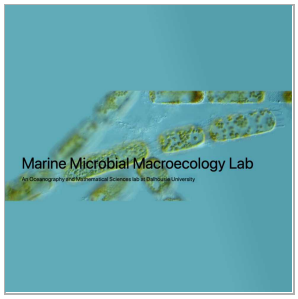

VERSION 11

FEB 20, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6qpvro85bvmk/v11

Protocol Citation: Ying-Yu Hu, Zoe V. Finkel 2023. Total RNA and DNA from Microalgae (12 samples per microplate) . **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvro85bvmk/v11 Version created by Ying-Yu Hu

Protocol status: Working
We use this protocol and it's working

Created: Oct 18, 2022

Last Modified: Feb 20, 2023

PROTOCOL integer ID: 71512

Keywords: RNA, DNA, SYBR Green II, DNase, RNase, microalgae, fluorescence

Total RNA and DNA from Microalgae (12 samples per microplate) V.11

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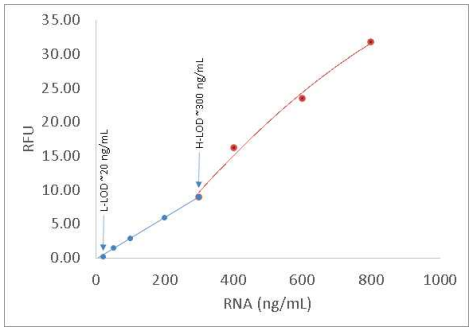
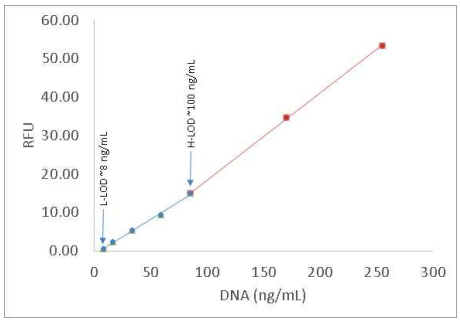
ABSTRACT

Here we describe a protocol for extracting and quantifying bulk RNA and DNA from microalgae, which is adapted from Berdalet E. et al. (2005).

RNA and DNA are extracted from microalgae samples and then quantified by fluorochrome SYBR Green II.

The level of sensitivity of this method is set at ca. 20 ~300 ng RNA and 10 ~ 100 ng DNA (ml assay)$^{-1}$.





CITATION

Berdalet E, Roldán C, Olivar MP, Lysnes K. Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part A. Optimisation of the assay. Scientia Marina.

LINK

https://doi.org/10.3989/scimar.2005.69n11

CITATION

Berdalet E, Roldán C, Olivar MP. Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part B. Quantification in natural samples. Scientia Marina.

LINK

https://doi.org/10.3989/scimar.2005.69n117

GUIDELINES

Estimation of RNA/DNA in the collected microalgae samples:

Under replete condition, RNA and DNA is about 5.7% and 1% in total dry mass, while Chl-a is bout 1.1% in total dry mass. Therefore, RNA_ug/L = Chl-a_ug/L X (5.7/1.1), DNA_ug/L = Chl-a_ug/L X (1/1.1).

Common dilution from sample collected on the filter to assay is 1/40.

MATERIALS

STEP MATERIALS

- Ribonuclease A from bovine pancreas **Sigma Aldrich Catalog #R6513-50MG**
- DEOXYRIBONUCLEASE1 RNase and Protease Free **Bioshop Catalog # DRB002.10**
- Magnesium chloride solution **Sigma Aldrich Catalog #63069-100ML**
- Calcium chloride solution **Sigma Aldrich Catalog #21115-100ML**
- SYBR™ Green II RNA Gel Stain, 10,000X concentrate in DMSO **Thermo Fisher Catalog #S7564**
- Tris(hydroxymethyl)aminomethane hydrochloride 1M pH 8.0 RNase free **Fisher Scientific Catalog #AAJ60080AK**
- Deoxyribonucleic acid from calf thymus **Sigma Aldrich Catalog #D4522-1MG**
- N-Lauroylsarosine sodium salt solution (20% RNase/DNase free) **Sigma Aldrich Catalog #L744-50mL**
- EDTA buffer (0.5M DNase/RNase free) **Bioshop Catalog #EDT333.100**
- UltraPure™ DNase/RNase-Free Distilled Water **Thermofisher Catalog #10977023**
- E. coli Total RNA **Thermo Fisher Scientific Catalog #AM7940**

