

PCR: 31/1/2020

prepare primers (Pre-PCR room)

1) get the materials

- tips 0.5-20 μ L

- tips 100-1000 μ L

- tips 2-200 μ L

- tubes

- tubes

2) put materials + pipettes in UV cabinet (10 min)

3) clean bench

- deionised water

- ethanol (80%)

- DNA away

4) clean pipettes (same as bench)

5) put 2 primers in rack

→ write names on caps

6) put 2 tubes in rack and write corresponding primer name on cap

→ careful not to touch the inside of the cap!

on the tube side: my name, date

Karla

7) centrifuge the primers to get powder in the bottom

- load centrifuge equally!

- 2 minutes, 10,000 rpm, 20°C

if hands get dirty, can use ethanol → make sure it's dry before touching anything again! It destroys PCR-products!

8) add DEPC treated water to primers

1.113 ml to G52-F

1.055 ml to G55-F

) keep everything closed
don't talk while pipetting

9) vortex primers (max speed, 1 minute)

10) spin primers a little bit (5-10 seconds)

11) dilute primers 10x

~ fill 1000 μ L DEPC water in tubes

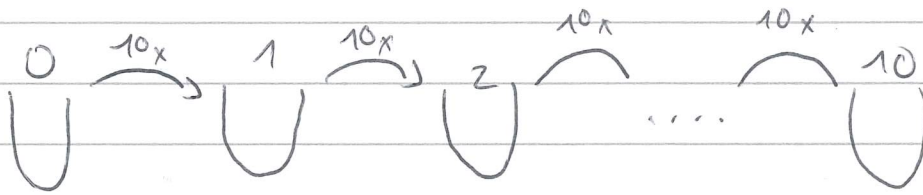
31/1/2020

- 12) to the tubes with 1000 μL DEPC water, add 100 μL primer each
- when adding primer, push it into the water (pipette tip into water, can mix it a bit)
- 13) vortex tubes (1 min)
- 14) spin (5 seconds)

Blanka also prepared 4 tubes with 1000 μL DEPC water for me (marked d H₂O)

Trash used pipette tips (yellow bin)
wash grey box and leave in drying rack

qPCR master mix: we will do dilution with the standard



and then have those in the 8 connected tubes. They can then go into the plates all at once:

each well: 15 μL

each tube: 25 μL

1	0	-	-	-	-
2	0	-	-	-	-
3	0	-	-	-	-
4	0	-	-	-	-
5	0	-	-	-	-
6	0	-	-	-	-
7	0	-	-	-	-
8	0	-	-	-	-

we need at least 375 μL $\rightarrow$ let's do 500 μL

3/2/2020

DNA extraction: Raphael's samples sample list:

strain	volume (ml)	cell density (pellet resuspended in)
1 NO018	50	2550 100 ul mqH2O
2 NO018	50	2960 100 ul mqH2O
3 NO018	50	2095 100 ul mqH2O
4 BO182	50	2055 100 ul mqH2O
5 BO182	50	1960 100 ul mqH2O
6 BO182	50	1255 100 ul mqH2O
7 VI013	50	3295 100 ul mqH2O
8 VI013	50	2880 100 ul mqH2O
9 VI013	50	2710 100 ul mqH2O
10 PA008	50	1125 100 ul mqH2O
11 PA008	50	990 100 ul mqH2O
12 PA008	50	1135 100 ul mqH2O

- 1) put equipment into UV cabinet
 - pipettes
 - tips (2-200, 0.5-20, 100-1000 μ L)
- 2) clean bench
 - deionised water, 80% ethanol, DNA-away
- 3) get extraction kit (Quick-DNA Fungal/Bacterial Miniprep Kit)
- 4) get samples from freezer room
 - a) let melt
 - b) vortex them (10-20 sec)
 - c) spin them (~5 sec)
 - don't, it accumulates again
- 5) prepare rack
 - sample tubes
 - ER Bashing Bead Lysis Tubes
- 6) add sample to ER BB Lysis Tube (50-100 mg wet weight of algae is ideal → we just used the whole sample (~200 μ L))
 - be careful, don't touch the inside of the lid of the ER BB Lysis Tubes
- 7) add 750 μ L of Bashing Bead Buffer to ER BB Lysis Tube
 - vortex buffer before using (in some protocols, it has to be heated)

3/2/2020

[...]

