Genomic DNA extraction protocol using DNeasy Blood & Tissue Kit (QIAGEN) optimized for Gram-Negative bacteria

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Abstract

We found many specific steps and conditions for gram-negative bacteria while working with DNeasy Blood & Tissue Kit. These details are described in DNeasy Blood & Tissue Handbook (https://www.qiagen.com/us/resources/resourcedetail?id=6b09dfb8-6319-464d-996c-79e8c7045a50&lang=en). However we decided to describe this protocol step by step pointing out in some critical steps. This protocol was successfully applied while genomic DNA extraction from Shigella spp. and Salmonella spp. strains for whole-genome sequencing.

Citation: Pavlova A.S.,Olneva T.A.,Konovalova T.A.,Guseva A.N.,Goptar I.A.,Podkolzin A.T.,Kuleshov K.V. Genomic DNA extraction protocol using DNeasy Blood & Tissue Kit (QIAGEN) optimized for Gram-Negative bacteria. **protocols.io** dx.doi.org/10.17504/protocols.io.paadiae

Published: 12 Apr 2018

Before start

Add **Ethanol 96%** to reagents of QIAGEN kit as described by vendor

Prepare CSB solution (Cell suspension buffer)

1 M Tris-HCl (pH 8.0) - 10 ml

0.5 M EDTA (pH 8.0) - 20 ml

Add deionized water to 1 Litre

Store at +4°C to +8°C

Prepare **Proteinase K solution (20 mg/ml)** in deionized water

Store at -20°C

Materials

QIAgen DNeasy Blood and Tissue Kit, 50 rxn 69504 by Qiagen

✓ Ethanol by Contributed by users

Proteinase K 0706-1G by Amresco

Protocol

Solutions to prepare in advance

Step 1.

Add Ethanol to reagents of QIAGEN kit as described by vendor

CSB solution (Cell suspension buffer)

1 M Tris-HCl (pH 8.0) - 10 ml

0.5 M EDTA (pH 8.0) - 20 ml

Add deionized water to 1 Litre

Store at +4°C to +8°C

Prepare **Proteinase K solution (20 mg/ml)** in deionized water

Store at -20°C

Ethanol 96%

Plating for confluent growth

Step 2.

Streak an isolated colony from test cultures onto **Trypticase Soy Agar** (**TSA**) plates for confluent growth.

Incubate aerobically at **37°C for 18-24 hours**.

🌡 TEMPERATURE

37 °C Additional info: incubation

Preparation of bacterial culture

Step 3.

24-hour culture is suspended in **2.2 ml CSB Buffer** and measured the optical density by taking a value of **D=7.0** (**21x10^{8} cells/ml**).

AMOUNT
2.2 ml Additional info: CSB Buffer

NOTES
Anastasia Pavlova 09 Apr 2018

Use a sterile polyester-fiber or cotton swab that has been moistened with sterile CSB to remove some of the growth from agar plate; suspend cells in CSB by spinning swab gently so cells will be evenly dispersed and formation of aerosols is minimized.

We used a densitometer (Densi-La-Meter ® II, Erba Lachema, Czech Republic) for the measurement of bacterial suspension optical density, subsequently brought to a value of **D=7.0**, which, according to McFarland standards, corresponds to **21x10^{8} cells/ml.**

Concentration of bacterial cells

Step 4.

Transfer **1 ml** of bacterial cell suspension into a microcentrifuge tube and centrifuging for **10 min at 7 500 rpm**. Carfully discard supernatant.

AMOUNT
1 ml Additional info: bacterial suspension

DNA Lysis Buffer

Step 5.

Add **180 µl Buffer ATL** (QIAGEN) to the pellet and carefully resuspense it.

AMOUNT
180 µl Additional info: Buffer ATL

REAGENTS
Buffer ATL 19076 by Qiagen

NOTES
Anastasia Pavlova 09 Apr 2018

You should use 1000 µl pipette tips to prevent cell injury.

Lysis Incubation

Step 6.