SAFETY WARNINGS

No data is available addressing the mutagenicity or toxicity of SYBR® Green II Nucleic Acid Gel Stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, solutions of SYBR Green II Nucleic Acid Gel Stain should be poured through activated charcoal before disposal or collected in waste container to be treated later. The charcoal must then be incinerated to destroy the dye.

Day 1: Freeze-dry samples

1 Freeze dry samples and blank filters. Freeze at -80 °C until processed.

Note

1. Freeze-drying should be as short as possible to reduce sample degradation.
2. The exact duration of freeze-drying depends on size of filter, quantity of sample and the size of container.

Equipment

FreeZone® 2.5 L Benchtop Freeze Dryers	NAME
Labconco®	BRAND
700202000	SKU

Day 1: Prepare primary solutions

2 Turn on UV light in biosafety cabinet for 00:15:00

3 Clean working surface with decontamination solution.

4 Prepare Tris buffer [M] 5 mM pH 8.0

4.1 Pour [M] 1 M pH 8.0 Tris into an RNase free 15 mL Falcon tube

Tris(hydroxymethyl)aminomethane hydrochloride 1M pH 8.0 RNase free **Fisher Scientific Catalog #AAJ60080AK**

Equipment

Falcon® Centrifuge Tubes	NAME
Polypropylene, Sterile, 15 mL	TYPE
Corning®	BRAND
352096	SKU

4.2 Directly add 2.5 mL [M] 1 M pH 8.0 Tris into 500 mL RNase free water in its original package.
UltraPure™ DNase/RNase-Free Distilled Water **Thermofisher Catalog #10977023**

Equipment	
BT Barrier Pipet Tips	NAME
Pre-Sterile	TYPE
Neptune®	BRAND
BT1250, BT100, BT10	SKU

5 RNA primary standard solution ([M] 200 ug/ml)

5.1 In the original package, the **frozen** E. Coli Total RNA is of 1 mg/mL, in which total RNA is 200 ug.
E. coli Total RNA **Thermo Fisher Scientific Catalog #AM7940**

Note

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/sp_7940.pdf

5.2 Uncap the original package of E. Coli Total RNA and directly add 800 µL Tris buffer ([M] 5 mM , pH 8.0) .
Cap the package and vortex for a thorough mix.

5.3 Aliquot 30 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at -80 °C

Equipment	
Finnpipette Stepper Pipette	NAME
Thermo Scientific™	BRAND
4540000	SKU

Equipment

Finntip stepper pipette tips	NAME
500 ul (sterile)	TYPE
Thermo Scientific	BRAND
Thermo Scientific™ 9404173	SKU

6 DNA primary standard solution (≈ [M] 500 ug/ml)

6.1 Uncap the original package of Deoxyribonucleic acid from calf thymus and add 2 mL Tris buffer ([M] 5 mM , pH 8.0).
Deoxyribonucleic acid from calf thymus **Sigma Aldrich Catalog #D4522-1MG**

Note

https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/d4522pis.pdf

6.2 Cap the package. Do not vortex or sonicate.

6.3 Keep the solution at 0 °C ~ 4 °C overnight to completely solubilize the DNA. Gentle reversion is recommended.

6.4 Aliquot 10 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at -80 °C

Equipment

Finntip stepper pipette tips	NAME
500 ul (sterile)	TYPE
Thermo Scientific	BRAND
Thermo Scientific™ 9404173	SKU