If pipette tip touches inside of tube, use new tip!

8) centrifuge

- 10,000 rpm, 1 minute, (20°C)

9) prepare rack

- collection tube with Zymo Spin tube

10) add 400 μ L supernatant to Zymo Spin tube

- careful not to touch the solid part with the broken cells

11) centrifuge Zymo spin tube

- 8,000 rpm, 1 minute, (20°C)

12) prepare rack

- collection tubes with Zymo Spin II C Column

13) ~~transfer 800~~ - vortex Genomic Lysis Buffer

prepare two pipettes

1) add 1200 μ L of Genomic Lysis Buffer to ^{Collection Tube with} Zymo spin tube with filtrate

- keep Zymo spin tube in hand

2) 2nd pipette: add 800 μ L from (B/1) - step to Zymo Spin Column tube

- put Zymo Spin tube on top of its Collection Tube again

→ This part is tough to understand in the kit's protocol! Be careful!

14) centrifuge Zymo Spin Column

- 10,000 rpm, 1 minute, (20°C)

15) Discard through-flow from collection tube (white bin)

16) repeat 13) and 14)

- can trash Zymo Spin Tubes now

- get all the remaining liquid, if less than 800 μ L

17) prepare new rack

- Collection Tube

18) put Zymo Spin Columns on new Collection Tubes

- can trash old ones now

3/2/2020

[...]

- 19) vortex DNA pre-wash buffer (10-20 sec)
- 20) add 200 μ L DNA pre-wash buffer to Zymo Spin Columns in new Collection Tubes
- 21) centrifuge
 - 10,000 rpm, 1 minute, (20°C)
- 22) vortex g-DNA wash buffer (10-20 sec)
- 23) add 500 μ L g-DNA Wash Buffer to Zymo Spin Column in new Collection Tubes
 - no need to discard filtrate from 20)
- 24) centrifuge
 - 10,000 rpm, 1 minute, (20°C)
- 25) vortex DNA Elution Buffer
- 26) prepare rack
 - 1.5 ml ~~Biosphere~~ Biosphere Safe Seal Tube
- 27) Put Zymo Spin Column in 1.5 ml tubes
 - can ~~discard~~^{trash} Collection Tubes
- 28) add 50 μ L DNA Elution Buffer to Zymo Spin Column
 - as close as possible to the white filter part
 - BUT: don't touch the white filter part!!!
- 29) for next centrifuge step, don't use the one in the pre-PCR room!
 - we can't close the tubes now, it will damage/dirty the centrifuge!
- 30) ~~take samples to~~
wait 3-4 minutes to get DNA out of column,
- 31) use centrifuge next door, window, left side
 - 10,000 rpm, 30 sec
 - close lid!

some lids broke (1, 2, 10, 12) can trash Zymo Spin Columns after 31)

→ we transferred the DNA from those into new 1.5 ml tubes

3/2/2020

Finished!

- put back DNA extraction kit
- clean bench
- put DNA-tubes into my qPCR box + freezer in qPCR room
- turn off centrifuge

Concern: no negative control

- should have started it at the same time as the other samples to trace any contamination
- think of it next time!
- this is method-testing, so it's not a huge deal

17/2/2020

DNA extraction: Skogaryd 2018

1) put equipment in UV-cabinet

- pipettes
- racks
- pipette tips
- pincer + scissor

2) clean bench

- water, ethanol, DNA-away

3) get rack + 2 falcon tubes (one for milliQ, one for ethanol)

~~gas~~ burner

Kim wipes

extraction kit (Qiagen DNeasy PowerSoil Kit)

