



FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM

Standard Operating Procedure

Title: DNA Extraction and Quantification in Preparation for Whole Genome Sequencing

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DNA Preparation for Whole Genome Sequencing

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SECTION 1

INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth **standard** guidelines for genomic DNA extraction from multiple genera, and the steps to quantify and submit this DNA in 96-well skirted plates for Nextera XT library preparation and Whole Genome Sequencing on the Illumina platform at the Cornell BRC/CORE.

1.2 Scope

This SOP applies to the Food Safety Lab and the Milk Quality Improvement Program.

1.3 Definitions

260/280 ratio: The ratio of: the wavelength at which DNA & RNA absorb light, over the wavelength at which aromatic amino acids absorb light. A spectrophotometric measure of nucleic acid purity.

260/230 ratio: The ratio of: the wavelength at which DNA absorbs light, over the wavelength at which organic contaminants (e.g., guanidine hydrochloride) absorb light. A spectrophotometric measure of nucleic acid purity.

1.4 Safety

Many of the microorganisms used in our lab (e.g., *Salmonella* and *L. monocytogenes*) are BSL-2 pathogens. If you are using this SOP with human pathogens, appropriate protective equipment should be worn and appropriate protective measures need to be taken. All waste from BSL-2 organisms must be treated appropriately.

SECTION 2

MATERIALS

- **DNA extraction kit:** Qiagen QiAmp, Qiagen DNEasy, or MO BIO PowerSoil kits can be used, depending on what has been optimized for the organism from which you are extracting DNA.
- **Tris-HCL, pH = 8:** for preparing lysozyme solution, and eluting, storing and diluting DNA during all downstream processes **when using Qiagen Extraction Kits**. You must prepare this (recipes are available in any of the lab library reference manuals). Note: MO BIO kits have their own elution buffer (also a 10mM Tris solution) that should be used for elution, and potentially for all downstream processes, also.
- **EDTA, pH = 8:** for preparing lysozyme solution **when using Qiagen Extraction Kits**. You must prepare this (recipes are available in any of the lab library reference manuals).



- **NanoDrop** Spectrophotometer: for 260/280 & 260/230 ratios (to check nucleic acid purity) prior to dsDNA quantification.
- **Qubit®** Fluorometer & Assay Kit (dye, buffer and equipment are all available and housed at the Cornell Biotechnology CORE/BRC): for quantifying dsDNA prior to sample submission.
- **Skirted 96-well plates**: for prep and submission to Cornell Biotechnology CORE/BRC.



SECTION 3 PROCEDURES

3.1. Extraction of genomic DNA

DNA extraction is performed using the QIAmp DNA Mini Kit; for detailed information on DNA extraction for WGS, please consult protocol “8.3.13-Genomic_DNA_Extraction_Qiagen”.

***NOTE:** We did not use the Qiagen DNA Easy Kit for development of this protocol; if DNA extraction of your organism is optimized with the DNA Easy kit, you should be cautious of potential incompatibilities, even though the solutions and processes are similar between the Qiagen kits.*

*Alternatively, the MO BIO “PowerSoil®” DNA Isolation Kit (see link in Reference Section) can be used, but **an RNase step should be added** at the first step; tech support mentions 1uL of a 25ug/mL, but the Qiagen 100ug/mL RNase A can be used with no ill effects.*

3.2 NanoDrop measurement of nucleic acid quality

*See “8.3.10-Quantification of Purified DNA by NanoDrop” for instructions on using the NanoDrop. Briefly, initiate with dH₂O, blank with your elution solution (e.g., 10mM Tris HCl), place 1-1.5uL of your sample on the pedestal, apply magnet and click “Measure”. Once the ‘clicks’ stop, your 260/280 and 260/230 ratios will display, along with the concentration and a curve of your sample’s optical reading. Besides the numbers, the curves are important in determining sample quality: running only **12 samples at a time** makes it easier to resolve between your curves when you print and evaluate your graph.*

NOTE: The minimum reliable concentration on the NanoDrop is **10ng/uL**. Anything lower may also mean your ratios are unreliable. Using published sources and anecdotal evidence, we have determined that **260/280 ratios between 1.7-2.1 (optimal ratio is 1.8), and 260/230 ratios between 1.4-2.4 (optimal ratio is 2-2.2)** produces good results. We also typically use Elution 2 for our downstream processes, unless Elution 1 returns better ratios. If your ratios deviate greatly from acceptable criteria, you may need to perform DNA extraction again (see Troubleshooting Section).

3.3 Qubit quantification

Though not used here, additional SOPs for quantification of dsDNA exist: “Quant iT broad range dsDNA Assay SOP” and “Pico Green dsDNA Assay SOP”. Use at your own discretion.

