A protocol for extraction and purification of high-quality and quantity bacterial DNA applicable for genome sequencing: a modified version of the Marmur procedure.

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Method Article

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Abstract

Here we present a modified version of the Marmur procedure \(Marmur, 1961) for extraction, isolation and purification of bacterial DNA. The protocol is effective in providing large amounts of high quality and high molecular weight genomic DNA from Gram-negative and Gram-positive bacteria, suitable for numerous downstream applications, including next-generation sequencing.

Introduction

Nucleic acids are the basis of the biology of every organism. Numerous methodologies have been developed to extract, study and analyse DNA, as it contains information providing insights into the lifestyles and intrinsic characteristics of any organism. In the field of microbiology, DNA has been used for decades as material for studying microorganisms at the highest level of genetic resolution, i.e., at the nucleotide level. For instance, genomic DNA-DNA similarity continues to be a genotypic “gold standard” methodology for confirming whether two bacterial strains belong to the same species. Additionally, the development of next-generation sequencing technologies has enabled the rapid and cost-effective determinations of entire genome sequences of microorganisms, revolutionizing microbiological research. However, the sequencing technologies require that the DNA is extracted in a form that meets particular quantity and quality requirements. In this protocol, we present a modified version of the Marmur procedure \(Marmur, 1961), a robust and straightforward method for successful extraction, isolation and purification of total DNA from most Gram-negative and Gram-positive bacteria. The protocol provides large amounts of high-quality DNA, sufficient and suitable for most downstream applications, including DNA-DNA hybridization, DNA fragment profiling, as well as for the most widely-used next-generation sequencing technologies, i.e., Illumina \(Illumina, Inc.), Ion Torrent \(Thermo Fisher Scientific, Inc.), PacBio \(Pacific Biosciences of California, Inc.) and MinION \(Oxford Nanopore Technologies). Bacterial DNA, using this extraction protocol, has been offered as a service at the Culture Collection University of Gothenburg \(CCUG) during the last 10 years.

Reagents

• EDTA-Saline \[C10H16N2O8 0.01 M and NaCl 0.15 M; pH = 8.0 x 800 µl]. • Lysozyme \[300 mg/ml x 10 µl]. • Mutanolysin \[1,000 U/ml x 10 µl]. • RNase A, DNase-free \[100 mg/ml x 7.0 µl]. • SDS \(Sodium Dodecyl Sulfate) \[NaC12H25SO4 25% \(w/v) x 80 µl]. • Sodium chloride \[NaCl 5 M x 250 µl]. • Chloroform:isoamyl alcohol \[CHCl3:C5H12O \(24:1) x 2.0 ml]. • Sodium acetate \[C2H9NaO5 3 M x 90 µl]. • Absolute isopropanol \[C3H8O x 600 µl]. • ‘Low’-TE \[Tris \(C4H11NO3) 1 mM and EDTA \(C10H16N2O8) 0.1 mM; pH = 7.0 – 8.0 x 100 µl].

Equipment

• Sterile 1.5 ml microcentrifuge tubes. • Sterile 2.0 ml microcentrifuge tubes. • Vortex. • Heating block or water bath. • Microcentrifuge. • Orbital shaker. • Glass rods.

Procedure

1. Suspend a fully-loaded inoculating loop of bacterial biomass in a 2.0 ml tube with 800 µl of EDTA-Saline; vortex at maximum speed to mix thoroughly. 2. Add 10 µl of lysozyme. 3. If Gram-positive bacteria, add 10 µl of mutanolysin. 4. Mix by vortexing for a few seconds to suspend the biomass. 5. Add 7 µl of RNase A. 6. Incubate at 37°C for at least 15 – 45 min; vortex every 15 min. 7. Add 80 µl of SDS. 8. Vortex at maximum speed for a few seconds; the viscosity is increased. 9. Incubate at 65°C for 10 min; vortex once during this time. 10. Spin down briefly and add 250 µl of sodium chloride 5 M. 11. Vortex at maximum speed for a few seconds and spin down briefly. 12. Add 400 µl of chloroform:isoamyl alcohol. 13. Vortex at maximum speed for a few seconds; shake for 15 min at 1,400 rpm on an orbital shaker. 14. Centrifuge at > 13,000 x g for 15 min. 15. Transfer the top-layer to a new tube \(2 ml); avoid the protein layer. 16. Add 400 µl of chloroform:isoamyl alcohol again; shake vigorously by hand and centrifuge again. 17. Repeat step 16 until there is no protein layer. 18. To 1 ml of solution, add 90 µl of sodium acetate 3 M. 19. To 1 ml of solution, add 600 µl of cold isopropanol \(4°C). 20. Precipitate the DNA by inverting the tube several times by hand, when threads of DNA are seen, shake the tube harder to clump the DNA threads. 21. Spool the DNA using a glass rod and leave it to dry completely, for at least 5 min at room temperature. 22. Alternatively, for steps 20 – 21, if there is no formation of threads, spin down at > 13,000 x g for 10 min, carefully discard the supernatant and let the DNA pellet dry completely at room temperature. 23. Suspend the spooled or pelleted DNA in 100 µl of ‘low’-TE. 24. Incubate the resuspended DNA over-night at 4ºC for complete resuspension.

Timing

The entire protocol takes approximately four - five hours.

Troubleshooting

Low amounts of DNA might be obtained for some taxa of bacteria that are highly-resistant to enzymatic and detergent lysis \(e.g., Mycobacterium spp.). Therefore, in some cases mechanical lysis \(e.g., bead beating) might be necessary.

Anticipated Results

The quantity and quality of the extracted and purified DNA will vary depending on the amount of starting material and the nature of each particular bacterium. In most cases, when eluted in 100 µl of ‘low’-TE, concentrations \(Qubit BR measurement) of several hundreds of nanograms per microliter \(i.e., tens of micrograms in total) and absorbance ratios of 1.8 – 2.0 \(260/280 nm) and 2.0 – 2.2 \(260/230 nm) can be expected to be recovered. DNA fragments larger than 60 kb can also be expected for most bacteria. Once dissolved in ‘low’-TE, the DNA is ready to be used for most downstream applications \(e.g., Oxford Nanopore sequencing). However, further purification protocols may be applied in cases where downstream applications are very sensitive to contaminants and impurities \(e.g., PacBio sequencing).

But, this modified protocol provides a standard, relatively simple protocol for obtaining the DNA for most cases of whole-genome sequence determinations.

References

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