4) take 16 PowerBead Tubes, put them into rack

mark 1 - 16

5) add filter DNA sample to PowerBead Tube with sterilised scissor (milliQ, ethanol, fire) and cut to tiny pieces

careful: pieces stick to scissor, get them into tube with the pincers

16 is the control-sample

17/02/2020

DNA extraction: Skogaryd 2018

sample list

sample	lake	depth	filtrate	id	year
1	3	1	100 ^m / _l	i-70118030101 DNA B 100 ml 0,2 um filt Erker	2018
2	3	2	100 ^m / _l	5-70118030102 DNA B 100 ml 0,2 um filt Erker	2018
3	4	1	100 ^m / _l	5-70118040101 DNA B 100 ml 0,2 um filt Erker	2018
4	4	2	100 ml	5-70118040102 DNA B 100 ml 0,2 um filt Erker	2018
5	5	1	80 ml	5-70118050101 DNA B 80 ml 0,2 um filt Erker	2018
6	5	2	80 ml	5-70118050102 DNA B 80 ml 0,2 um filt Erker	2018
7	6	1	?	5-70118060101 DNA B 0,2 um filt Erker	2018
8	6	2	?	5-70118060102 DNA B 0,2 um filt Erker	2018
9	7	1	?	5-70118070101 DNA B 0,2 um filt Erker	2018
10	8	1	100 ml	5-70118080101 DNA B 100 ml 0,2 um filt Erker	2018
11	8	2	100 ml	5-70118080102 DNA B 100 ml 0,2 um filt Erker	2018
12	9	1	100 ml	5-70118090101 DNA B 100 ml 0,2 um filt Erker	2018
13	9	2	100 ml	5-70118090102 DNA B 100 ml 0,2 um filt Erker	2018
14	11	1	?	5-70118110101 DNA B 0,2 um filt Erker	2018
15	11	2	?	5-70118110102 DNA B 0,2 um filt Erker	2018

16 K Control: nothing added

17/2/2020

[...]

- 6) vortex Solution C1 (5-10 seconds)
- 7) to each Power Bead Tube, add 60 μL of Solution C1
- 8) vortex Power Bead Tubes at max. speed for 20 minutes
- 9) centrifuge 30s (10,000 rpm)
 - if it's still milky, centrifuge some more
- 10) transfer supernatant ($\sim 500 \mu\text{L}$) to clean 2ml collection tubes
 - can put pipette tip into the solid part and dig around for the liquid
 - no worries if you get some particles, we're cleaning it afterwards
- 11) vortex C2 solution 5-10 seconds
- 12) add 250 μL of C2 solution.
- 13) vortex for 5 seconds
- 14) incubate at 4°C for 5 minutes (fridge)
- 15) centrifuge 10,000 rpp for 1 minute
- 16) transfer 600 μL of supernatant to clean ^{2ml} collection tubes
 - careful not to disturb the pellet!
- 17) add 200 μL of C3 solution
- 18) vortex 5 seconds
- 19) incubate at 4°C for 5 minutes (fridge)
- 20) centrifuge 10,000 rpp for 1 minute
- 21) transfer 700 μL of supernatant to clean 2ml collection tubes
 - watch for pellet
- 22) shake C4 solution
- 23) ~~transfer 700 μL of supernatant to clean 2ml collection tubes~~
- 21) - watch for pellets: start high up in tube and slowly go down, pipette slowly!
 - important: if we have particles left, they will stick to the DNA filter in the next step... so be careful!

17/2/2020

[...]

- if you get a bubble, pipette it back + centrifuge it again!

24) add 1200 μ L C4 solution

25) vortex 5 seconds

→ tubes are completely full, vortex tubes on the side!

26) transfer 675 μ L onto the MB Spin Column

27) centrifuge 10,000 rpm for 1 minutes

28) Discard throughflow

- keep the tube + close it again!

29) repeat 26-28 ^{more} two times

- at last transfer, some liquid can be trapped in the lid, get that, too!

30) vortex C5 solution

31) add 500 μ L of C5 solution

32) centrifuge 10,000 rpm for ~~1 minute~~ 30 seconds

33) discard throughflow

34) centrifuge 10,000 rpp for 1 minute

- get it out without hitting it, otherwise centrifuge again to get rid of the ethanol → it will degrade the DNA later!

35) carefully place the MB Spin Column into a clean 2ml collection tube

- avoid splashing any C5 solution onto the column!

36) ~~add 50 μ L C5~~ vortex C6 solution

37) add 50 μ L C6 solution to the center of the white filter membrane

- as close as possible to white filter membrane

- BUT: don't touch the white filter membrane!