Precise quantification of double-stranded DNA is performed on the Qubit 3.0 Fluorometer in Rm 140 Biotech. Email Jennifer Mosher (jdm323@cornell.edu) at least 2 days ahead of the time you want to quantify, since it will take anywhere from 2-6 hours, depending on the number of samples and people present (approximately 2 hours with 3 people measuring 96 samples) –



PLEASE let them know when you are coming! You need to bring your own 10uL (or 2.5uL), 200uL and 1000uL micropipettes, corresponding filter tips, a 15mL Falcon tube (to prepare Master Mix), nitrile gloves, and all your samples **diluted to 10ng/uL** (calculated from the NanoDrop concentration), for a **final volume of 20uL** each (use $C_1V_1 = C_2V_2$). Also bring a pen, fine-tipped Sharpie, and a large Eppendorf tube rack. Clear-sided, 0.6mL sample tubes are provided at the BRC/CORE.

Set-Up

Once there, follow the instructions posted for starting the instrument. Use the “High Sensitivity” assay and reagents. You do not need to calibrate it, but if desired, you can prepare and run the standards, though it is not necessary, as the BRC does both. You will need to prepare a Master Mix (fluorescent dye and buffer) at a ratio of [1uL dye + 199uL Buffer per sample; e.g., 24uL dye + 4,776uL Buffer for 24 samples]. **DO NOT prepare more than 24 samples’ worth** of the Master Mix, as the dye is light-sensitive. Vortex the mixture and make sure there are no bubbles. **The dye must not be exposed to light for long periods of time (>30min)**. The standards, dye and buffer are all at the CORE facility; if there are reagents missing from Room 140, ask one of the techs and they will get you a new tube.

Assay Preparation

Handle the tubes as little as possible, do not touch the sides, and never with bare fingers. For each sample: make sure they are at room temp, vortex your DNA, add 2uL of your DNA to 198uL of the prepared Master Mix in the clear 0.6mL Eppendorf tubes. Vortex and spin down briefly to make sure there are no bubbles. Incubate at room temperature for 1-2 minutes, then follow the screenshots below. Record the displayed concentration in your WGS spreadsheet; there is no way to print the results. The Qubit will store numeric data and build graphs, if desired, but that is beyond the scope of the present needs.



Note: Incubate the samples for the appropriate amount of time after mixing them with the working solution (2 minutes for the Qubit® DNA and RNA assays, 15 minutes for the Qubit® protein assay).

1. In the *Read standard* screen, touch Run samples



2. In the *Sample volume* screen, select the sample volume and units:
 - a. Touch the + or – buttons on the wheel to select the sample volume added to the assay tube (between 1 and 20 μ L).
 - b. From the dropdown menu, select the units for the output sample concentration.





3. Insert a sample tube into the sample chamber, close the lid, and then touch **Read tube**. The reading takes approximately 3 seconds.



4. The software displays the results.



To read multiple samples for the same assay:

1. Remove the current sample, and insert a new sample.
2. To change the sample volume, swipe right or press the left arrow.
3. Touch **Read tube**.
4. Repeat step 2 and step 3 (above).

3.4 Preparing Sample Plates for Submission

Dilute your samples to **0.2-1 ng/uL** each in 10mM Tris-HCl and load them into a 96-well plate, making sure to add the correct sample to the correct well. Prepare the dilution directly in the 96-well plate wells, as this minimizes loss (you are working with very small volumes!). You will need to submit a spreadsheet with the Sample Name, the Final Concentration and the Well Number with your plate to the BRC. The 96-well plate should be filled column-wise (A1, B1, C1 etc.). We have had success submitting at **1 ng/uL**, but you can choose to submit at any concentration within the specified range. If any sample is $0.2\text{ng/uL} < x < 1\text{ng/uL}$, you can submit



it with that concentration, but make sure that is noted clearly in your submission form, and that you've discussed with someone at the CORE/BRC. If a significant number are $<1\text{ng/uL}$, check with the CORE/BRC to see if you should dilute everything to a lower concentration, as this may affect library prep.

3.5 Submitting Plates to BRC

Go to <http://www.biotech.cornell.edu/brc/genomics-facility>, click "Sample Submission" and log in:

The image shows a screenshot of the Cornell University Biotechnology Resource Center (BRC) Genomics Facility website. The page header includes the Cornell University logo, the text "CORNELL UNIVERSITY INSTITUTE OF BIOTECHNOLOGY", a search bar, and navigation links for "User Login" and "Create User Account". The main navigation menu includes "Home", "About", "BRC", "Services", "Resources", "News & Events", and "Contact". The breadcrumb trail reads "institute of biotechnology >> brc >> genomics facility". The page title is "Biotechnology Resource Center Genomics Facility". A sidebar on the left lists "DNA Sequencing" with sub-items: "Next generation sequencing" and "Sanger sequencing and fragment analysis". A red button labeled "Sample Submission" is circled with a dashed line. A large black arrow points from this button to a "Login Form" on the right. The login form includes fields for "E-mail" and "Password", a "LOGIN" button, and links for "Create a new account" and "Reset your password".



