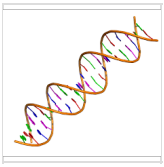

Aug 14, 2020

Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit

Julie Haendiges[1], Ruth Timme[1], George Kastanis[1], Maria Balkey[1]

[1]US Food and Drug Administration

1 Works for me dx.doi.org/10.17504/protocols.io.bi4dkgs6

GenomeTrakr
Tech. support email: genomeTrakr@fda.hhs.gov

Julie Haendiges
US Food and Drug Administration

ABSTRACT

This SOP outlines the procedure for manual extraction of genomic DNA from live bacterial cells using the Qiagen Qiagen DNeasy Blood and Tissue Kit followed by quality control of purified extracts using the Qubit for purposes of whole genome sequencing.

This SOP is applicable to all GenomeTrakr laboratories with the intent of obtaining high quality, purified genomic DNA from gram-negative or gram-positive bacterial cells for use in subsequent sequencing protocols.

Complete in order:

1. DNA Extraction (included protocol *or* Automated DNA Extraction using the Qiacube)

- Step-by-step procedures to obtain high quality DNA from isolates in TSB for whole genome sequencing

2. DNA Quantitation

- Quantitation of extracted DNA using the Qubit Flourometer

3. Library Preparation for WGS

- Library preparation using NexteraXT *or* Illumina DNA Prep (previously Nextera DNA Flex)

4. Sequencing using Illumina MiSeq

5. Data Quality Checks and NCBI Submission

DOI

dx.doi.org/10.17504/protocols.io.bi4dkgs6

PROTOCOL CITATION

Julie Haendiges, Ruth Timme, George Kastanis, Maria Balkey 2020. Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bi4dkgs6

KEYWORDS

DNA Extraction, Qiagen Blood and Tissue Kit, GenomeTrakr, Whole Genome Sequencing

CREATED

Jul 28, 2020

LAST MODIFIED

Aug 14, 2020

PROTOCOL INTEGER ID

39781

GUIDELINES

The Qiagen DNeasy Blood and Tissue Kit contains:

- AL Buffer
- ATL Buffer
- Proteinase K
- AW1 Buffer (Must be diluted prior to use, see "Before Start" section)
- AW2 Buffer (Must be diluted prior to use, see "Before Start" section)
- Collection Tubes
- Spin Tubes

DNA Extractions used in WGS library preparation and sequencing must not contain EDTA. This chemical inhibits critical processes during library prep (ex. Tagmentation) and is found in the elution buffer contained in this kit. For this reason, Buffer EB should be ordered separately and used for final elution.

MATERIALS

NAME	CATALOG #	VENDOR
Buffer EB	19086	Qiagen
DNeasy Blood & Tissue Kit, QIAGEN	Cat No./ID: 69504	
Proteinase K (2 ml)	19131	Qiagen
DNA LoBind Tubes	#022431021	Eppendorf
1M Tris-HCl (pH 8.0)	15568025	Thermo Fisher Scientific
200 Proof Ethanol pure	E7023	Sigma Aldrich
Lysostaphin - Bioultra	L4402	Sigma – Aldrich
Collection Tubes	19201	Qiagen
Lysozyme from chicken egg white BioUltra lyophilized powder	L4919	Sigma Aldrich
RNase A	19101	Qiagen
Triton X-100	MTX15681	Fisher Scientific
0.5 M EDTA	50-983-251	Fisher Scientific

MATERIALS TEXT

Equipment:

- Eppendorf 5430 Microcentrifuge (Eppendorf Catalog# 022620557 or equivalent)
- Incubator with 2.0 ml tube block attachment (Eppendorf Thermomixer Catalog# 2231000574 or a heatblock equivalent)
- Vortex
- Pipettes and tips

SAFETY WARNINGS

Biosafety Warning: Foodborne pathogens are capable of causing serious disease. Always use a minimum of BSL2 practices, and use extreme caution when transferring and handling strains of this type. Wear appropriate PPE when handling infectious organisms. Work in a BSC when handling organisms with a low infectious dose. Disinfect or dispose of all plastic ware and glassware that are exposed to the bacterial cultures.

Chemical Safety Warning: Take the required precautions, and wear appropriate PPE when handling potentially hazardous chemicals. Ensure that chemicals, spent containers, and unused contents are disposed of in accordance with governmental safety standards.

RNase A has a GHS Category 1 classification as a skin and respiratory sensitization reagent. See the SDS for more information.

Qiagen DNeasy Kit Components: See Qiagen's SDS for additional information.

Citation: Julie Haendiges, Ruth Timme, George Kastanis, Maria Balkey (08/14/2020). Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit. https://dx.doi.org/10.17504/protocols.io.bi4dkgs6

AL Buffer: GHS Category 2 for skin & eye irritant. GHS Category 1 for skin sensitization. Contains a chaotropic salt and is not compatible with agents containing bleach.

AW1 Buffer: GHS Category 4 for oral & inhalation acute toxicity. GHS Category 2 for skin & eye irritation. Contains a chaotropic salt and is not compatible with agents containing bleach.

Proteinase K: GHS Category 1 for respiratory sensitization.