38) wait 3-4 minutes

39) centrifuge (other room, with lid) 10,000 rpm, 30 seconds

- broken lids: 1

- trash columns and close collection tubes

- transferred sample 1 to new 1.5 ml ~~collection~~ tube

18/2/2020

sample list: Skogaryd 2018 and 2017

<u>sample</u>	<u>lake</u>	<u>depth</u>	<u>filtrate</u>	<u>year</u>
16	1	1	80 ml	2018
16				
17	1	2	80 ml	2018
18	2	1	?	2018
19	2	2	80 ml	2018
20	7	2	?	2018
21	12	1	100 ml	2018
22	12	2	100 ml	2018
23	4	1	150 ml	2017
24	4	2	150 ml	2017
25	5	1	100 ml	2017
26	5	2	100 ml	2017
27	6	1	150 ml	2017
28	6	2	100 ml	2017
29	17	1	?	2017
30	17	2	100 ml	2017

L Control: nothing added

5-70118070102

Skogaryd Lake Year Sampling Site
DNA B
0,2 um filt



Erker

5-70118120101

Skogaryd Lake Year Sampling Site
DNA B 100 ml
0,2 um filt



Erker

5-70118120102

Skogaryd Lake Year Sampling Site
DNA B 100 ml
0,2 um filt



Erker

18/2/2020

DNA extraction: rest of Skogaryd 2018, beginning Skogaryd 2017

procedure: same as 17/2/2020

changes: C1 solution should be heated (60°C) before use, to make sure it's completely liquid

After the last centrifuging (3g), the cap of sample 17 broke off. I transferred ~~at~~ the sample to a clean 1.5 ml tube.

Ask Josefine about filtration volume.

19/2/2020

DNA extraction: rest of Skogaryd 2017

procedure: same as 18/2/2020

After the last centrifuging (3g), the cap of sample M broke off. I transferred the sample into a clean 1.5 ml tube.

Sample list is on p. 62.

19/12/2020

sample list: Skogaryd 2017

sample	lake	depth	filtrate	year
31	7	1	80 ml	2017

5-7011707010101
DNA B
Vol: 80ml

5-7011707010102
DNA B
Vol: 80ml

32	7	2	80 ml	2017
----	---	---	-------	------

5-7011708010101
DNA B
Vol: 80ml

5-7011708010102
DNA B
Vol: 80ml

33	8	1	80 ml	2017
----	---	---	-------	------

34	8	2	80 ml	2017
----	---	---	-------	------

35	9	1	150 ml	2017
----	---	---	--------	------

5-7011709010101
Skogaryd Lake Year Sampling Site
DNA B
Vol: 150ml
Gbg

36	9	2	150 ml	2017
----	---	---	--------	------

5-7011709010102
Skogaryd Lake Year Sampling Site
DNA B
Vol: 150ml
Gbg

37	11	1	100 ml	2017
----	----	---	--------	------

DNA B
File: 100ml
5-701171010101
ERKEN

38	11	2	100 ml	2017
----	----	---	--------	------

5-7011711010102
Skogaryd Lake Year Sampling Site
DNA B 100 ml
Vol: 100 ml
Gbg

39	12	1	80 ml	2017
----	----	---	-------	------

5-7011712010101
DNA B
Vol: 80ml

40	12	2	80 ml	2017
----	----	---	-------	------

5-7011712010102
DNA B
Vol: 80ml

41	14	1	80 ml	2017
----	----	---	-------	------

42	14	2	80 ml	2017
----	----	---	-------	------

43	15	1	80 ml	2017
----	----	---	-------	------

44	15	2	80 ml	2017
----	----	---	-------	------

M control: nothing added

N control: nothing added

24/2/2020

DNA quantification with Pico Green

Samples measured: Skogaryd 2017 + 2018, Raphael's samples (1-12)
(1-44, K, L, M)

Protocol used: DNA quantification with Pico Green by
Anna Székely

Calculations for buffer, standards, Pico Green:

standards: low $2\mu\text{L st} + 18\mu\text{L TE}$



medium $2\mu\text{L} + 398\mu\text{L TE}$ ← $2 \times (4\mu\text{L} + 796\text{TE})$
 $2\mu\text{L st} + 398\mu\text{L TE}$ ← $2 \times (4\mu\text{L} + 796\text{TE})$

Pico Green: $125\mu\text{L PicoGreen} + 25\text{mL TE}$ this is how we did

TE ~~buffer~~: 16 mL for plates
1.61 mL for standards
25 mL for Pico Green
22.61 mL

