ABS

Brief Notes

Successful purification of DNA from PFGE agarose plugs for whole genome sequencing

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Abstract

Whole-genome sequencing (WGS) has replaced Pulsed-field gel electrophoresis (PFGE)-based bacterial genotyping as the reference genotyping method. We investigated the suitability of purified genomic DNA extracted from PFGE agarose plugs stored in a laboratory collection for WGS in cases where bacterial isolates are no longer available. Our study has shown that bacterial WGS can be successfully performed on DNA extracted from PFGE agarose plugs.

Keywords

DNA purification; PFGE plugs; genotyping; bacterial isolates, WGS

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Uspešno čiščenje DNK iz agaroznih čepkov PFGE za sekvenciranje celotnega genoma

Izvleček

Sekvenciranje celotnega genoma (WGS) je nadomestilo tipizacijo bakterij na osnovi gelske pulzne elektroforeze (PFGE) kot referenčne metode genotipizacije. Raziskali smo primernost genomske DNA, očiščene iz agaroznih čepkov PFGE, shranjenih v laboratorijski zbirki, za WGS v primerih, ko bakterijski izolati niso več na voljo. Naša študija je pokazala, da je bakterijski WGS mogoče uspešno izvesti na DNA, očiščeni iz agaroznih čepkov PFGE.

Ključne besede

čiščenje DNA, čepki PFGE, genotipizacija, bakterijski izolati, WGS

Introduction

For decades, pulsed-field gel electrophoresis (PFGE) genotyping has been central to bacterial genome fingerprinting analysis, providing invaluable insights into microbial epidemiology, transmission routes and outbreak investigations. However, the widespread adoption of whole-genome sequencing (WGS) has significantly diminished the relevance of classical genotyping methods such as PFGE, rendering them more or less obsolete. WGS has revolutionised the approach to genotyping bacterial isolates by providing comprehensive genetic information in a single analysis.

If the WGS genotyping includes isolates over an extended period of time, older isolates may no longer be available for various reasons (e.g., equipment failure planned removal of old frozen bacterial samples to make room for newer isolates in the laboratory freezers). In cases where older bacterial isolates are no longer available but have undergone PFGE genotyping, their DNA incorporated into PFGE agarose plugs may still be stored at 4°C.

The sample preparation procedure for both PFGE and WGS genotyping begins with a pure bacterial culture obtained by subculturing a single colony. PFGE agarose plugs contain total bacterial DNA, but different methods have to be used to analyse chromosomal and plasmid DNA separately using the PFGE genotyping method (Barton et al., 1995; Goering, 2010; Matushek et al., 1996). Classical molecular techniques (e.g. polymerase chain reaction, PCR) frequently involve the use of agarose gel electrophoresis to separate DNA fragments (one or more) by their molecular mass and visualise them as band(s) under ultraviolet light by staining the DNA with fluorescent dyes (Hamelin & Yelle, 1990). Various methods for extracting DNA from agarose gels have been described, although purification of DNA from the agarose gel is not always necessary for some PCR reactions (Gao et al., 2021). To the best of our knowledge, there is no publication on the use of preserved purified bacterial DNA from PFGE agarose plugs for WGS.

The aim of our study was to perform WGS directly from bacterial DNA incorporated into PFGE agarose plugs of a number of old *Acinetobacter baumannii* strains. The agarose plugs were between seven and eleven years old, and the original isolates were no longer available.

Materials and Methods

PFGE agarose plugs

Forty-five samples of *A. baumannii* genomic DNA stored in the PFGE agarose in a TE buffer at 4°C between seven and 11 years were used. Originally, the stored PFGE agarose plugs were prepared using the following procedure: Using a sterile swab, a standardised suspension of each *A. baumannii* isolate in a buffer (e.g., SE) was prepared. To ensure a sufficient amount of DNA in the agarose plug, the cell density was measured using a spectrophotometer (typically 0.5 and 1.5 A610) or a nephelometer (typically McFarland standard ≥3). A small volume of the cell suspension was mixed with an equal volume of low melting point agarose (typically at 50–55°C) and immediately pipetted into the plug moulds and allowed to solidify. The bacterial cells incorporated into the agarose plugs were lysed using cell lysis buffer with proteinase K (20 mg/ml) at 54 to 55°C with shaking (150 to 175 rpm), usually for 2 to 4 hours. After

lysis, the plugs were first washed with molecular biology grade water and then with TE buffer (5-6 times in total) to remove contaminant compounds. Plugs containing purified whole DNA were transferred to fresh TE buffer and stored at 4°C (Goering, 2010; Leber, 2016; Matushek et al., 1996).

Purification of DNA from stored PFGE agarose plugs for WGS

For DNA purification, different numbers of agarose plugs were used: protocol 1 used three agarose plugs, protocol 2 used five agarose plugs and protocol three was performed with three agarose plugs.

