SOPs for DNA extraction and library preparation when using the Illumina sequencing platform

Introduction

With rapid development of whole genome sequencing (WGS) analysis, many bacterial DNA extraction procedures have been tested. Here, we review three methods commonly used to extract bacterial DNA and library preparation methods for WGS.

DNA extraction methods

APHA uses an automated MagNA pure system (Roche Life Science) for routine DNA extraction. MagNA Pure LC 2.0 Instrument performs a majority of the extraction steps, including binding of DNA to magnetic glass particles, washing steps and elution of pure DNA. The purified DNA was analysed with respect to DNA integrity, recovery, purity and ability to amplify target sequence with LightCycler® 480 and LightCycler® (Roche Life Science) Carousel-Based Instruments. The product has since been withdrawn. Related products can be found the Roche website *(https://lifescience.roche.com/en_gb/products/magna-pure-24-instrument.html*).

In addition, APHA tested boilate method for bacterial DNA extraction for WGS. The boilate method developed for PCR templates (Wimalarathna et al., 2013; Queipo-Ortuno et al., 2008) has proven to be suitable for the preparation of libraries for WGS of *Mycobacterium bovis* at the APHA sequencing unit, however, testing by comparison to sequencing extracted DNA using MagNA Pure extraction method showed the boilates method not to be suitable for sequencing *Salmonella* spp. and *E. coli* genomes. The advantages of the boilate method include no requirements for special equipment or reagents, rapid preparation and a safe way to transport pathogenic isolates for sequencing and thus further method development outside this project will be carried out for potential use in *Salmonella* sequencing.

Since the start of the project, the MagNA Pure extraction system has been discontinued from production by Roche Life Sciences and therefore APHA adopted a similar magnetic separation protocol using KingFisher™ Duo Prime Magnetic Particle Processor (Thermofisher) (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/KingFisher_Duo_Prime_User_Manual_5400110.pdf).

At DTU, genomic DNA extraction is carried out using Easy-DNA™ Kit (Invitrogen, Thermofisher). The extraction method yields high-quality DNA with an average size between 100 kb and 200 kb, which is suitable for PCR, DNA hybridization, genomic DNA library construction, and other applications. The extraction procedure contains only 4 steps with no special equipment required.

(https://tools.thermofisher.com/content/sfs/brochures/713_021456_easydnapr_bro.pdf)

At PHE, genomic DNA extraction is carried out using an automated method to extract DNA from bacterial cells. The QIAsymphony DNA Investigator Kit (Qiagen) enables automated purification of genomic DNA from up to 96 samples from a wide range of starting material such as swabs, filters, casework, crime-scene samples and blood. Purification is fast and efficient, and purified DNA performs well in downstream analyses. This method requires QIAsymphony SP/AS instrument.

(https://www.qiagen.com/gb/resources/resourcedetail?id=b0c38b97-2200-4102-a2d5-ba99648fc9d5&lang=en)

Sequencing library preparation

DTU, APHA and PHE follow the Illumina NexteraXT library preparation manual (Illumina) (*https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nexteradna/nextera-dna-library-prep-reference-guide-15027987-01.pdf*).

Sequencing method

DTU, APHA and PHE use Illumina sequencing platforms including MiSeq, HiSeq and NextSeq.

Reference

1. Wimalarathna HM, Richardson JF, Lawson AJ, Elson R, Meldrum R, Little CL, Maiden MC, McCarthy ND, Sheppard SK: **Widespread acquisition of antimicrobial resistance among Campylobacter isolates from UK retail poultry and evidence for clonal expansion of resistant lineages**. *BMC microbiology* 2013, **13**:160.

2. Queipo-Ortuno MI, De Dios Colmenero J, Macias M, Bravo MJ, Morata P: **Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis**. *Clinical and vaccine immunology : CVI* 2008, **15**(2):293-296.

APPENDIX C.2 STANDARD OPERATING PROCEDURE – DNA extraction (bacteria), DTU Food

Easy-DNA™ Kit (Invitrogen)

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SOP-version: 1

Introduction

This protocol describes a bacterial DNA extraction method to extract DNA. Easy-DNA™ (Invitrogen) extraction method yields high-quality DNA with an average size between 100 kb and 200 kb, which is suitable for PCR, DNA hybridization, genomic DNA library construction, and other applications. In this method, protein and lipids are precipitated and extracted by the addition of solution B and chloroform. The solution is then centrifuged to separate it into two phases with a solid interface in between the phases. The DNA is in the upper. The extraction procedure contains only four steps with no special equipment required.