7 RNase primary stock solution ([M] 10 mg/ml)

7.1 Uncap the original package of Ribonuclease A from bovin pancreas and add 5 mL Tris buffer ([M] 5 mM , pH 8.0).
Cap the package and vortex for a thorough mix.
Ribonuclease A from bovine pancreas **Sigma Aldrich Catalog #R6513-50MG**

7.2 Aliquot 30 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at -20 °C

Equipment	
Finntip stepper pipette tips	NAME
500 ul (sterile)	TYPE
Thermo Scientific	BRAND
Thermo Scientific™ 9404173	SKU

Equipment	
Finntip™ Stepper Pipette Tips	NAME
500 ul (Sterile)	TYPE
Thermo Scientific	BRAND
21-377-149	SKU

8 DNase primary stock solution ([M] 5 mg/ml = 10,000 U/mL)

8.1 Uncap the original package of Deoxyribonuclease1 and add 1 mL Tris buffer ([M] 5 mM , pH 8.0) .
Cap the package and vortex for a thorough mix.
DEOXYRIBONUCLEASE1 RNase and Protease Free **Bioshop Catalog # DRB002.10**

8.2 Aliquot 70 uL to 600 uL RNase free microtubes (every assay required 60 uL). Keep frozen at -20 °C .

Day 2: Exact RNA and DNA

9 Turn on UV light in biosafety cabinet for 00:15:00

10 Clean working surface with decontamination solution.

11 Prepare falcon tubes and tube rack in biosafety cabinet

Volume of the tube (mL)	Content in the tube
5	0.5 M EDTA
5	20% sarcosine
50	5 mM Tris
15 or 50	1% STEB

Equipment

Falcon® Centrifuge Tubes

NAME

Polypropylene, Sterile, 15 mL

TYPE

Corning®

BRAND

352096

SKU

Equipment

Falcon® Centrifuge Tubes

NAME

Polypropylene, Sterile, 50 mL

TYPE

Corning®

BRAND

352070

SKU

12 Prepare STEB ([M] 1 %)

Note

Use the following formula to determine the total volume of 1% STEB required:
(# samples + # blank filters) X (500 ul) + (500 ul) = total volume of 1% STEB required

12.1 Pour sarcosine ([M] 20 %) into an RNase free 5 mL falcon tube.
N-Lauroylsarosine sodium salt solution (20% RNase/DNase free) **Sigma Aldrich Catalog #L744-50mL**

12.2 Pour EDTA ([M] 0.5 M) into an RNase free 5 mL falcon tube.
EDTA buffer (0.5M DNase/RNase free) **Bioshop Catalog #EDT333.100**

12.3 Pour Tris buffer ([M] 5 mM , pH 8.0) into an RNase free 50 mL falcon tube.

12.4 Mix 500 µL sarcosine ([M] 20 %) , 10 µL EDTA ([M] 0.5 M) and 9 mL + 490 µL Tris buffer ([M] 5 mM , pH 8.0) to obtain STEB ([M] 1 %).

