**FOOD SAFETY LABORATORY**
**CORNELL UNIVERSITY**

# ***Name of Protocol***

## FILE NAME: Genomic DNA Extraction (Qiagen)

**Authored by: Steven Warchocki**
**Last Modified on: 4/28/2016**
**Approved by:**

EFFECTIVE DATE: Date of Approval

APPROVED BY:

_____ _____
Dr. Martin Wiedmann (date)

AUTHORED BY:

_____Steven Warchocki_____ _____
(Name) (date)

TABLE OF CONTENTS

# SECTION 1 INTRODUCTION

## 1.1 Purpose

The purpose of this document is to provide detailed instructions on isolating high quality genomic DNA from bacteria for Illumina Nextera XT preps and subsequent full genome sequencing. However, this protocol can be used for other applications where high quality genomic DNA is required.

## 1.2 Scope

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

## 1.3 Definitions

Lysozyme – Enzyme that attacks and damages peptidoglycans in bacterial cell walls. Used to improve lysis of gram-positive bacteria.

RNase – Nuclease that catalyzes the degradation of RNA into its respective subunits.

Proteinase K – Broad-spectrum serine protease. Used to inactivate and destroy DNases and RNases in cell lysates.

A260/A280 ratio – "Used as a measure of purity in both nucleic acid and protein extractions. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA."

A260/A230 ratio – "A secondary measure of nucleic acid purity in DNA/RNA extractions. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230 nm."

## 1.4 Safety

If isolating DNA from BSL-2 organisms (e.g. *Listeria*), appropriate protective measures need to be taken. All BSL-2 waste needs to be treated accordingly.

## SECTION 2 MATERIALS

Gram Positive

- Lysozyme
- Stock EDTA
- Triton X-100

Gram Positive and Negative

- Stock Tris·HCl (pH 8.0)
- 1.5 mL microcentrifuge tube
- QIAamp DNA MiniKit
- RNase A (100 mg/mL; cat. no. 19101)
- Ethanol (96-100%)

If growing cultures on agar plates:

- Phosphate Buffered Saline (pH 7.4)
- Sterile inoculating loop or swab

# SECTION 3 PROCEDURES

*Note: The protocol shown below is taken directly from the QIAamp DNA Mini & Blood Mini Handbook with modifications made where necessary. Modifications are in italics. Only applicable steps and excerpts from the handbook are shown. The full version is available through* www.qiagen.com

## 3.1 Tips before starting

- If harvesting cells from plate cultures, do not use too many cells. Too large of a pellet can result in incomplete lysis and clogging of the column in subsequent steps.
- Be gentle when vortexing and do not vortex more than instructed. You do not want to over-shear the DNA.
- For steps #8-10, rotate the microcentrifuge tubes 180° for each spin. This will help prevent wash buffer from collecting on the inner rim of the tube and contaminating your eluted sample.

## 3.2 Isolation of genomic DNA from Gram-positive bacteria

1. *Harvest cells from 1.8 mL of an overnight culture. If bacteria do not grow well in liquid culture:*
    a. *Isolate bacteria from fresh culture plate using an inoculation loop/sterile swab*
    b. *Homogenize bacteria in 1.8 mL PBS*
2. Pellet bacteria in 2 mL microcentrifuge tube by centrifugation at *7,500 rpm (5,000 x g) for 10 min (can do higher speed for shorter time).* Remove all supernatant without disturbing pellet.
3. Suspend bacterial pellet in 180 µl of the appropriate enzyme solution (20 mg/ml lysozyme or 200 µg/ml lysostaphin; 20 mM Tris·HCl, pH 8.0; 2 mM EDTA; 1.2% Triton). *Pipet to homogenize the bacterial suspension. FSL and MQIP use lysozyme.*
4. *Incubate for 30-75 min at 37°C (see references). Gently flick tubes to mix solution after 15 min of incubation.*
5. Add 20 µl proteinase K (from kit) and gently mix by pulse-vortexing for 15 s. *Centrifuge for a few seconds to collect liquid. If RNA-free genomic DNA is required, incubate at 56°C for 10 min and proceed to step 6. If RNA-free genomic DNA is not required, incubate at 56°C for 45 min. and proceed to step 7.*
6. *Let liquid cool to room temperature. Add 4µl RNase (100mg/ml). Gently mix by pulse-vortexing for 15 s and centrifuge for a few seconds to collect liquid. Incubate at room temperature for 10 min.*
7. Add 200 µl Buffer AL and mix by pulse-vortexing for 15 s. *If RNase treatment was performed, complete the proteolytic digestion with an additional incubation at 57℃ for 30 min.*
8. Incubate at 70°C for 10 min to inactivate any remaining enzymes. *Cool to room temperature and centrifuge for a few seconds to collect liquid.*
9. Follow the "Protocol: DNA Purification from Tissues" from step 6 (page 34 and below).

## 3.3 Isolation of genomic DNA from known Gram-negative bacteria

Isolation of genomic DNA from bacterial suspension cultures

1. Pipet *1.0 ml* of bacterial culture into a *1.5 ml* microcentrifuge tube, and centrifuge for 5 min at 5000 x *g* (7500 rpm). Remove all supernatant without disturbing pellet.
2. Calculate the volume of the pellet or concentrate and add Buffer ATL (supplied in the QIAamp DNA Mini Kit) to a total volume of 180 µL *(OK to just add 180 µL of buffer).*
3. Follow the "Protocol: DNA Purification from Tissues" from step 3 (page 33 and below).

Isolation of genomic DNA from bacterial plate cultures

1. Remove bacteria from culture plate with an inoculation loop or sterile swab and suspend in 180 µl of Buffer ATL (supplied in the QIAamp DNA Mini Kit) by vigorous stirring.
2. Follow the "Protocol: DNA Purification from Tissues" from step 3 (page 33 and below).