↳ we made 50 mL: 2.63 mL TE + 50 mL milliQ water
buffer

Protocol changes: In 6 - Prepare plate - add DNA samples
we added $2\mu\text{L}$ of sample to each well

24/2/2020

Plate list

PLATE 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	St medium	St medium	1	1	9	9	17	17	25	25	33	33
B	St medium	St medium	2	2	10	10	18	18	26	26	34	34
C	St medium	St medium	3	3	11	11	19	19	27	27	35	35
D	St medium	St medium	4	4	12	12	20	20	28	28	36	36
E	St low	St low	5	5	13	13	21	21	29	29	37	37
F	St low	St low	6	6	14	14	22	22	30	30	38	38
G	St low	St low	7	7	15	15	23	23	31	31	39	39
H	St low	St low	8	8	16	16	24	24	32	32	40	40

PLATE 2

= Raphael's algae samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	St medium	St medium	41	41	1	1	9	9				
B	St medium	St medium	42	42	2	2	10	10				
C	St medium	St medium	43	43	3	3	11	11				
D	St medium	St medium	44	44	4	4	12	12				
E	St low	St low	K	K	5	5						
F	St low	St low	L	L	6	6						
G	St low	St low	M	M	7	7						
H	St low	St low			8	8						

26/2/2020

received from Raphael: 4x 15ml MWC + Se medium
2x 15ml NO-018 culture (US)
2x 15ml SE-001 culture (Poland)

Transferred to culture bottles:

SE-001	NO-018
26-2-20	26-2-20
Karla	Karla
2x	3x
10ml medium	10 ml medium
+ 15 ml culture	+ 15 ml culture
	3rd: ~ 5 ml medium
	+ 7 ml culture

Stored in: incubator fish lab

Bottles have to lie down!

3/3/2020

qPCR test run

Test run with Raphael's algae samples and 3 of the Skogaryd samples in 3 dilutions (x10, x20, x50).

Samples: 60 (7x3 standards, 1x3 water, 2x3 Raphael's samples, 1x sample 2, 27, 34, 9x3 diluted samples).

Sample dilutions:	DNA	water	
undiluted	5 μ L	0 μ L	take from DNA extract
x10 dilution	3 μ L	27 μ L	take from DNA extract
x20 dilution	10 μ L	10 μ L	take from x10 dilution
x50 dilution	3 μ L	117 μ L	take from 20 x10 dilution

3/3/2020

Master Mix: 60 samples, prepare enough for 70 reactions
20 μL master mix per well needed

	μL	μL
water	6	420
fwd primer	0.75	52.5
rev primer	0.75	52.5
SYBR Green	12.5	875
DNA or standard	5	



for 1 reaction

for 70 reactions

To each well, we add 20 μL Master mix and 5 μL DNA or Standard

Standard: going to make 7 standards (20 μL per standard, as we'll need 3 x 5 μL for the wells on the plate) to the stock

1. We calculated the amount of water to add (646.29 μL)^v

→ see page 67 and Folder "Errsjön" → "qPCR"

numbers used from the IDT delivery sheet:

Amount delivered = 4 μg

Molecular weight = 1862948.3

2. To the stock tube, we added 323.15 μL water (too little space for whole amount), transferred it to a new tube (labelled ST) and added the other 323.15 μL water.

3. Diluted the stock 10x: 64.63 μL stock + 581.66 μL water (labelled ST x 10)

3/3/2020

Plasmid dilution

Delivered from IDT

4 ug

plasmid weight	4.00E-06	g
molar weight (M)	1862948.3	g/mole
Avogadro	6.02E+23	1/mole
plasmid amount (n)	2.15E-12	mole
plasmid amount (pieces)	1.29E+12	targets

Stock dilution

water to add	646.29	ul
target concentration	2.00E+09	target/ul

Working stock dilutions

number tubes	10	
target from stock	64.63	ul
water to add	581.66	ul
total V	646.29	ul
target concentration	2.00E+08	target/ul

Reaction aliquots	25	ul
Total of aliquots	approx 300	

Reaction

max conc. standard tube		
amount of working stock/tube	5	ul
amount of plasmids	1.00E+09	target/reaction

Standards: we diluted from STx10 by 10 seven times.

standard	dilution	standard to add (ul)	water to add (ul)
st 1	x 10	2	18
st 2	x 100	2	18
st 3	x 1000	2	18
st 4	x 10000	2	18
st 5	x 100000	2	18
st 6	x 1000000	2	18
st 7	x 10000000	2	18

For st 1, we added STx10, for st 2, we added st 1, etc.