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Service Order Form

The BRC will no longer accept credit cards as an option for placing an order after March 1, 2016
 Customers from outside of Cornell University must provide a valid Purchase Order and will be invoiced after services are rendered. Payment cards will still be accepted for invoice payments. You may also pay with checks and wire transfers. Our complete payment policies and procedures are located [here](#).

What are projects and experiments?
 Projects and experiments are a way to group orders within or among facilities and share results and ordering privileges for those projects with your collaborators. Projects identify a collection of orders spread among different facilities. Experiments identify a subset of orders from a project and consist of orders for single service. Go ahead and click [[add/manage](#)] if you are interested in using these features.

Project [[add/manage](#)]: None ▾

*Facility: Illumina Sequencing ▾

*Service: Illumina sequencing ▾

Experiment [[add/manage](#)]: None ▾

* denotes required field

←

Choose “Illumina Sequencing” for both “Facility” and “Service” options

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Illumina Sequencing

The BRC no longer accepts Cornell procurement cards as an option for placing an order as of June 30, 2015
 External customers will be able to provide payment cards for payment until a date to be determined in 2015. After that time, all external customers will need to provide a valid Purchase Order and will be invoiced after services are rendered. Payment cards will still be accepted for invoice payments along with checks and wire transfers. Our complete payment policies are located here: <http://www.biotech.cornell.edu/brc/brc/services/terms-and-policies#Payment>

Select a Stored Payment Method

Submit

Or Enter New Billing Information

Use this new billing information

Select a payment option:

Select One... ▾

Use stored payment or enter new billing information



Fill out the form (left) to look like the on the right (the Instrument and Run length may vary depending on what type of a sequencing run you decide on; the other options stay the same):

Fill out next page as below:



New Payment Information Summary

Payment Information: Cornell Account: 1438146	Billing Address: 412 Stocking Hall Ithaca, NY 14853 United States	Phone: Billing: 607-255-1266
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You will be able to change your billing information before submitting your order.

Send a copy of invoice to additional e-mail address(es):

Invoice Comments
 OPTIONAL: Enter any additional information you would like displayed on your invoice:

General Comments
 OPTIONAL: If you have any other comments, please insert them here:
 We would like the Core to:
 i) prepare Nextera XT libraries for all 96 samples and;
 ii) run Bioanalyzer QC for 20 randomly selected samples (marked with "Bioanalyzer" in submission form;
 iii) perform bead-based normalization for all 96 samples.
 NOTE: All samples are submitted in concentration 1 ng/ul (determined by Qubit).
 If you need any assistance filling out this form, please contact us at (607) 255-2307.

Order Options

Instrument: MiSeq
 Run Length: 2x200 Paired End
 Sample Preparation: DNA/RNA Library Prep by Genomics Facility
 Digital PCR: No
 Prep Option: Nextera XT
 Organism: Prokaryotic

[Change Order Options](#)

Sample Submission Requirements

Use only letters, numbers, hyphens (-), underscores (_), and number symbols (#) for sample names. Each sample name must be unique with a 40 character maximum length.

Upload text file with sample information: No file selected.

Sample Name	Lane	Number of Lanes	Volume (µl)	Conc. (ng/µl)	Comments
1	None				
2	None				
3	None				
4	None				
5	None				
6	None				
7	None				
8	None				

Sample Summary

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Nancy (nrs13@cornell.edu) needs a copy of the invoice

Enter this: "We would like the Core to:
 i) prepare Nextera XT libraries for all {x number} samples and;
 ii) run Bioanalyzer QC for {y number} randomly selected samples (marked with "Bioanalyzer" in submission form;
 iii) perform bead-based normalization for all {x number} samples.
 NOTE: All samples are submitted in concentration 1 ng/ul (as determined by Qubit).

This doesn't work, so fill in one Sample Name, Lane = 1, Volume = 10, and Conc. = 1, then see instructions following.

Make sure you specify you want the CORE lab to **prepare the Nextera XT libraries** and what type of quality control (QC) steps they should perform. You should also let the CORE laboratory know you would like them to perform **bead-based normalization**, which is part of



the Nextera XT library preparation protocol. The alternative is digital PCR, which results in substantial additional costs.

YOU MUST SUBMIT AN .xls FILE via email to Jennifer Mosher (jdm323@cornell.edu) [please cc Peter Schweitzer (pas48@cornell.edu)] with: Sample Names with corresponding 96-well plate well numbers; Pool {A}; Lane {1}; Volume submitted; Concentration submitted. Indicate which samples should be run on BioAnalyzer in the comments column. The CORE/BRC **NEEDS** these, minimum (see example below), but you can add extra information (e.g., Qubit concentration) if desired.

