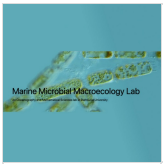

Version 2

Oct 15, 2020

Total RNA and DNA in Microalgae V.2

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1 *Works for me* This protocol is published without a DOI.

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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.

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

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PROTOCOL CITATION

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Version created by Yingyu Hu

KEYWORDS

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

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LAST MODIFIED

Oct 15, 2020

PROTOCOL INTEGER ID

42130

GUIDELINES

The optimized linear range in this assay for DNA is 0~100 ng/mL and RNA is 0~300 ng/mL in the microplate, respectively. Samples are diluted to 1/40 in the assay, therefore, maximum DNA and RNA required in the assay is around 4 ug and 12 ug per sample. Assuming total DNA and RNA is 1% and 5.7% of total biomass at replete condition, carbon per sample required in this assay is about 80 ug.

Microalgae samples are collected on polycarbonate filter and kept frozen under -80 ºC.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Nuclease decontamination solution	11-05-03-01	IDT
Ribonuclease A from bovine pancreas	R6513-50MG	Sigma Aldrich
DEOXYRIBONUCLEASE1 RNase and Protease Free	DRB002.10	Bioshop
Magnesium chloride solution	63069-100ML	Sigma Aldrich
Calcium chloride solution	21115-100ML	Sigma Aldrich
SYBR™ Green II RNA Gel Stain, 10,000X concentrate in DMSO	S7564	Thermo Fisher
Tris(hydroxymethyl)aminomethane hydrochloride 1M pH 8.0 RNase free	AAJ60080AK	Fisher Scientific
Deoxyribonucleic acid from calf thymus	D4522-1MG	Sigma Aldrich
N-Lauroylsarosine sodium salt solution (20% RNase/DNase free)	L744-50mL	Sigma Aldrich
EDTA buffer (0.5M DNase/RNase free)	EDT333.100	Bioshop
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermofisher
E. coli Total RNA	AM7940	Thermo Fisher Scientific

EQUIPMENT

NAME	CATALOG #	VENDOR
VWR ANALOG VORTEX MIXER	10153-838	VWR international Ltd
Fastprep-24 5G™ Sample Preparation Instrument	116005500	VWR international Ltd
SHAKING INCUBATOR	6753	
LYSING TUBES	116913500/116933050	VWR international Ltd
Falcon® Centrifuge Tubes	352096	VWR international Ltd
Screw-Cap Centrifuge Tube	10002-738	VWR international Ltd
CENTRIFUGE 5430 R	MP2231000510	
FreeZone® 2.5 L Benchtop Freeze Dryers	700202000	VWR international Ltd
BT Barrier Pipet Tips	BT1250, BT100, BT10	VWR international Ltd
Falcon® Centrifuge Tubes	352070	VWR international Ltd
Filter forceps	XX6200006P	Emdmillipore
Finntip stepper pipette tips	Thermo Scientific™ 9404173	Fisher Scientific
Finnpipette Stepper Pipette	4540000	Fisher Scientific
Microcentrifuge Tubes	MCT-175-C/MCT-060-L-C	VWR international Ltd
Finntip stepper pipette tips	Thermo Scientific™ 9404183	Fisher Scientific
Finntip™ Stepper Pipette Tips	21-377-149	Fisher Scientific
Finntip™ Stepper Pipette Tips	Thermo Scientific™ 9404193	Fisher Scientific
Finntip™ Stepper Pipette Tips	9404170	Fisher Scientific
96-Well Black Microplates	655076	
Black Vinyl Films for Fluorescence and Photoprotection	89087-692	
Microplate Lids	07000288	Fisher Scientific
Varioskan LUX Multimode Microplate Reader	VL0L00D0	
µDrop™ Plates	N12391	Thermo Scientific

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

Day 1: Freeze-dry samples

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

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.