Add **20 µl** Proteinase K (QIAGEN) or **2 µl** of self-prepared **Proteinase K** (20 mg/ml) (see 'Reagents section'). Mix thoroughly by vortexing, and incubate **1 hours at 56°C**.

AMOUNT
2 µl Additional info: Proteinase K

TEMPERATURE
56 °C Additional info: incubation

REAGENTS

Proteinase K [0706-1G](#) by [Amresco](#)

NOTES

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It is crucial that Proteinase K should be freshly prepared or stored at -20^{0}C before use.

DNA precipitation

Step 7.

Vortex for 15 s. Add **200 µl Buffer AL** (QIAGEN) to the sample, and pipetting thoroughly up and down to yield homogeneous solution.

AMOUNT

200 µl Additional info: Buffer AL

REAGENTS

Buffer AL (lysis buffer) by [Qiagen](#)

NOTES

Anastasia Pavlova 09 Apr 2018

A white gelatinous lysate may form on addition of Buffer AL and ethanol. This lysate *can clog membrane* pores when the mixture will be placed into the column. To avoid this effect pipette sample thoroughly up and down to yield homogeneous solution.

DNA precipitation

Step 8.

Then add **200 µl ethanol 96%**, and again thoroughly pipetting. Mix the sample by vortexing.

AMOUNT

200 µl Additional info: Ethanol 96%

REAGENTS

ethanol by Contributed by users

DNA extraction

Step 9.

Pipet the mixture (including any precipitate) into the **DNeasy Mini spin column** (QIAGEN) placed in a 2 ml collection tube. Centrifuge at **8 000 rpm for 1 min**.

Wash

Step 10.

Discard flow-through and collection tube. Place the DNeasy Mini spin column in a new collection tube.

Add **500 µl Buffer AW1** to the column and centrifuge at **8 000 rpm for 1 min**.

AMOUNT

500 µl Additional info: Buffer AW1

REAGENTS

Buffer AW1 19081 by Qiagen

Wash

Step 11.

Again discard flow-through and collection tube and place the DNeasy Mini spin column in a new collection tube.

Add **500 µl Buffer AW2** to the column. Centrifuge at **14 000 rpm for 3 min**.

AMOUNT

500 µl Additional info: Buffer AW2

REAGENTS

Buffer AW2 19072 by Qiagen

DNA elution

Step 12.

Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube and pipet **100 µl Buffer AE** directly onto the DNeasy membrane.

Incubate the sample at room temperature (**23-25°C**) **for 2-5 min,** centrifuge at **8 000 rpm for 1 min** to elute the DNA solution.

The first elution should contain aproximately 80-90 µl DNA.

AMOUNT

100 µl Additional info: Buffer AE

TEMPERATURE

25 °C Additional info: incubation

REAGENTS

Buffer AE 19077 by Qiagen

DNA elution

Step 13.

Place the DNeasy Mini spin column in another clean 1.5 ml microcentrifuge tube and again pipet **100 µl Buffer AE** directly onto the DNeasy membrane.

Incubate the sample at room temperature (**23-25°C**) **for 2-5 min** and then centrifuge at **8 000 rpm for 1 min**.

The second elution contains 100-110 µl DNA.

AMOUNT

100 µl Additional info: Buffer AE

TEMPERATURE

25 °C Additional info: incubation

REAGENTS

Buffer AE 19077 by Qiagen

Warnings

Always use Biosafety Level 2 practices (at a minimum) and extreme caution when transferring and handling strains of these genera. Work in a biological safety cabinet when handling large amounts of cells. Disinfect or dispose of all plasticware and glassware that come in contact with the cultures in a safe manner.

It is recommended to plate cultures, prepare cell suspensions, and cast plugs in a Class II Biosafety Cabinet (BSC), if available. Treat all plasticware, glassware, pipets, spatulas, etc. that come in contact with the cell suspensions or plugs as contaminated materials and dispose of or disinfect according to your institutional guidelines.