The DNeasy Blood and Tissue Kit (Qiagen) with slight modifications was used for DNA purification. Three to five agarose plugs per sample were placed in a sterile tube (Table 1); 200 µl AL buffer (Qiagen) was added and incubated at 56°C to melt the agarose gel. After the agarose was completely dissolved, purification was performed with ethanol precipitation and washing according to the manufacturer's instructions. The DNA was eluted in 200 µl (eluted twice with 100 µl, protocol 1) or in 100 µl (reloading of the eluat on the membrane, protocols 2 and 3) of buffer AE (Qiagen). After purification, the concentration and purity of eluted DNA were measured with Qubit dsDNA HS Assay Kit on Qubit 3.0 Fluorometer (Thermo Fischer Scientific) and Nanodrop 2000/2000c Spectrophotometer (Thermo Fischer Scientific), respectively.

WGS protocol

Before sequencing, the size of the fragments was evaluated using a high-sensitivity DNA kit (Agilent). Short-read sequencing genomic libraries were prepared using Nextera XT Library Preparation Kit (Illumina). Isolates were sequenced on the NextSeq 550 System (Illumina) using 2×149bp paired-end reads chemistry. Fastp v0.23.2 was used (Chen et al., 2018) to trim raw reads of adapter sequences and low-quality reads using the parameters »--correction –cut_right –length_required 30«. The quality of both raw and trimmed reads was assessed using FastQC v0.11.9 (Andrews, 2010). Assembly of trimmed reads into contigs was done with SPAdes v3.15.3 (Bankevich et al., 2012) using the default Kmer values and the "--careful" parameters. Quast v5.2.0 (Mikheenko et al., 2018) and BUSCO (Manni et al., 2021) were used for the quality assessment of the assemblies.

Reference genome

Reference strain for *A. baumannii* K09-14 (accession number - GCF_008632635.1) was used for WGS analysis (chromosome size 3.972.439 bp).

Quality control

After sequencing, quality control parameters were checked according to the EURGenRefLabCap protocol »Agreed common WGS-based genome analysis methods and standard protocols for national CCRE surveillance and integrated outbreak investigations« (EURGen-RefLabCap, 2022), including phred quality score Q30, average read size, number of contigs (<500), N50 (>15.000) and genome size.

Results

DNA was purified from 45 bacterial genomes incorporated into PFGE agarose plugs using three different protocols - protocol 1 (23 samples), protocol 2 (7 samples) and protocol 3 (15 samples). After whole-genome sequencing (WGS), the quality parameters were evaluated (Table 1). The number of agarose plugs has no influence on the purity of the eluted DNA. Therefore, the agarose is efficiently removed. When using a higher number of plugs and a correspondingly higher amount of DNA, as well as lower elution volume, the DNA concentration and some NGS quality parameters (average number of contigs and N50) are higher.

Discussion

WGS provides comprehensive bacterial genotyping information in a single analysis and is often required for molecular biology research and outbreak investigations. When performing genotyping over an extended period of time, bacterial isolates are sometimes no longer available. If PFGE genotyping has been performed in the past, the DNA agarose plugs of the isolates may still be stored. To determine whether bacterial DNA preserved in PFGE agarose plugs is suitable for WGS, we purified genomic DNA from the plugs using three different approaches varying the number of plugs per sample and the elution volumes. The quality control of the sequencing parameters was consistent for all three purification protocols, and the

Table 1. WGS quality control parameters follow the purification of DNA from PFGE agarose plugs according to three different protocols.

Tabela 1. Parametri kontrole kvalitete WGS po čiščenju DNK iz agaroznih čepkov glede na tri različne protokole.

Quality control parameters	Protocol 1	Protocol 2	Protocol 3
N of agarose plugs	3	5	3
Volume of elution (µl)	200	100	100
N of samples	23	7	15
Average concentration (ng/µl)	1,0	6,4	2,5
Average purity (A260/280)	1,8	1,9	1,9
Average fragment size (bp)	524	549	517
Reads passing filter (%)	92.9	93.9	92.9
Q30 after filtering (%)	~94	~94	~94
Average read size before filtering (bp)	133	134,72	135
Average read size after filtering (bp)	~128	~130	~130
Genome size compared to reference⁺ genome (bp)	99.6	99.2	100
Average number of contigs	277	297	275
N50 (bp)	95.950	106.394	76.738
BUSCO completeness (%)	99.7	99.7	99.7

Legend: ⁺ reference strain *Acinetobacter baumannii* K09-14 (accession number - GCF_008632635.1)

purified DNA was of high quality and suitable for effective genome assembly (Table 1).

In summary, all three protocols showed high efficiency in terms of read quality and genome assembly, with minor differences in read size, contig number and N50 values. Protocol 2 yielded the highest N50 value, while Protocol 3 achieved the full expected genome size (EURGen-RefLab-Cap, 2022). All protocols had excellent BUSCO completeness scores, indicating high-quality assemblies.

Conclusions

Our study has demonstrated that WGS can be successfully performed on bacterial genomic DNA preserved in PFGE agarose plugs, provided an appropriate purification protocol is used to remove inhibitory compounds. This finding expands the possibilities for studying older bacterial isolates, even when viable cultures are no longer available.

Author Contributions

KSS, TT and MP participated in the conception and design of the study; KSS performed DNA extraction, writing and revision of the manuscript; ACŠ performed sequencing and analysis of sequencing data, revision and editing of the manuscript; MP contributed bacterial genomes in PFGE agarose plugs, writing, revision and editing of the manuscript; TT contributed revision and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

Authors declare no competing interest.

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