FreeZone® 2.5 L Benchtop Freeze Dryers

Labconco® **700202000**

Day 1: Prepare primary solutions

2 Turn on UV light in biosafety cabinet for **00:15:00** and clean working surface with decontamination solution.

Nuclease decontamination solution

by IDT

Catalog #: 11-05-03-01

3 Prepare Tris buffer **[M]5 mM** **pH8.0**

3.1 Pour [M]1 M pH8.0 Tris into an RNase free 15 mL Falcon tube

Tris(hydroxymethyl)aminomethane hydrochloride 1M pH 8.0 RNase free
by Fisher Scientific
Catalog #: AAJ60080AK
CAS Number: 1185-53-1

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 15 mL
Corning® 352096

3.2 Directly add 2.5 mL [M]1 M pH8.0 Tris into 500 mL RNase free water in its original package.

UltraPure™ DNase/RNase-Free Distilled Water
by Thermofisher
Catalog #: 10977023

BT Barrier Pipet Tips
Pre-Sterile
Neptune® BT1250, BT100, BT10

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

4.1 In the original package, the E. Coli Total RNA is of 1 mg/mL, in which total RNA is 200 ug.

E. coli Total RNA
by Thermo Fisher Scientific
Catalog #: AM7940

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

4.2 Uncap the original package of E. Coli Total RNA and directly add **800 µl** Tris buffer (**5 mM** , **8.0**) .
Cap the package and vortex for a thorough mix.

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

Finnpipette Stepper Pipette
Thermo Scientific™ 4540000

Finntip stepper pipette tips
500 ul (sterile)
Thermo Scientific Thermo Scientific™ 9404173

Microcentrifuge Tubes
1.7 mL/0.6 mL
Axygen Scientific MCT-175-C/MCT-060-L-C

5 DNA primary standard solution (≈ **500 ug/ml**)

5.1 Uncap the original package of Deoxyribonucleic acid from calf thymus and add **2 mL** Tris buffer (**5 mM** , **8.0**).

Deoxyribonucleic acid from calf thymus
by Sigma Aldrich
Catalog #: D4522-1MG

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

5.2 Cap the package. Do not vortex or sonicate.

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

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

Finntip stepper pipette tips
500 ul (sterile)
Thermo Scientific Thermo Scientific™ 9404173

5.5 Dilute 5 ul primary DNA standard solution with 95 ul Tris buffer (**5 mM** , **8.0**) in a microtube (600 ul).
Measure DNA concentration by using udrop plate (sample volume: 4 ul)

µDrop™ Plates
Thermo Scientific N12391

Varioskan LUX Multimode Microplate Reader
Thermo Fisher VL0L00D0

5.6 DNA concentration (µg/ml) = Abs_{260}x 50 µg/ml x (10mm/0.5 mm) X DF = Abs_{260} x 50 x 20 X10

6 RNase primary stock solution (**10 mg/ml**)

6.1 Uncap the original package of Ribonuclease A from bovin pancreas and add **5 mL** Tris buffer (**5 mM** , **8.0**).
Cap the package and vortex for a thorough mix.

Ribonuclease A from bovine pancreas
by Sigma Aldrich
Catalog #: R6513-50MG

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

Finntip stepper pipette tips
500 ul (sterile)
Thermo Scientific Thermo Scientific™ 9404173

Finntip™ Stepper Pipette Tips
500 ul (Sterile)
Thermo Scientific 21-377-149

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

7.1 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
by Bioshop
Catalog #: DRB002.10

7.2 Aliquot 100 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at **-20 °C** .

Finntip stepper pipette tips
1250 ul (sterile)
Thermo Scientific Thermo Scientific™ 9404183

Day 2: Exact RNA and DNA

8 Turn on UV light in biosafety cabinet for **00:15:00** and clean working surface with decontamination solution.

9 Prepare falcon tubes and tube rack in biosafety cabinet

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

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 15 mL
Corning® **352096**

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 50 mL
Corning® **352070**

10 Prepare STEB ([M]**1 %**)

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

10.1 Pour sarcosine ([M]**20 %**) into an RNase free 5 mL falcon tube.

N-Lauroylsarosine sodium salt solution (20% RNase/DNase free)
by Sigma Aldrich
Catalog #: L744-50mL

10.2 Pour EDTA ([M]**0.5 M**) into an RNase free 5 mL falcon tube.

EDTA buffer (0.5M DNase/RNase free)
by Bioshop
Catalog #: EDT333.100

10.3 Pour Tris buffer ([M] **5 mM** , pH **8.0**) into an RNase free 15 mL falcon tube.

10.4 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 %**).