Sample Material

Bacterial culture

Equipment

- Heating block capable of maintaining 65°C
- Microcentrifuge
- Vortex Mixer

Reagents

- Easy-DNA™ Kit, cat.no. K1800-01 (Invitrogen)

Literature

Order info: *https://www.fishersci.com/shop/products/kit-easy-dna/k180001*

Procedure, DNA extraction (bacteria), DTU Food (Easy-DNA™ Kit (Invitrogen))

1. Resuspend a 10 µl loopful of bacterial cells streaked on blood agar in 200 µl PBS.
2. Add 350 µl of Solution A and incubate at 65°C for 10 min.
3. Add 350 µl of Solution B and vortex vigorously.
4. Add 500 µl of chloroform and vortex.
5. Spin at 20,000 g for 10 min at 4°C.
6. Transfer 300-500 µl of the upper phase into 1 ml of cooled ethanol.
7. Incubate on ice for 30 min.
8. Centrifuge at 20,000 g for 10-15 min at 4°C.
9. Wash with 500 µl of cooled 80% ethanol.
10. Centrifuge at maximum speed for 3-5 min at 4°C to remove ethanol.
11. Resuspend pellets in 100 µl of Tris-RNase (40 µg/ml) and incubate for 1 h at 37°C.
12. Store DNA samples at -20°C until required.

APPENDIX C.3 STANDARD OPERATING PROCEDURE – DNA extraction (bacteria), PHE

QiaSymphony DNA (Qiagen) extraction

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Introduction

This protocol describes an automated method to extract DNA from bacterial cells. The QIAsymphony DNA Investigator Kit (Qiagen) enables automated purification of genomic DNA from 1–96 samples from a wide range of starting material, such as swabs, filters, casework or crime-scene samples, and blood on the QIAsymphony SP. Purification is fast and efficient, and purified DNA performs well in downstream analyses.

Sample Material

Bacterial culture

Equipment

- Heating block capable of maintaining 95°C
- Microcentrifuge tubes
- Vortex Mixer
- QIAsymphony SP/AS instrument

Reagents

- QIAsymphony DNA Investigator Kit

Literature

Application note:
https://www.qiagen.com/gb/resources/resourcedetail?id=b0c38b97-2200-4102-a2d5-ba99648fc9d5&lang=en

Procedure, DNA extraction (bacteria), PHE (QiaSymphony DNA extraction)

1. Transfer 700 µl of overnight culture into a Fortitude 96 well plate.
2. Spin at 3500 rpm for 20 min to collect bacterial cells.
3. Lyse cells with ATL buffer and Proteinase K.
4. Add 4 µl of RNase.
5. Heat inactivate for 95°C for 10 mins.
6. Transfer plate onto the QiaSymphony extractor.
7. Perform automated DNA extraction.
8. Store DNA samples at -20°C until required.

APPENDIX C.4 STANDARD OPERATING PROCEDURE – DNA extraction (bacteria), APHA

Bacterial DNA extraction with the MagNA Pure LC (Roche Life Science) system

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SOP-version: 1

Introduction

This protocol describes an automated method to extract DNA from bacterial cells. The isolation procedure is based on magnetic-bead technology. The samples are lysed by incubation with a special buffer containing chaotropic salts and Proteinase K. Magnetic Glass Particles are added and the DNA is bound to their surfaces. Unbound substances are removed by several washing steps, then the purified DNA is eluted. The MagNA Pure LC automatically performs all isolation and purification steps such as addition of Lysis/Binding buffer and magnetic glass particles (MGPs), binding of DNA to the MGPs, washing steps, elution of the pure DNA, and transfer to a cooled storage cartridge.

Sample Material

Bacterial culture

Equipment

- Heating block capable of maintaining 65°C
- Microcentrifuge tubes
- Vortex Mixer
- MagNA Pure LC 2.0 Instrument for 8-32 samples per run

Reagents

- MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)

Literature

Application note:
MagNA Pure LC 2.0 Instrument has since been withdrawn. Related products can be found the Roche website *(https://lifescience.roche.com/en_gb/products/magna-pure-24-instrument.html*).

Procedure, DNA extraction (bacteria), APHA (bacterial DNA extraction with the MagNA Pure LC system)

1. Prepare 1.5 ml overnight cultures in LB broth from a single colony.
2. Spin to collect bacterial cells.
3. Wash cells with 500 µl of TE buffer.
4. Re-suspend cells in 100 µl of TE buffer.
5. Add 130 µl of Bacterial Lysis Buffer and 20 µl of Proteinase K.
6. Incubate at 65°C for 10 minutes.
7. Place 100 µl of sample mix in a sample cartridge.
8. Perform automated DNA extraction.
9. Store DNA samples at -20°C until required.

