 60°C in the gel room.

Sample preparation

In a PCR 8-well rack mix:

- For the DNA, I mix 6 µL of DNA sample with 4 µL of loading buffer (which is 15 ng of DNA)
- For the RNA, I mix 2.5 µL of RNA sample with 7.5 µL of loading buffer (which is 100 ng of RNA)
- Tap gently pipette mix

Electrophoresis

For this electrophoresis migration, we run:

- 10 µL of each sample
- 5 µL of the 1 kb ladder
- 5 µL of the 2-log ladder

I leave empty lanes between ladders and samples.

1. Place the agarose gel into the gel box (electrophoresis unit) containing TAE buffer
2. Load 5 µL of molecular weight ladder into the first and last lane of the gel
3. Load 9 µL of each sample into the additional wells of the gel
4. Run for 60 min with the EPS 301 at 80 V, 400 mA
5. Remove gel from the migration tank and place in the staining container (a recycled tip box).

SYBR® Gold Staining

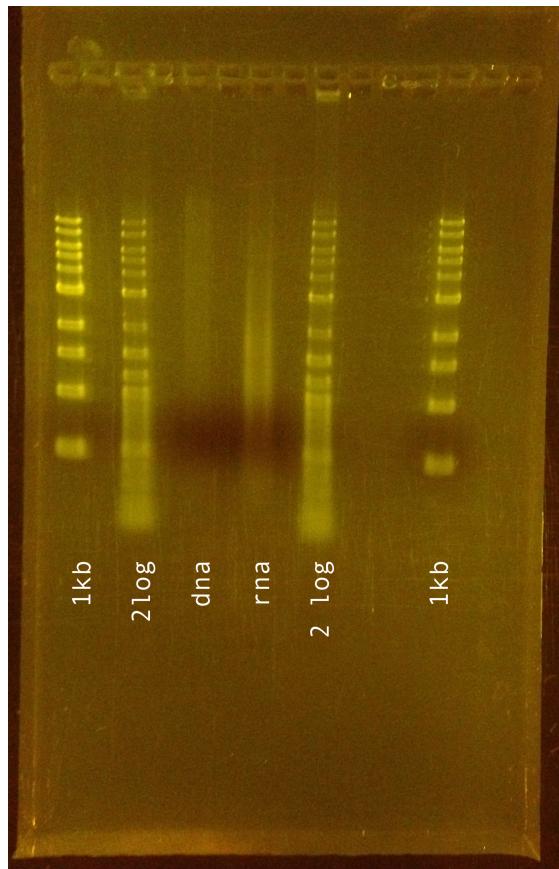
1. Ensure SYBR® gold is properly thawed.
2. Ensure the pH of Tris-acetate-EDTA (TAE) buffer is between 7.5 and 8
3. Prepare the staining solution at 1X SYBR® Gold in TAE buffer.

4. Transfer 100 mL of staining solution in the staining container.
5. Incubate under agitation for 30 min.
6. Visualize your nucleic acid fragments with UV lights.

The charge coupled device (CCD) camera by BioRad does not work anymore. In the meanwhile, we use transilluminator and take the picture with my cell phone.

Results

Figure 1.15: Picture of the gel after migration



Compared to the previous gels I obtained with SYBR® Gold, the genomic DNA does not show a clean band. This can be explained by the fact that I was working with cultures before which means one size of genomic DNA was very abundant compared to others while this DNA was obtained from a Sterivex™ filter containing a variety of species. Same thing can explain the fact that the rRNA bands seems a lot more blurry than previously.

https://www.researchgate.net/post/Can_anyone_help_me_with_gel_electrophoresis_of_total_RNA

Discussion

After quick search on the internet, the fact that I am unable to identity specific bands for the RNA sample makes sense. For *E.coli*, the 16S rRNA molecule is 1.5 kb and the 23S rRNA molecule weights 2.9 kb. For yeast, the 18S rRNA molecule is 2.0 kb and the 26S rRNA molecule weights 3.8 kb. And there are also more smaller RNA molecules like messenger-RNA (mRNA) and transfer-RNA (tRNA) (and maybe miRNA?). So with rRNA molecules from a wide variety of organisms, it is not surprising to observe a long smearing lane. Also, it is important to keep in mind that this gel does not allow me to determine the sizes of the RNA molecules because RNA will not run on agarose gel exactly according to its size: the migration of RNA molecules will be affected by the secondary and tertiary structures of the RNA molecules. Therefore, it is necessary to use denaturing gel (formaldehyde, urea, methyl mercury, etc.) and compare it to a RNA ladder!