11 Prepare ice bath

12 Remove freeze-dried samples from -80ºC freezer and place them **On ice** .

13 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** .

LYSING TUBES
MATRIX D 2 mL/15 mL

MP BIOMEDICALS	116913500/1169330 50

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

Filter forceps
blunt end, stainless steel

Millipore	XX6200006P

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

16 Vortex immediately then put back **On ice** . 20s

VWR ANALOG VORTEX MIXER

VWR	10153-838

With tube insert

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

Fastprep-24 5G™ Sample Preparation Instrument

MP Biomedicals 116005500

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

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

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

Room temperature

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

25 In the biosafety cabinet, aliquot 150 uL of homogenates into RNase free 600 uL tubes.

Finntip™ Stepper Pipette Tips
2.5 mL (sterile)

Thermo Scientific Thermo Scientific™ 9404193

26 Freeze at **-80 °C** until analyzed.

Day 3: Run the assay

27 Prepare ice bath.

28 Turn on UV light in biosafety cabinet for **00:15:00** and clean working surface with decontamination solution.

29 Prepare falcon tubes, microtubes and tube racks in biosafety cabinet
* Maximum number of samples (including blanks) per assay is 20.

Number of tubes	Type of tubes	Contents
6	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), Sybr Green II working solution (SG-II WS)
2	15 mL falcon tubes	5 mM Tris buffer, 0.05% STEB
3	600 uL RNase free tubes	RNase working solution, 900 mM MgCl2, 900 mM CaCl2
2	1.7 mL RNase free tubes	Secondary RNA standard solution, Secondary DNA standard solution
33	1.7 mL RNase free tubes	RNA standard solutions for RNA standard curves, DNA standard soutions for DNA standard curves
N= total number of samples and blanks	1.7 mL RNase free tubes	Samples and blanks
3XN	1.7 mL RNase free tubes	Diluted samples and blanks
5	Microtube racks	Tubes of 600 uL and 1.7 mL
1	Tube racks	Falcon tubes

Screw-Cap Centrifuge Tube
5 mL
VWR 10002-738

30 Organize and label the tubes as shown below

Set 1:
In microtube rack, label 1.7 mL tubes for samples and blanks to be further diluted.

1	2	3	4	5	6	7	8	9	…	Blank

Set 2:
In microtube rack, label 1.7 mL tubes for RNA (marked in pink) and DNA (marked in blue) standard solutions to be used as standard curves.
Tubes A is for standard solutions treated with working solution A (WS-A)
Tubes B is for standard solutions treated with working solution B (WS-B)
Tubes C is for standard solutions treated with working solution C (WS-C)

Tubes A	R1A	R2A	R3A	R4A	R5A	R6A	D1A	D2A	D3A	D4A	D5A
Tubes B	R1B	R2B	R3B	R4B	R5B	R6B	D1B	D2B	D3B	D4B	D5B
Tubes C	R1C	R2C	R3C	R4C	R5C	R6C	D1C	D2C	D3C	D4C	D5C

Set 3:
In microtube rack, label 1.7 mL tubes for diluted samples and blanks.
Tubes A is for diluted samples and blanks treated with working solution A (WS-A)
Tubes B is for diluted samples and blanks treated with working solution B (WS-B)
Tubes C is for diluted samples and blanks treated with working solution C (WS-C)

Tubes A	1A	2A	3A	4A	5A	6A	7A	8A	9A	...	BlankA
Tubes B	1B	2B	3B	4B	5B	6B	7B	8B	9B	...	BlankB
Tubes C	1C	2C	3C	4C	5C	6C	7C	8C	9C	...	BlankC