13 Prepare ice bath

14 Remove freeze-dried samples from -80°C freezer and place them On ice .

15 Add 500 µL Tris buffer ([M] 5 mM , pH 8.0) and 500 µL STEB ([M] 1 %) to the bead tube. Place tubes On ice .

Equipment

LYSING TUBES	NAME
MATRIX D 2 mL/15 mL	TYPE
MP BIOMEDICALS	BRAND
116913500/116933050	SKU

16 Rinse forceps by [M] 70 % volume ethanol and air dry.

Equipment

Filter forceps

NAME

blunt end, stainless steel

TYPE

Millipore

BRAND

XX6200006P

SKU

17 Transfer sample/blank filter into the bead tube by using clean forceps.

18 Invert immediately then put back On ice. 20s

19 Disrupt samples on the bead mill at 6.5 m/s. 30s

Equipment

Fastprep-24 5G™ Sample Preparation Instrument

NAME

MP Biomedicals

BRAND

116005500

SKU

20 Keep tubes On ice. Check the label on each tube, restore the label if it fades. 1m 30s

21 Disrupt samples on the bead mill at 6.5 m/s. 30s

22 Keep tubes On ice. Check the label on each tube, restore the label if it fades. 1m 30s

23 Disrupt samples on the bead mill at 6.5 m/s 30s

24 Keep tubes On ice . Check the label on each tube, restore the label if it fades. 1m 30s

25 Disrupt samples on the bead mill at 6.5 m/s. 30s

26 Continuously shake homogenate in a multi-head vortex at the highest speed for 01:00:00 Room temperature 1h

Note

Votex mixer should be able to remain stable on the bench under this vortex speed.

27 In the biosafety cabinet, transfer all homogenate into RNase free 2 mL micro-tube.

28 Freeze at -80 °C until analyzed.

Day 3: Run the assay

29 Prepare ice bath.

30 Turn on UV light in biosafety cabinet for 00:15:00

31 Clean working surface with decontamination solution.

32 Prepare falcon tubes, microtubes and tube racks in biosafety cabinet

Number of tubes	Type of tubes	Contents
5	5 mL falcon tubes	1 M MgCl2
		1 M CaCl2
		Working solution A (WS-A)
		Working solution B (WS-B)
		Working solution C (WS-C)
1	50 mL falcon tube	5 mM Tris buffer
1	15 mL falcon tubes	0.05% STEB
6	2 mL RNase free tubes	RNase working solution
		RNA secondary standard solution
		DNA tertiary standard solution
		900 mM MgCl2
		900 mM CaCl2
		Sybr green working solution (SG-II WS)
1	600 uL RNase free tube	DNA secondary standard
24	2 mL RNase free tubes	RNA standard solutions for RNA standard curves
		DNA standard solutions for DNA standard curves
N= total number of samples and blanks	2 mL RNase free tubes	Samples and blanks
3XN	2 mL RNase free tubes	Diluted samples and blanks
4	Microtube racks	Tubes of 2 mL in Set 1
		Tubes of 2 mL in Set A
		Tubes of 2 mL in Set B
		Tubes of 2 mL in Set C
1	Tube racks	Falcon tubes

Equipment

Screw-Cap Centrifuge Tube	NAME
5 mL	TYPE
VWR	BRAND
10002-738	SKU

Day 3: Run the assay (Caution: It is a long procedure!)

33 Organize and label the tubes as shown below

Set 1:
In microtube rack, label 2 mL tubes for samples and blanks to be further diluted.

Blk	1	2	3	4	5	6	7	8	9	10	11

Set A, B and C:
In microtube rack, label 2 mL tubes for RNA (marked in pink), DNA (marked in blue) standard solutions and samples (marked in yellow)
Set A is for working solution A (WS-A) treatment, i.e. treated with DNase
Set B is for working solution B (WS-B) treatment, i.e. treated with RNase
Set C is for working solution A (WS-A) and C (WS-C) treatment, i.e. treated with DNase and RNase

Set A	R1A	R2A	R3A	R4A	R5A	D1A	D2A	D3A				
	BlkA	1A	2A	3A	4A	5A	6A	7A	8A	9A	10A	11A

Set B	R1B	R2B	R3B	R4B	R5B	D1B	D2B	D3B				
	BlkB	1B	2B	3B	4B	5B	6B	7B	8B	9B	10B	11B

Set C	R1C	R2C	R3C	R4C	R5C	D1C	D2C	D3C				
	BlkC	1C	2C	3C	4C	5C	6C	7C	8C	9C	10C	11C

34 Label tubes for reagents as following.
Follow the sheet, add Tris buffer ([M] 5 mM , pH 8.0) to the reagent tubes:

	Content	5 mM Tris (uL)
	SG-II WS	1000+250
	WS-A	2X1000+820
	WS-B	2X1000+820
	WS-C	2X1000+940
	RNase	380
	900 mM MgCl2	40
	900 mM CaCl2	40
	RNA secondary	990+495
	DNA secondary	95
	DNA tertiary	960
	0.05% STEB	9X1000 + 500