1.17 March 22, 2018

1.17.1 Amplification by PCR with OneTaq® polymerase and EMP primers of DNA extracted with modified AllPrep® method

Description of the DNA templates

1. APB: DNA extracted from micro-algae cultures using the AllPrep® kit with a harsh bead beating step.
2. APb: DNA extracted from micro-algae cultures using the AllPrep® kit with a gentle bead beating step.
3. APb: DNA extracted from micro-algae cultures using the MasterPure™ kit with a gentle bead beating step.
4. SAPb: DNA extracted from a lost Sterivex™ filter using the AllPrep® kit with a gentle bead beating step.

Tag(s):
Laboratory (lab)
Polymerase chain reaction (pcr)
DNA (dna)
Earth Microbiome Project (emp)

Author(s):
Clara Jegousse (cj)

Description of controls

My controls are:

- + diluted DNA from *Thermoanaerobacterium sp.* (Bacteria)
- + DNA from *Thermococcus barophilus* 1 ng/ µL (Archaea)
- DNA from Cod 1 ng/ µL (Eukaryote)
- Autoclaved MilliQ water

Description of the polymerase

I will use the OneTaq® Hot Start DNA polymerase by New England BioLabs with the EMP primers.

Preliminary setup

1. Defrost all reagents and samples on ice (1h).
2. Place all required material in LabCair PCR Workstation and turn on UV light for at least 20 min.

It is very important that reagents (especially primers and dNTPs) are fully defrosted otherwise the concentration will not be accurate.

Methods

1. Work in LabCair PCR Workstation
2. Multiply the volume of each reagent by the number of individual PCR reactions you wish to perform (including the positive and negative controls) and add 2 extra to account for pipetting error (see table 1.13).

I have 4 samples, 4 controls, and 2 extra, which means I must prepare a master mix for 12 reactions.

3. In a single 2mL-Eppendorf tube combine the following:

- Sterile dH₂O
- 5X OneTaq Reaction Buffer
- OneTaq GC Enhancers
- dNTP mix (10 mM each nt)
- primers (EMP primers)
- OneTaq® Hot Start DNA Polymerase

4. Mix the contents by gently pipetting up and down several times (keep tube on ice)

I use a new tube of OneTaq® Hot Start DNA Polymerase that I received few weeks ago.

5. Transfer 22.5 µL of Master Mix into small PCR tubes
6. add 10 µL of DNA template in each PCR tube (on the pre-PCR bench) following the layout shown in figure 1.16.
7. Secure the tops to the PCR tubes
8. Tap gently tubes to mix up everything
9. Centrifuge briefly to bring all the liquid to the bottom before placing it in the PCR machine

I am not sure if the last well of the PCR rack actually contains 20 µL ... it seems that even with 2 extra, I did not have enough MasterMix.

Figure 1.16: Samples organisation in PCR tubes

	EMP							
	A	B	C	D	E	F	G	H
#12	+ARCH	+BACT	-COD	-H2O	APB	APb	MPb	SAPb

I decreased the denaturation/annealing/extension temperatures from 68 to 68 because it is still within the range recommended for the OneTaq® polymerase and it will give more time for the annealing (the downside is that it increases the chances of mismatch). Also I increased the number of cycles from 30 to 40 cycles which results in a total time of 1H30 in the thermocycler.