Order #: 10359865							
Sample	Pool	Lane	Concentration (Qubit; ng/ul)	Volume (ul)	Comments	Position in 96 well plate	
1 FSL W10-0001	A	1	1	10		A1_plate1	
2 FSL W10-0002	A	1	1	10		B1_plate1	
3 FSL W10-0003	A	1	1	10		C1_plate1	
4 FSL W10-0004	A	1	1	10		D1_plate1	
5 FSL W10-0005	A	1	1	10		E1_plate1	
6 FSL W10-0006	A	1	1	10		F1_plate1	
7 FSL W10-0007	A	1	1	10		G1_plate1	
8 FSL W10-0008	A	1	1	10	Bioanalyzer QC	H1_plate1	
9 FSL W10-0009	A	1	1	10		A2_plate1	
10 FSL W10-0010	A	1	1	10		B2_plate1	
11 FSL W10-0011	A	1	1	10		C2_plate1	
12 FSL W10-0012	A	1	1	10		D2_plate1	
13 FSL W10-0013	A	1	1	10		E2_plate1	
14 FSL W10-0015	A	1	1	10	Bioanalyzer QC	F2_plate1	
15 FSL W10-0014	A	1	1	10		G2_plate1	
16 FSL W10-0016	A	1	1	10		H2_plate1	
17 FSL W10-0017	A	1	1	10		A3_plate1	
18 FSL W10-0018	A	1	1	10		B3_plate1	
19 FSL W10-0019	A	1	1	10		C3_plate1	
20 FSL W10-0020	A	1	1	10		D3_plate1	

Once you have completed and submitted the order, you will receive an order number. **Write this Order Number on your plate**, and use it as your subject line when you send Jennifer the email, and make sure it is on every hardcopy form you bring down to the BRC. When you take samples to the BRC, make sure they have the correct library, QC, sequencing run and sample concentration information.

Create a submission .xls file containing the order number, date of submission and names of people involved in organizing a run in a file header. This file should be saved in a subfolder of a “WGS” folder on the BoorWiedmannLab server, together with all QC files and basic sequence QC information and location of fastq.gz files received after the sequencing run is completed.

Make sure all samples and files are in the correct format: “FSL {user ID}-{4-digit number}”.



3.6 Quality Control and BioAnalyzer

For HiSeq runs, randomly select 10% of your samples for Bioanalyzer QC after the tagmentations step. If 90% of these samples pass the QC control (i.e., the majority of fragments are **700-1200 bp** long, there is a **single peak** in a size distribution curve, and **curves look smooth**), you can let the CORE lab know they can proceed with library preparation. If samples pass the Bioanalyzer QC, the Core laboratory will proceed with Nextera XT library preparation and will perform bead-based normalization for all samples prior to library pooling.

If more than 10% of samples fail the Bioanalyzer QC, perform Bioanalyzer QC for **ALL** samples and **repeat** the DNA extraction and tagmentation steps for samples of poor quality. **DO NOT PROCEED with sequencing if a large percentage of your samples fail!** If a low percentage fail Bioanalyzer, you can exclude them from the current sequencing run and proceed with fewer samples. However, this may affect the cost (\$\$) of the sequencing run, so there are other factors to consider when deciding whether or not to proceed.



SECTION 4 TROUBLESHOOTING

Problems potentially encountered with this procedure include:

1. **The 260/230 Ratio is too low:** Most of the time, this is due to guanidine contamination (guanidine compounds are part of the binding solution and absorb at 230nm). Qiagen recommends re-running the entire extraction procedure from the start, but there is a shortcut: re-bind the DNA to the Qiagen columns and re-wash the DNA. *Only attempt this if you have the option to re-extract the DNA if it does not work – you could completely lose all your DNA!* Start from the addition of Buffer AL and repeat the protocol from that step onward, using 700uL (vs. 500uL) AW1 and AW2 each time the DNA is washed (once with AW1 and twice with AW2). This has resulted in decreased absorbance at 230nm.

SECTION 5 REFERENCES

1. QiAmp DNA Spin Mini Kit Manual:
<https://www.qiagen.com/resources/download.aspx?id=67893a91-946f-49b5-8033-394fa5d752ea&lang=en>
2. MO BIO PowerSoil® DNA Isolation Kit Manual:
<https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf>
3. NanoDrop User Guide: <http://www.nanodrop.com/library/nd-1000-v3.7-users-manual-8.5x11.pdf>
4. Qubit® dsDNA HS Assay Manual:
https://tools.thermofisher.com/content/sfs/manuals/qubit_3_fluorometer_man.pdf