35 Thaw Sybr green II at room temperature

SYBR™ Green II RNA Gel Stain, 10,000X concentrate in DMSO **Thermo Fisher Catalog #S7564**

36 Add 900 µL Tris buffer ([M] 5 mM , pH 8.0) to each tube in **Set 1**

> Note
>
> Depending on the dilution of extracted sample

37 Follow the sheet, add Tris buffer ([M] 5 mM , pH 8.0) to each tube in **Set A, B and C**. The unit of volume is uL.

Set A	650	640	600	550	500	640	610	550				
	650	650	650	650	650	650	650	650	650	650	650	650

Set B	650	640	600	550	500	640	610	550				
	650	650	650	650	650	650	650	650	650	650	650	650

Set C	600	590	550	500	450	590	560	500				
	600	600	600	600	600	600	600	600	600	600	600	600

38 Prepare STEB ([M] 0.05 %)

Add 500 µL STEB ([M] 1 %) to 0.05% STEB tube, and vortex.

39 Add 250 µL STEB ([M] 0.05 %) to RNA and DNA standards in **Set A, B and C** by reverse pipetting.

Set A, B, C	250	250	250	250	250	250	250	250				

40 Place RNase and DNase primary stock solutions, RNA and DNA primary standard solutions and samples On ice .

41 Turn on refrigerated centrifuge and set the temperature to 4 °C .

Equipment	
CENTRIFUGE 5430 R	NAME
Eppendorf	BRAND
MP2231000510	SKU

42 Turn on shaker/incubator and set temperature to 37 °C.

Equipment	
SHAKING INCUBATOR	NAME
71L	TYPE
Corning® LSE™	BRAND
6753	SKU

43 Prepare [M] 900 mM $MgCl_2$

43.1 Pour [M] 1 M $MgCl_2$ solution into 5 mL RNase free Falcon tube

Magnesium chloride solution **Sigma Aldrich Catalog #63069-100ML**

43.2 Transfer 360 µL [M] 1 M $MgCl_2$ solution into 900 mM $MgCl_2$ tube

44 Add 60 µL [M] 900 mM $MgCl_2$ to WS-A

45 Add 60 µL [M] 900 mM $MgCl_2$ to WS-B

46 Prepare [M] 900 mM $CaCl_2$

46.1 Pour [M] 1 M $CaCl_2$ solution into 5 mL RNase free Falcon tube
Calcium chloride solution **Sigma Aldrich Catalog #21115-100ML**

46.2 Transfer 360 µL [M] 1 M $CaCl_2$ solution into 900 mM $CaCl_2$ tube

47 Add 60 µL [M] 900 mM $CaCl_2$ to WS-A

48 Add 60 µL [M] 900 mM $CaCl_2$ to WS-B

49 Prepare SG-II WS

49.1 Centrifuge one tube of SG-II concentrate at Room temperature 13000 rpm, 00:05:00 to deposit DMSO.

49.2 Wrap SG-II WS tube with foil, transfer ***supernatant*** of SYBR Green II 10,000X concentrate to SG-II WS tube in biosafety cabinet ([M] 8.75 ul per 1.25 mL Tris)

Note

Any step involving SYBR Green II should be operated in dark room or at least dim light.
Prepare Sybr green II WS at this step to allow enough time for stabilization.

50

Note

Lunch break!

51 Prepare RNase working solution [M] 0.5 mg/ml
Add 20 µL RNase primary stock solution ([M] 10 mg/ml) to RNase tube

52 Add 60 µL [M] 0.5 mg/ml RNase to WS-B.
Keep WS-B On ice .

53 Add 60 µL [M] 0.5 mg/ml RNase to WS-C.
Keep WS-C On ice .

54 Add 60 µL DNase primary stock solution ([M] 5 mg/ml) to WS-A.
Keep WS-A On ice .

55 Centrifuge extracted samples 10000 x g, 4°C, 00:04:00

56 Prepare RNA secondary standard solution [M] 2 ug/ml
Add 15 µL RNA primary standard solution to RNA standard tube and mix.
Keep On ice .