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

Reagent	5 mM Tris (uL)
0.05% STEB	9X1000+500
RNase	190
900 mM $MgCl_2$	20
900 mM $CaCl_2$	20
WS-A	2X1000+820
WS-B	2X1000+820
WS-C	2X1000+940
RNA	990+495
DNA	998

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

33 Follow the sheet, add Tris buffer ([M] **5 mM** , pH **8.0**) to each tube in **Set 2**. The unit of volume is uL.

Tubes A	650	640	625	600	550	500	640	630	610	580	550
Tubes B	650	640	625	600	550	500	640	630	610	580	550
Tubes C	600	590	575	550	500	450	590	580	560	530	500

34 Follow the sheet, add Tris buffer ([M]**5 mM** , pH**8.0**) to each tube in **Set 3**. The unit of volume is uL.

Tubes A	650	650	650	650	650	650	650	650	650	…	650
Tubes B	650	650	650	650	650	650	650	650	650	…	650
Tubes C	600	600	600	600	600	600	600	600	600	…	600

35 Prepare STEB ([M]**0.05 %**)

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

36 Add **250 µl** STEB ([M]**0.05 %**) to each tube in **Set 2**.

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

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

CENTRIFUGE 5430 R

Eppendorf MP2231000510

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

SHAKING INCUBATOR

71L

Corning® LSE™ 6753

40 Prepare [M]**900 mM** $MgCl_2$

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

Magnesium chloride solution

by Sigma Aldrich

Catalog #: 63069-100ML

40.2 Transfer **180 µl** [M]**1 M** $MgCl_2$ solution into 900 mM $MgCl_2$ tube

41 Add **60 µl** **900 mM** $MgCl_2$ to WS-A and WS-B

42 Prepare **900 mM** $CaCl_2$

42.1 Pour **1 M** $CaCl_2$ solution into 5 mL RNase free Falcon tube

Calcium chloride solution
by Sigma Aldrich
Catalog #: 21115-100ML

42.2 Transfer **180 µl** **1 M** $CaCl_2$ solution into 900 mM $CaCl_2$ tube

43 Add **60 µl** **900 mM** $CaCl_2$ to WS-A and WS-B

44 Prepare RNase working solution **0.5 mg/ml**

Add **10 µl** RNase primary stock solution (**10 mg/ml**) to RNase tube

45 Add **60 µl** **0.5 mg/ml** RNase to WS-B and WS-C.

Keep WS-B and WS-C **On ice** .

46 Add **60 µl** DNase primary stock solution (**5 mg/ml**) to WS-A.

Keep WS-A **On ice** .

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

48 Prepare RNA secondary standard solution **2 ug/ml**

Add **15 µl** RNA primary standard solution to RNA standard tube and vortex.

Keep **On ice** .

49 Prepare DNA secondary standard solution **1 ug/ml**

Add **2 µl** DNA primary standard solution to DNA standard tube and vortex.

Keep **On ice** .

50 Load **50 µl** WS-A to Tubes A in **Set 2** and **Set 3**.

51 Load **50 µl** WS-A to Tubes C in **Set 2** and **Set 3**.

52 Load **50 µl** WS-B to Tubes B in **Set 2** and **Set 3**.

53 Load **50 µl** WS-C to Tubes C in **Set 2** and **Set 3**.

54 Add **100 µl** centrifuged samples to its corresponding tubes in **Set 1**.
Vortex each tube.

55 From Set 1, transfer **250 µl** of diluted samples to each corresponding tubes in **Set 3**.

56 Follow the sheet:
Add RNA secondary standard to tubes (marked in pink) in Set 2.
Add DNA secondary standard to tubes (marked in blue) in Set 2.
The unit of volume is uL.