APPENDIX C.5 STANDARD OPERATING PROCEDURE – DNA extraction (bacteria), APHA

Preparation of cell boilates to extract DNA suitable for sequencing library preparation

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SOP-version: 1

Introduction

This protocol describes a rapid and inexpensive method to extract DNA from bacterial cells. This crude extract has proven to be suitable for the preparation of libraries for WGS of some bacteria such as Mycobacterium bovis.

Sample Material

Bacterial culture (single colony or pellet following centrifugation of broth culture)

Equipment

- Heating block capable of maintaining 95°C
- Microcentrifuge tubes
- Vortex Mixer

Reagents

- Molecular Biology Grade Water

General remarks

All bacterial cultures and boilates should be handled at the appropriate containment level. Once the inactivation of the bacteria by the heating process has been properly assessed and validated, boilates can be transferred to a lower containment level.

Literature

None

Procedure, DNA extraction (bacteria), APHA (preparation of cell boilates to extract DNA suitable for sequencing library preparation)

1. Dispense 100 µl of Molecular Biology Grade Water into Microcentrifuge tube.
2. Resuspend a single colony of bacteria (~3 mm^2) in the water.
3. Vortex for 15 s.
4. Briefly spin down to collect the liquid in the bottom of the tube.
5. Heat tube at 95°C for 10 minutes.
6. Spin down at 3500 rpm for 2 minutes to pellet cell debris.
7. Transfer supernatant to a fresh centrifuge tube (or well of a 96 well plate).
8. Store at -20°C until required.

APPENDIX C.6 STANDARD OPERATING PROCEDURE – Sequencing library preparation, APHA

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SOP-version: 1

Introduction

This protocol describes Nextera XT DNA Library Preparation (Illumina) with genomic DNA samples. The principle is that genomic DNA is randomly broken into small fragments (typically less than 1000 bp), before ligating sequencing primers to each end. Each of these ligated fragments are immobilized and clonally amplified, before denaturing. As complimentary bases are sequentially added to the single stranded template the sequence of nucleotides for each template is determined.

Sample Material

Genomic DNA samples

Equipment

- Heating block capable of maintaining 65°C
- 96-well microtiter plates
- Plate sealing film
- Centrifuge (capable of spinning 96-well plates between 100 x g and 1100 x g, room temperature)
- Thermocycler for 96-well plates
- Vortex mixer

Reagents

- Nextera XT library kit

Literature

Application note
http://emea.support.illumina.com/sequencing/sequencing_kits/nextera_xt_dna_kit/documentation.html

Procedure, Sequencing library preparation, APHA

1. Fragment DNA and then tags the DNA with adapter sequences in a single step.
2. Normalize gDNA.
3. Amplify libraries with 12 cycles of PCR.
4. Clean up libraries with AMPure XP beads.
5. Run 1 µl of undiluted library on an Agilent Technology 2100 Bioanalyzer to check libraries.
6. Normalize libraries to ensure equal representation.
7. Combine equal volumes of normalized libraries in a single tube for pooling libraries.

APPENDIX C.7 STANDARD OPERATING PROCEDURE – DNA sequencing using the MiSeq Instrument, APHA

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Institution: APHA, UK
SOP-version: 1

Introduction

This procedure describes the steps required for the preparation of pooled Nextera® XT libraries for loading onto an Illumina® MiSeq Sequencing Platform. The Illumina MiSeq® system combines proven sequencing by synthesis (SBS) technology with a revolutionary workflow that enables you to go from DNA to analyzed data in as little as 8 hours. The MiSeq integrates cluster generation, sequencing, and data analysis on a single instrument.

Sample Material

Pooled libraries

Equipment

- The Illumina MiSeq desktop sequencer
- The Illumina Sequence Analysis Viewer software
- Vortex mixer

Reagents

- MiSeq Sequencing Kit v2 300 cycles (Illumina)

Literature

User guide:
http://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-system-guide-15027617-01.pdf

Procedure, DNA sequencing using the MiSeq Instrument, APHA

1. Mix 2.5 µl of diluted NaOH and 7.5 µl of a library pool for 5 min at RT.
2. Add 940 µl of hybridisation buffer to the denatured library and 50 µl PhiX Control.
3. Load 600 µl of the library mix onto the reagent cartridge.
4. Run the MiSeq system.
5. Wash the instrument with PR2 buffer.
6. Inspect cluster density for the data output quality.

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