57 Prepare DNA secondary standard solution ([M] 25 ug/ml)
Add 5 µL primary DNA standard solution ([M] 500 ug/ml) to DNA secondary tube and mix.

Note

DNA secondary standard will also be used to verify the actual concentration of DNA by using µdrop plate.

Note

Avoid vortexing DNA standard. Mix with pipette tip by aspiring up and down several times.

58 Prepare DNA tertiary standard solution [M] 1 ug/ml
Add 40 µL DNA secondary solution ([M] 25 ug/ml) to DNA tertiary standard tube and mix.
Keep On ice .

59 Load 50 µL WS-A to tubes in **Set A**.

Note

From 59 to 62: Reverse pipetting
Decontaminate pipet between different WS.

60 Load 50 µL WS-A to tubes **Set C**.

61 Load 50 µL WS-B to tubes in **Set B**.

62 Load 50 µL WS-C to tubes in **Set C**.

63 Add 100 µL centrifuged samples to its corresponding tubes in **Set 1**.
Gently invert the tube to mix sample.

64 From Set 1, transfer 250 µL of diluted samples to each corresponding tubes (marked in yellow) in **Set A, B and C**.
Add RNA secondary standard to tubes (marked in pink) in **Set A, B and C**.
Add DNA secondary standard to tubes (marked in blue) in **Set A, B and C**.
The unit of volume is uL.

Set A, B, C	0	10	50	100	150	10	40	100				
	250	250	250	250	250	250	250	250	250	250	250	250

Note

In order to avoid cross contamination from RNase or DNase, use one tip for each dispensing.
Pipette solution in the tube up and down for mixing.

65 Invert each tube to mix well and place all tubes into the shaker/incubator at 37 °C, continuously shaking at 200 RPM for 00:20:00. 20m

Note

Incubation time is critical. Temperature might be disturbed by door open/close. Don't start the timer until temperature returns to 37°C.

66 After incubation, invert each tube for mixing and then place into the fridge to stop the reaction.

Day 3: Verify DNA concentration and SG-II absorbance

67 Measure DNA secondary concentration by using µdrop plate (sample volume: 4 ul)
Use Tris buffer ([M] 5 mM , pH 8.0) as blank.

Equipment

µDrop™ Plates NAME

Thermo Scientific BRAND

N12391 SKU

Equipment

Varioskan LUX Multimode Microplate Reader NAME

Thermo Fisher BRAND

VL0L00D0 SKU

68 DNA_primary concentration (µg/ml) = (Abs_{260}-$Abs_{260\ (blank)}$)x 50 µg/ml x (10mm/0.5 mm) X DF

Where, DF=20

Note

The DNA concentration is around 500 ug/mL but can be much lower (since the small volume of DNA primary aliquot is hard to be mixed), use the measured DNA value to calculate the DNA primary concentration.

69 Check absorbance of SG-II WS:

69.1 In a transparent microplate, load
(1) 200 uL Tris buffer as blank
(2) 10 uL SG-II WS and 190 uL Tris buffer

69.2 Read absorbance at 480 nm, the value after subtracted by blank shall be no higher than 0.21

Day 3: Read fluorescence

70 Remove samples out of the fridge and allow to reach Room temperature for 00:02:00 before loading the microplate.

Note

Since fluorescence decreases with increasing temperature, with percentage changes depending on the fluorophore (Bashford, 1987), the SG-II WS must be kept dark at RT (22ºC) and the samples must be equilibrated at RT (c. 2 min).

71 Adhere black film on the top of a microplate lid.

Equipment

Black Vinyl Films for Fluorescence and Photoprotection NAME

VWR BRAND

89087-692 SKU

Equipment

Microplate Lids NAME

Polystyrene TYPE

Greiner Bio-One BRAND

07000288 SKU

72 Load 10 µL SG-II WS to each well in the microplate with 0.5 mL tip of stepper, and cover the plate with the black-film lid.