Tubes A	0	10	25	50	100	150	10	20	40	70	100
Tubes B	0	10	25	50	100	150	10	20	40	70	100
Tubes C	0	10	25	50	100	150	10	20	40	70	100

57 Vortex each tube for **00:00:02** and place all tubes into the shaker/incubator at **37 °C** , continuously shaking at 200 RPM for **00:20:00** . 20m

Read fluorescence

58 Prepare SYBR Green II working solution (SG-II WS)

58.1 Each 96-well microplate requires 1 mL of SG-II WS.

58.2 Wrap 5 mL tube with foil, add **7 µl** SYBR Green II 10,000X concentrate to each one mL Tris buffer (**5 mM** , **8.0**) in biosafety cabinet.

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

58.3 Load **10 µl** SG-II WS to each well in the microplate with 0.5 mL tip of stepper.

Finntip™ Stepper Pipette Tips
500 uL
Thermo Scientific™ **9404170**

96-Well Black Microplates
Polystyrene
Greiner Bio-One **655076**

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

58.4 Adhere black film on the top of a microplate lid and cover the plate with this lid.

Black Vinyl Films for Fluorescence and Photoprotection
VWR **89087-692**

Microplate Lids
Polystyrene
Greiner Bio-One **07000288**

59 After incubation, vortex each tube for **00:00:02** and then place into the fridge to stop the reaction.

60 Allow samples to reach **Room temperature** for **00:02:00** before loading the microplate.

45m

61 Load **190 µl** working sample to the microplate.

Standards must be included in each microplate.
Pink area: RNA standard solutions for RNA standard curves
Blue area: DNA standard solutions for DNA standard curves
Yellow area: Samples and blanks

	1	2	3	4	5	6	7	8	9	10	11	12
A	R1A	R2A	R3A	R4A	R5A	R6A	1A	1A	1B	1B	1C	1C
B	R1B	R2B	R3B	R4B	R5B	R6B	2A	2A	2B	2B	2C	2C
C	R1C	R2C	R3C	R4C	R5C	R6C	3A	3A	3B	3B	3C	3C
D	D1A	D2A	D3A	D4A	D5A		4A	4A	4B	4B	4C	4C
E	D1B	D2B	D3B	D4B	D5B		5A	5A	5B	5B	5C	5C
F	D1C	D2C	D3C	D4C	D5C		6A	6A	6B	6B	6C	6C
G	9A	9A	9B	9B	9C	9C	7A	7A	7B	7B	7C	7C
H	Blank A	Blank A	Blank B	Blank B	Blank C	Blank C	8A	8A	8B	8B	8C	8C

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

10m

62 Shake black film covered microplate at **Room temperature** for **00:10:00**

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

Varioskan LUX Multimode Microplate Reader
Thermo Fisher **VL0L00D0**

64 Read fluorescence and export data to excel sheet.

Calculate

65 RNA standard curve

65.1 Concentrations of RNA standards in the microplate

Standard	2 ug/mL (uL)	Tris+WS (uL)	0.05% STEB (uL)	SG II WS (uL)	Final (ng/mL)
R1	0	700	250	50	0
R2	10	690	250	50	20
R3	25	675	250	50	50
R4	50	650	250	50	100
R5	100	600	250	50	200
R6	150	550	250	50	300

65.2 Slope of fluorescence in Tube A vs concentration of RNA standard gives $m_{RNA+DNase}$
Slope of fluorescence in Tube B vs concentration of RNA standard gives $m_{RNA+RNase}$

65.3 Calculate ρ

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

66 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.

67 DNA standard curve

67.1 Concentrations of DNA standards in the microplate

Standard	1 ug/mL (uL)	Tris+WS (uL)	0.05% STEB (uL)	SG II WS (uL)	Final (ng/mL)
D1	10	690	250	50	10
D2	20	680	250	50	20
D3	40	660	250	50	40
D4	70	630	250	50	70
D5	100	580	250	50	100

67.2 Slope of fluorescence in Tube A vs concentration of DNA standard gives $m_{DNA+DNase}$
Slope of fluorescence in Tube B vs concentration of DNA standard gives $m_{DNA+RNase}$

67.3 Calculate δ

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

68 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.

69 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