BEFORE STARTING

1. **Bacterial Isolate Preparation**: Sequencing should be preformed on pure isolated colonies only. Inoculate TSB with a single colony and incubate at 37^{o}C for 16-24 hours.

2. **Preparation of AW1 and AW2:** These buffers need to be diluted with 100% Ethanol prior to use for the first time. Add the appropriate volumes as indicated on the bottle and write the date on the bottle.

3. **Preheat incubator or heatblock to 56^{o}C**

Collect Cells

1 Removed the inoculated TSB samples from the incubator.

2 Resuspend cells in the bacterial culture by pulse vortexing 3-5 times on low setting or by pipetting.

3 Transfer **1.0 mL** bacterial culture into a 2.0 ml sterile Eppendorf tube.

4 Pellet cells by centrifuging at **7500 rpm** for **00:05:00** minutes.

5 Discard supernatant into a 50 ml conical tube with 10% commercial bleach marked “biohazard waste".

Lysing Bacterial Cells

6 Select the appropriate lysing conditions based on the type of bacteria:
Step 6 includes a Step case.
Gram-Positive Bacteria
Gram-Negative Bacteria

step case

Gram-Positive Bacteria

7 Prepare enzymatic lysis buffer (ELB) containing 20 mM Tris-HCl, 2 mM EDTA, and 1.2% Triton. A 250ml stock will be prepared. This stock buffer will last for 1 year at room temperature.

Mix **5 mL** of 1 M Tris-HCl, **1 mL** of 0.5 M EDTA, **3 mL** of Triton X-100 and enough molecular-grade water to bring the volume up to **250 mL** . Mix gently to incorporate the Triton.

Citation: Julie Haendiges, Ruth Timme, George Kastanis, Maria Balkey (08/14/2020). Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit. https://dx.doi.org/10.17504/protocols.io.bi4dkgs6

8 Add **20 mg/ml** of lysozyme to appropriate amount of lysis buffer within 24 hours of use.

Num. of samples + 1 extra	Total ELB volume (µl)	Lysozyme (mg)
(5+1) = 6	1080	21.6
(10+1) = 11	1980	39.6
(18+1) = 19	3420	68.4
(20+1) = 21	3780	75.6

Note: for *Staphyloccocus* spp., add 1 mg of lysostaphin for every 2 ml of enzymatic lysis buffer, regardless of ELB preparation method.

9 Add **180 µl** of enzymatic lysis buffer (ELB) + Enzyme to sample tube.
Resuspend bacterial cell pellet by vortexing.

10 Incubate at **56 °C** for **00:30:00** .

11 Add **4 µl** of RNase A. Mix by vortexing and incubate at room temperature for **00:05:00**

12 Add **25 µl** of Proteinase K. Add **200 µl** of Buffer AL. Vortex on high setting for 5 seconds to mix.

13 Incubate at **56 °C** for at least **00:30:00**

14 Add **200 µl** of 100% Ethanol and mix by vortexing.

DNA Purification

15 Label lid of a DNeasy spin column with a unique, recorded identifier for each sample.

16 Discard the collection tube and its contents.

17 Using a P1000 pipette, transfer full volume of pre-treated bacterial cells to the corresponding spin column.

Citation: Julie Haendiges, Ruth Timme, George Kastanis, Maria Balkey (08/14/2020). Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit. https://dx.doi.org/10.17504/protocols.io.bi4dkgs6

18 Centrifuge at **8000 rpm** (> 6000 x g) for **00:01:00**

19 Place DNeasy spin column into a new 2.0 ml collection tube.

20 Add **500 µl** of Buffer AW1.

21 Centrifuge at **8000 rpm** (> 6000 x g) for **00:01:00**

22 Discard the collection tube and its contents.

23 Place DNeasy spin column into a new 2.0 ml collection tube.

24 Add **500 µl** of Buffer AW2.

25 Centrifuge at **13000 rpm** (>20000 x g) for **00:03:00** .

26 Discard the collection tube and its contents.

27 Examine the spin column to ensure that no liquid remains on the spin column.

If there is liquid present, until it is clean.

28 Place spin column in a new, sterile 1.5 ml elution tube labeled with a unique, recorded identifier and/or FDA accession (CFSANxxxxxx) number.

29 Add **100 µl** of Buffer EB to the spin column.

Optional: Incubate Buffer EB at 37°C to improve elution.

Citation: Julie Haendiges, Ruth Timme, George Kastanis, Maria Balkey (08/14/2020). Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit. https://dx.doi.org/10.17504/protocols.io.bi4dkgs6

30 Incubate at room temperature for **00:05:00** .

31 Centrifuge at **8000 rpm** (>6000 x g) for **00:01:00**

32 DO NOT discard eluate, it is the purified DNA. DNA can be stored at **4 °C** for short-term storage and **-20 °C** or **-80 °C** for long-term storage.

Citation: Julie Haendiges, Ruth Timme, George Kastanis, Maria Balkey (08/14/2020). Manual DNA Extraction using Qiagen DNeasy Blood and Tissue Kit. https://dx.doi.org/10.17504/protocols.io.bi4dkgs6