Equipment

Finntip™ Stepper Pipette Tips NAME

500 uL TYPE

Thermo Scientific™ BRAND

9404170 SKU

Equipment

96-Well Black Microplates	NAME
Polystyrene	TYPE
Greiner Bio-One	BRAND
655076	SKU

Note

Wipe or dab the liquid drop on the outside of the tip, avoid wiping the tip open before dispensing the liquid.

73 Organize tubes in 96-well microtube rack in the same order as how microplates are loaded.

R1A	R1B	R1C	BlkA	BlkB	BlkC	4A	4B	4C	8A	8B	8C
R2A	R2B	R2C									
R3A	R3B	R3C	1A	1B	1C	5A	5B	5C	9A	9B	9C
R4A	R4B	R4C									
R5A	R5B	R5C	2A	2B	2C	6A	6B	6C	10A	10B	10C
D1A	D1B	D1C									
D2A	D2B	D2C	3A	3B	3C	7A	7B	7C	11A	11B	11C
D3A	D3B	D3C									

74 Load 190 µL working sample to the microplate by reverse pipetting. 45m

	1	2	3	4	5	6	7	8	9	10	11	12
A	R1A	R1B	R1C	BlkA	BlkB	BlkC	4A	4B	4C	8A	8B	8C
B	R2A	R2B	R2C	BlkA	BlkB	BlkC	4A	4B	4C	8A	8B	8C
C	R3A	R3B	R3C	1A	1B	1C	5A	5B	5C	9A	9B	9C
D	R4A	R4B	R4C	1A	1B	1C	5A	5B	5C	9A	9B	9C
E	R5A	R5B	R5C	2A	2B	2C	6A	6B	6C	10A	10B	10C
F	D1A	D1B	D1C	2A	2B	2C	6A	6B	6C	10A	10B	10C
G	D2A	D2B	D2C	3A	3B	3C	7A	7B	7C	11A	11B	11C
H	D3A	D3B	D3C	3A	3B	3C	7A	7B	7C	11A	11B	11C

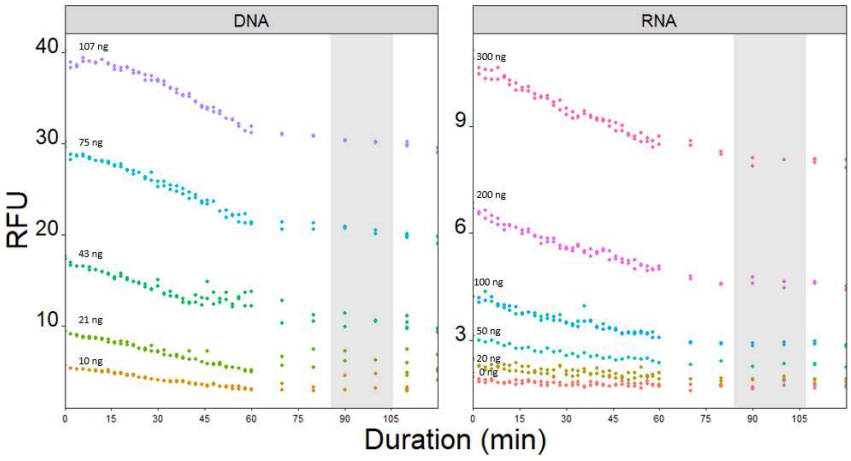
Note

Wipe or dab the liquid drop on the outside of the tip, avoid wiping the tip open before dispensing the liquid.

75 Shake black film covered microplate at Room temperature for 01:30:00 1h 30m

Note

Read fluorescence right after 1h30 incubation at room temperature.