Table 1.13: Master Mix

Primers	EMP	
	Volumes (μL) for	
	1 reaction	10 reactions
dH ₂ O	10.875 μL	130.50 μL
5X OneTaq Reaction Buffer	5.00 μL	60.00 μL
GC Enhancers	2.50 μL	30.00 μL
dNTPs (10mM)	0.50 μL	6.00 μL
Forward primer (10 μM)	0.50 μL	6.00 μL
Reverse primer (10 μM)	0.50 μL	6.00 μL
OneTaq [®] DNA Polymerase	0.125 μL	1.50 μL
Template DNA	5 μL	-
V_f (μL)	25	-

Table 1.14: Thermocycler settings

Steps	EMP	
	T($^{\circ}\text{C}$)	Time
Initial denaturation	94	30 sec
Denaturation	94	30 sec
Annealing	60	40 sec
Extension	60	20 sec
Final extension	60	2 min
Infinite hold	4	-

1.17.2 Migration of nucleic acids in agarose gel

1% Agarose gel preparation

1. Measure with erlenmeyer 50 mL of 1% agarose gel.
2. Warm up the erlenmeyer for 15s in microwave: agarose will be more fluid and it will avoid bubbles when casting the gel
3. Add 2.5 µL of SYBR Safe to the agarose while swirling
4. Cast the gel with the comb (for 15 wells)
5. Let the gel set for 20 min

Tag(s):
Laboratory (lab)
Polymerase chain reaction (pcr)
DNA (dna)

Author(s):
Clara Jegousse (cj)

The 1% agarose gel is kept at 60°C in the gel room.

SYBR® Safe by Invitrogen,
10,000X in DMSO

PCR products preparation

With a multichanel pipette:

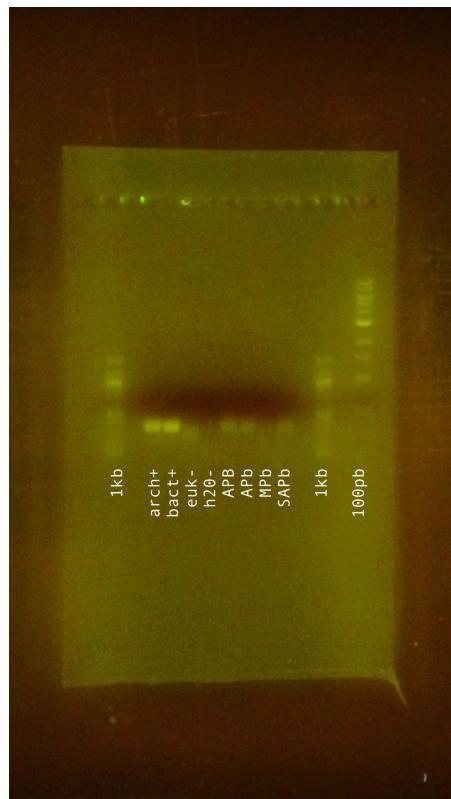
- 5 µL of each sample of PCR product
- 5 µL of blue dye (bromothymol blue)
- Tap gently tubes to mix up everything
- Centrifuge briefly to bring all the liquid to the bottom

Electrophoresis

For this electrophoresis migration, we run:

- 10 µL of each sample of PCR products
 - 5 µL of the 1 kb ladder
 - 5 µL of the 100 pb ladder
1. Place the agarose gel into the gel box (electrophoresis unit) containing TAE buffer
 2. Load 5 µL of molecular weight ladder into the first and last lane of the gel
 3. Load 10 µL of each sample into the additional wells of the gel
 4. Run for 50 min with the EPS 301 at 90 V, 400 mA
 5. Visualize your DNA fragments with UV lights

Figure 1.17: Picture of 1% agarose gel after 50 minute-long electrophoresis migration of PCR products obtained with EMP primers and DNA extracted from micro-algae cultures and surface seawater samples.