3/3/2020

qPCR plate

1) we added 20 μ L Master Mix to all 60 wells.

2) we added 5 μ L of standard to 1 A-G

2 A-G

3 A-G

→ see plate labelling below

3) we added 5 μ L water to H1-3

4) we added ^{5 μ L} Raphael's undiluted samples to A 4-5

B 4-5

5) we added ^{5 μ L} undiluted Skogaryd samples to C 4-6

6) we added ~~5~~ 5 μ L of diluted Skogaryd samples to

D 4-6 and A 7-9

¹
H 4-6

¹
D 7-9

When adding everything, go all the way down with the pipette tip. Don't do the extra-pressing to get air out of the pipette, we don't want to splash or create bubbles! When releasing liquid, slowly move the tip upward to avoid bubbles.

<>	1	2	3	4	5	6	7	8	9
A	st 1	st 1	st 1	as 1	as 1	as 1	27-50	27-50	27-50
B	st 2	st 2	st 2	as 6	as 6	as 6	34-10	34-10	34-10
C	st 3	st 3	st 3	2-0	27-0	34-0	34-20	34-20	34-20
D	st 4	st 4	st 4	2-10	2-10	2-10	34-50	34-50	34-50
E	st 5	st 5	st 5	2-20	2-20	2-20			
F	st 6	st 6	st 6	2-50	2-50	2-50			
G	st 7	st 7	st 7	27-10	27-10	27-10			
H	water ✓	water ✓	water ✓	27-20	27-20	27-20			

Check plate from below, same amount in all wells?

Careful: Master Mix, water and sample DNA is added in the pre-PCR room! Standards are made and added in the post-PCR room!

3/3/2020

Don't forget to vortex everything before using! Spin afterwards to get liquid to the bottom of the tubes.

When diluting, vortex to mix + spin before use!

When all wells are filled, close the plate with the plastic cover sticker. Use a bank card or similar to get it on evenly, then stretch out with it. Close the sides with the cards.

Go to qPCR room.

Use the computer right of the printer. Start + open the program Bio-Rad CFX.

Never touch the qPCR machine, operate it via the PC!

We modified the qPCR protocol from Johansson et al. 2015 according to Bianka's experience.

Johansson conditions

10 min at 90°C
40 cycles at 95°C for 15 s
and 60°C for 60 s

melt curve initiated by 95°C for 60 s

ramp from 55°C to 95°C
with 30 s at each degree

our conditions

2 min at 90°C
40 cycles at 95°C for 15 s
and 60°C
and 72°C

Saved protocol in my folder "Karla" as
Left it to run overnight.

4/3/2020

qPCR results: standards worked well, we got a very nice curve!

samples worked, DNA detected.

10x diluted samples: not too close together
20x and 50x diluted samples: close together
10x and 20x results related well to each other (20x were ~half of 10x)
50x seemed a bit inhibited

→ we decided to run the qPCR with those standards and x20 diluted samples at our modified qPCR conditions

Preparations: make sample dilutions (all Skogaryd samples) and store them in 8x strips (8 strips)

x20 dilutions: 1.5 ml tubes in rack, labelled

1) add 190 μ L water

2) add 10 μ L of ~~sample~~ DNA extracts (vortex ^{DNA extracts} before!)

strips: 8x strips in small racks (4 per rack, 2 empty rows between them)

label: 1-8 17-24 33-40
(x8) 9-16 25-32 41-44

1) vortex x20 dilutions

2) add 20 μ L to each correspondingly labelled tube of the 8x strips

Keep x20 dilutions + 8x strips in freezer in the qPCR room.

4/3/2020

Moved to post-PCR room.

Standards: ST x 10 from freezer $\rightarrow$ vortex

for 8xstrips (5x)

standard	dilution	standard added (μ L)	water added
st 1	x 10	14	126
st 2	x 100	14	126
st 3	x 1000	14	126
st 4	x 10000	14	126
st 5	x 100000	14	126
st 6	x 1000000	14	126
st 7	x 10000000	14	126

For st 1, we ~~used~~ added ST x 10, for st 2, we added st 1, etc.
Vortex standard before taking from it for next dilution!