## 3.4 DNA Purification from Tissues (QIAamp DNA Mini Kit)

**3. Add 20 µl proteinase K, mix by *pulse* vortexing, and incubate at 56°C until the tissue is completely lysed (*45-60 min*). Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform.**
**Note**: Proteinase K must be used. QIAGEN Protease has reduced activity in the presence of Buffer ATL. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. Lysis overnight is possible and does not influence the preparation. To ensure efficient lysis, a shaking water bath or a rocking platform should be used. If not available, vortexing 2–3 times per hour during incubation is recommended.

**4. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.**

**5. If RNA-free genomic DNA is required, follow step 5a. Otherwise, follow step 5b.**
RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

**5a. First, add 4 µl RNase A (100 mg/ml), gently mix by pulse-vortexing for 15 s, *centrifuge for a few seconds to collect liquid,* and incubate for 10min at room temperature (15–25°C). Add 200 µl Buffer AL to the sample. Mix again by pulse-vortexing for 15 s, briefly centrifuge the *1.5 ml* microcentrifuge tube to remove drops from inside the lid, and incubate at 70°C for 10 min.**
It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL. In most cases it will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

**5b. Add 200 µl Buffer AL to the sample, mix by pulse-vortexing for 15 s, *briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid,* and incubate at 70°C for 10 min.**

It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL, which in most cases will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

**6. Add 200 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the *1.5 ml* microcentrifuge tube to remove drops from inside the lid.**
It is essential that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the QIAamp Mini spin column. This precipitate does not interfere with the QIAamp procedure or with any subsequent application. Do not use alcohols other than ethanol since this may result in reduced yields.

**7. Carefully apply the mixture from step 6 (including the precipitate) to the QIAamp Mini spin column (in a 2 ml collection tube) <u>without wetting the rim</u>. Close the cap, and centrifuge at *full speed (20,000 x g 14,000 rpm)* for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.***
Close each spin column to avoid aerosol formation during centrifugation. It is essential to apply all of the precipitate to the QIAamp Mini spin column.
* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 of handbook for safety information.

**8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 <u>without wetting the rim</u>. Close the cap, and centrifuge at *full speed (20,000 x g; 14,000 rpm)* for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (not provided), and discard the collection tube containing the filtrate.***

**9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 <u>without wetting the rim</u>. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (not provided), and discard the collection tube containing the filtrate.***

**10. Repeat Step 9, using another 500 µl Buffer AW2. Centrifuge for 3 min.**

**11. Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 2 min.**
This step helps to eliminate the chance of possible Buffer AW2 carryover.

**12. Repeat Step 11, using a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

**13. *Pre*-warm *Tris-HCL (to be used for elution) at 37°C.* Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add *50*µl**

**Tris-*HCL* directly to the column. Incubate at room temperature for *4 min*, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min. *This is your first elution.***

**14. Repeat step 11 using new 1.5 microcentrifuge tube. *This is your second elution.***
A 5 min incubation of the QIAamp Mini spin column loaded with *Tris-HCL* before centrifugation generally increases DNA yield. A third elution step with a further 200 µl *Tris-HCL* AE will increase yields by up to 15%.

Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation. Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield (see Table 5, page 25). Eluting with 4 x 100 µl instead of 2 x 200 µl does not increase elution efficiency.

****For long-term storage of DNA, eluting in *Tris-HCL* and placing at –20°C is recommended**, since DNA stored in water is subject to acid hydrolysis. Yields of DNA will depend both on the amount and the type of tissue processed. 25 mg of tissue will yield approximately 10–30 µg of DNA in 400 µl of water (25–75 ng/µl), with an A260/A280 ratio of 1.7–1.9. For more information about elution and how to determine DNA yield, length, and purity, refer to pages 24–25 and Appendix A, page 50 of the QIAamp DNA Mini & Blood Mini Handbook.

## SECTION 4 TROUBLESHOOTING

- Elution: Water, Tris-HCL, or Tris-EDTA (Buffer AE) can be used for eluting DNA. Water pH can vary which leaves the DNA susceptible to acid hydrolysis during freeze-thaw cycles. Tris-HCL and Tris-EDTA are prepared to an optimal pH for DNA storage. EDTA, a chelator, can buffer primer degradation if contaminant nucleases are present. However, EDTA has the potential to inhibit downstream enzymatic processes. Due to these reasons, Tris-HCL is an ideal eluting option, especially if long term storage is required.
- When quantifying DNA, make sure to gently mix and warm up samples if they have sat in the cold room. Genomic DNA can precipitate out of solution.
- Low A260/A280 ratios may be caused by: Carryover of protein, salt, and other reagents associated with the extraction protocol and/or very low concentration (> 10 ng/ul) of nucleic acid.
- Low A260/230 ratios may be caused by: Carryover of salt, carbohydrates, peptides, phenol, TRIzol, guanidine HCL (DNA isolations), guanidine isothiocyanate (RNA isolations), or aromatic compounds in general.
- DNA from the 2nd elution has been shown to have a higher molecular weight. (See Steven Warchocki "DNA Prep For Full Genome" notebook page 16)

## SECTION 5 REFERENCES

*QIAamp DNA Mini & Blood Mini Handbook*

*http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf*

*http://www.bio.davidson.edu/projects/gcat/protocols/NanoDrop_tip.pdf*

*http://www.qiagen.com/knowledge-and-support/faq/?ID=c59936fb-4f1e-4191-9c16-ff083cb24574*

*http://www.ncbi.nlm.nih.gov/pmc/articles/PMC252029/pdf/jbacter00400-0140.pdf*