76 Setup microplate reader:
Plate: Greiner F bottom chimney well PP 96 well;
Shake: Continuous 5s at 600 rpm
Bandwidth: 5 nm
Endpoint reading: Ex 490 nm/Em 520 nm;

Equipment	
Varioskan LUX Multimode Microplate Reader	NAME
Thermo Fisher	BRAND
VL0L00D0	SKU

77 Read fluorescence and export data to excel sheet.

78 In the fume hood, dispose any waste with SG-II into fluorescence stain waste container (some stain waste has DMSO solvent).

Calculate

79 RNA standard curve

79.1 Concentrations of RNA standards in the microplate

	RNA standard	Secondary 2 ug/mL (uL)	Tris (uL)	STEB (uL)	WS (uL)	Sample in microplate (uL)	SG-II (uL)	Conc in microplate (ng/mL)
	R1	0.00	650.00	250.00	50.00	190.00	10.00	0.00
	R2	10.00	640.00	250.00	50.00	190.00	10.00	20.00
	R3	50.00	600.00	250.00	50.00	190.00	10.00	100.00
	R4	100.00	550.00	250.00	50.00	190.00	10.00	200.00
	R5	150.00	500.00	250.00	50.00	190.00	10.00	300.00

79.2 Slope of fluorescence in Set A vs concentration of RNA standard gives $m_{RNA+DNase}$ (≈0.03)
Slope of fluorescence in Set B vs concentration of RNA standard gives $m_{RNA+RNase}$

79.3 Calculate ρ

$$\rho = \frac{m_{RNA+RNase}}{m_{RNA+DNase}}$$

80 Total RNA of the samples

$$ug\ Total\ RNA\ (ml\ assay)^{-1} = 0.001 \times \frac{(RFU_A - RFU_{A_{Blank}}) - (RFU_C - RFU_{C_{Blank}})}{(1-\rho)} / m_{RNA+DNase}$$

Where,
RFU_A and RFU_C are the fluorescence in Tube A and Tube C of the same sample.
RFU_{ABlank} and RFU_{CBlank} are the fluorescence in Tube A and Tube $_C$ of the blank.

81 DNA standard curve

81.1 Concentrations of DNA standards in the microplate: Use measured DNA primary concentration instead of 500 ug/mL:

	DNA primary Conc (ug/mL)	DNA primary (uL)	Tris (uL)	Conc. DNA secondary (ug/mL)
		5	95	

	DNA secondary Conc. (ug/mL)	DNA secondary (uL)	Tris (uL)	Conc. DNA tertiary (ug/mL)
		40	960	

	DNA standard	DNA tertiary (uL)	Tris (uL)	STEB (uL)	WS (uL)	Sample in microplate (uL)	SG-II (uL)	Conc. in microplate (ng/mL)
	R1	0	650	250	50	190	10	0
	D1	10	640	250	50	190	10	~10
	D2	40	610	250	50	190	10	~40
	D3	100	550	250	50	190	10	~100

81.2 Slope of fluorescence in Set A vs concentration of DNA standard gives $m_{DNA+DNase}$
Slope of fluorescence in Set B vs concentration of DNA standard gives $m_{DNA+RNase}$ (≈0.12)

81.3 Calculate δ

$$\delta = \frac{m_{DNA+DNase}}{m_{DNA+RNase}}$$

82 Total DNA of the samples

$$ug\ Total\ DNA\ (ml\ assay)^{-1} = 0.001 \times \frac{(RFU_B - RFU_{B_{Blank}}) - (RFU_C - RFU_{C_{Blank}})}{(1-\delta)} / m_{DNA+RNase}$$

Where,
RFU_B and RFU_C are the fluorescence in Tube B and Tube C of the same sample
RFU_{BBlank} and RFU_{CBlank} are the fluorescence in Tube B and Tube $_C$ of the blank.

83 Dilution factor=40
If,

- Sample is extracted by 1 mL extraction reagent
- In Set 1, sample is diluted to 1/10
- In Set 3, diluted by Tris and all working solutions to 250/950
- In microplate, diluted by SG-II WS to 190/200