1.17.3 Woman in science

Tag(s):
Meeting (meet)

Author(s):
Clara Jegousse (cj)

Bibliography

- Zoe V Finkel, Mick J Follows, Justin D Liefer, Chris M Brown, Ina Benner, and Andrew J Irwin. Phylogenetic diversity in the macromolecular composition of microalgae. *PLoS one*, 11(5):e0155977, 2016.
- Michael J. Follows and Stephanie Dutkiewicz. Modeling diverse communities of marine microbes. *Annual Review of Marine Science*, 3(1):427–451, 2011. doi: 10.1146/annurev-marine-120709-142848. URL <https://doi.org/10.1146/annurev-marine-120709-142848>. PMID: 21329212.
- Anita Mäki, Pauliina Salmi, Anu Mikkonen, Anke Kremp, and Marja Tirola. Sample preservation, dna or rna extraction and data analysis for high-throughput phytoplankton community sequencing. *Frontiers in microbiology*, 8:1848, 2017.
- Ron Milo, Paul Jorgensen, Uri Moran, Griffin Weber, and Michael Springer. Bionumbers: the database of key numbers in molecular and cell biology. *Nucleic Acids Research*, 38:D750–D753, 2010. doi: 10.1093/nar/gkp889.
- Oliver J Williams, Rachel E Beckett, and David L Maxwell. Marine phytoplankton preservation with lugol's: a comparison of solutions. *Journal of applied phycology*, 28(3):1705–1712, 2016.

Abbreviations

CCD charge coupled device. 39

DMSO dimethyl sulfoxide. 43

DNA deoxyribonucleic acid. 26, 41, 43, 44

EMP Earth Microbiom Project. 40, 44

EPS electrophoresis power supply. 25, 28, 30, 38, 43

mRNA messenger-RNA. 40

PCR polymerase chain reaction. 8, 18, 19, 26, 27, 29, 38, 41, 43, 44

RNA ribonucleic acid. 26

SIP stable-isotope probing. 34

TAE Tris-acetate-EDTA. 38

tRNA transfer-RNA. 40

UV ultra violet. 25, 26, 28, 30, 39, 41, 43

People Index

Alexandra Leeper, 16
Anouk Lyver, 15, 33

Beata Wawiernia, 33

Elísabet Eik Guðmundsdóttir, 33

Gregory Farrant, 33

Halldóra Viðarsdóttir, 33

Hildur Inga Sveinsdóttir, 33

Hróar Hugosson, 15

Jérémy Courtin, 33

Kristinn Guðmundsson, 11

Laurent Bopp, 32

Magrét Geirsdóttir, 33

Marilaure Grégoire, 32

Mia Cerfonteyn, 11, 33

Natasa Desnica, 33

Nicolas Martin, 32

Pascal-Jean Lopez, 32

Pauline Bergsten, 33

Sigurlaug Skirnisdóttir, 33

Snædís Björnsdóttir, 33

Tómas Ármann Hafsteinsson, 11

File Index

/mime/scripts/py/ndv_to_latex_tab.py, 36

ndv_to_latex_tab.py, 19

Author Index

Clara Jegousse (cj), 3, 5, 7–9, 11–16, 19,
21, 23, 24, 26, 29, 32–34, 36–38,
40, 43, 44

Tag Index

DNA (dna), 3, 5, 7, 9, 16, 19, 23, 24, 26, 34, 36–38, 40, 43

Earth Microbiom Project (emp), 40
Excel (xls), 16
Extraction (extr), 3, 16, 34

Háskóli Íslands (hi), 11

Laboratory (lab), 3, 5, 7, 8, 11–16, 19, 21, 23, 24, 26, 29, 34, 36–38, 40, 43

Literature (lit), 13, 15, 23, 32

Meeting (meet), 15, 33, 44

Polymerase chain reaction (pcr), 40, 43

Quantification (qnt), 5, 7, 8, 19, 21, 36, 37

R (r), 9

RNA (rna), 3, 5, 8, 9, 16, 19, 21, 23, 24, 26, 29, 34, 36–38

Samples (smp), 11, 16, 21, 23

Selvogsbanki (sb), 12, 13

Sterivex (svx), 12, 34

Stokksnes (st), 13