Prepare small racks with 8xstrips (5x).

Add 20 μ L of standard to correspondingly labelled vial in the 8x-strips.

Make sure all standards were vortexed before transferring!

Store in the Limno-freezer in the post-PCR lab (top shelf)

Labelled: Karla, stock + standard, 3/3/2020, PI: Eva Lindström

5/3/2020

running 2 qPCR-plates with all Skogaryd samples

PLATE 1

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	st 1	st 1	st 1	1	1	9	9	9	9	10	11	12
B	st 2	st 2	st 2	2	2	10	10	10	10	17	17	17
C	st 3	st 3	st 3	3	3	11	11	11	11	18	18	18
D	st 4	st 4	st 4	4	4	12	12	12	12	19	19	19
E	st 5	st 5	st 5	5	5	13	13	13	13	20	20	20
F	st 6	st 6	st 6	6	6	14	14	14	14	21	21	21
G	st 7	st 7	st 7	7	7	15	15	15	15	22	22	22
H	water	water	water	8	8	16	16	16	16	23	23	23
										24	24	24

PLATE 2

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	st 1	st 1	st 1	25	25	33	33	33	33	41	41	41
B	st 2	st 2	st 2	26	26	34	34	34	34	42	42	42
C	st 3	st 3	st 3	27	27	35	35	35	35	43	43	43
D	st 4	st 4	st 4	28	28	36	36	36	36	44	44	44
E	st 5	st 5	st 5	29	29	37	37	37	37	19	19	19
F	st 6	st 6	st 6	30	30	38	38	38	38	26	26	26
G	st 7	st 7	st 7	31	31	39	39	39	39	23	23	23
H	water	water	water	32	32	40	40	40	40	27	27	27

Plate 2, samples
19, 26, 27
→ those are the
same as on plate 1,
they are there for
comparison

samples: 96 x2

5/3/2020

Morning: pre qPCR-room

1) made Master Mix for plate 1 → see recipe below

• needed: 1920 μL

• made: 2400 μL (separately in 2 tubes à 1200 μL)

Recipe

1 reaction	$\mu\text{g/L}$	60 reactions	$\mu\text{g/l}$	mix for 1 tube	• --> we make 2 tubes add to 1 well from reaction stock
water	6		720	360	20
fwd prime	0.75		90	45	
rev primer	0.75		90	45	
SYBR green	12.5		1500	750	
DNA or sta	5				
			2400	1200	

needed for one plate
1920

→ vortex! + spin

2) transferred Master Mix into 8x strips

- 2x 8strips

- 150 μL Master Mix in each tube

→ go all the way to the bottom, release slowly

→ afterwards: vortex + spin

Prepared plate 1

1) add 20 μL Master Mix to ~~all~~ all wells

→ multipipette

→ go all the way to bottom, release slowly, don't do the last press to push out air

2) add 5 μL water (from freezer) to H 1-3)

3) take sample 8x strips (1-8, 9-16, 17-24) from freezer

- defreeze, vortex, spin

4) add 5 μL of sample to respective wells

→ multipipette

5/3/2020

Morning: post-PCR room

move plate to post-PCR room

- 1) take standard 8x strip from freezer
 - defreeze, vortex, spin
- 2) add 5 μ L standard to respective wells A 1-3
 - multipipette
- 3) put plastic sticker cover on
 - card to do it without air bubbles
 - straighten, close sides
 - remove end parts

qPCR room

- 1) start qPCR machine + PC
- 2) open program
- 3) load our protocol
- 4) load + edit our plate set-up (sample numbers)
↳ save
- 5) check sample volume (25 μ L)
- 6) open machine, put in plate, close machine
- 7) start run
↳ save data in my folder "plate 1 - date - sample 1-24"

results: we got a good curve and the samples are distributed along it, too. Success!

afternoon: repeated the steps from this morning (p. 73-74) for plate 2

standards: the multipipette became a bit imprecise, so we added 3 A-Q with a single pipette. If the standard triplets are inconsistent, that one is the best to rely on.