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Genomic DNA purification protocol for whole genome sequencing from unknown bacteria

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We use this protocol and it's working



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Abstract

This protocol outlines the steps for genomic DNA extraction and purification from bacterial samples, including an enhanced bead-beating method for improved DNA yield and quality. The procedure incorporates bacterial growth in nutrient broth, lysis with a heat block, DNA precipitation, and quality control using spectrophotometry. Additionally, bead-beating is employed to mechanically disrupt bacterial lysosomes in challenging samples.

The expected results are high-quality DNA samples with a nucleic acid concentration ≥ 200 ng and a purity ratio (A260/A280) between 1.8 and 2.0, suitable for downstream genomic applications. The inclusion of the additional bead-beating protocol enhances DNA recovery from difficult samples. However, it is not always necessary; in a previous study this step was only needed for two out of eight unknown bacterial DNA samples.

Materials

General Laboratory Equipment

Autoclave—For sterilising tools and reagents.

Incubator—Set to 36°C for bacterial sample growth.

Centrifuge—capable of operating at 5000 × g and 10,000 rpm (~9400 × g).

NanoDrop Spectrophotometer – For DNA quantification and purity analysis.

Magnetic Shaker – With multi-Eppendorf tube attachment for bead-beating.

Heat Block—capable of maintaining a temperature of 65°C.

Vortex Mixer – For resuspending and mixing solutions.

Tweezers—at least two pairs, autoclaved, for handling glass beads.

Pipettes—adjustable volumes ranging from 10 µl to 1000 µl.

Pipette Tips—Sterile and disposable.

Consumables

1. Eppendorf Tubes—Sterile, 1.5 ml capacity.
2. Glass Beads—Sterilised by autoclaving, 2 per sample.
3. Oxoid Nutrient Broth—prepared according to the manufacturer's guidelines (13 g in 1 L of distilled water).
4. Deionised Water—Sterile, used for preparing solutions.
5. TE Buffer—For resuspending frozen samples.
6. Lysis Solution – As specified in the NovoGene protocol.
7. Precipitation Solution—Consisting of:
Sterile deionised water (720 µl per sample).
10-Fold Concentrated Precipitation Solution (80 µl per sample).
8. NaCl Solution—For dissolving the DNA pellet (100 µl per sample).
9. RNase A—stock solution (#EN0531) to achieve a final concentration of 0.2 mg/ml.
10. Molecular Grade Water – For NanoDrop calibration.

Personal Protective Equipment (PPE)

1. Gloves—disposable and powder-free.
2. Lab Coat—To prevent contamination.
3. Safety Goggles – To protect against accidental splashes.

Additional Notes

- Ensure all consumables and tools are sterilised or prepared under aseptic conditions to minimise contamination.
- Maintain a clean and organised workspace throughout the procedure.

Genomic DNA purification protocol for whole genome sequencing from unknown bacteria. Supplied by NovoGene (Termofisher, 2012).

1 Preparation of samples for DNA processing

Before conducting the genomic DNA purification, the samples were prepared by taking a sample of bacteria (from the isolated sample plates) and growing it in 0.5 ml of autoclaved Oxoid brand nutrient broth (following the manufacturer's guidelines of 13 g/1 L of distilled water) in a (previously autoclaved) Eppendorf vial. To grow these samples, they were placed in a 36°C incubator for 48 hours to reach the terminal growth point.

After incubation and before starting the genomic DNA purification protocol, the samples were centrifuged for 10 minutes at 5000 x g. If the sample had been frozen before this step, then it was resuspended in 200 µl of TE buffer and then centrifuged.

2 Genomic DNA purification and extraction protocol

- 2.1 **Step 1.** Combine 200 µl of the sample with 400 µl of the lysis solution and let it incubate in a heat block at 65°C for 10 minutes. Take each sample out and gently invert it every 1-2 minutes until it becomes clear. This is to begin the breakdown of the DNA. If a frozen sample is being used, then the lysis solution needs to be added as soon as the samples are out of the freezer, before thawing, and then follow the sample heat block incubation procedure for a fresh sample, but adding five minutes onto the total incubation time.
- 2.2 **Step 2.** Mix 200 µl of the sample with 400 µl of the lysis solution and allow it to incubate at 65°C for 5 minutes. If using a frozen sample, add the lysis solution before thawing. Then, let the sample incubate at 65°C for 10 minutes, gently inverting the tube from time to time.
- 2.3 **Step 3.** Prepare the precipitation solution by combining 720 µl of sterile deionized water with 80 µl of the supplied 10-fold concentrated precipitation solution.
- 2.4 **Step 4.** Move the upper aqueous phase, which holds the DNA, to a fresh tube. Add 800 µl of freshly prepared precipitation solution. Gently mix by inverting the Eppendorf tube several times at room temperature for 1-2 minutes. Then, centrifuge at 10,000 rpm (~9400 x g) for 2 minutes.
- 2.5 **Step 5.** Carefully decant the supernatant, ensuring not to overdry the pellet. Dissolve the DNA pellet in 100 µl of NaCl solution by gently vortexing, ensuring complete dissolution of the pellet.
- 2.6 **Step 6.** After completing step 5, introduce RNase A (#EN0531) to achieve a final concentration of 0.2 mg/ml. Ensure thorough mixing by vortexing for approximately 30

seconds at 1800 rpm, followed by a 10-minute incubation at 37°C.

- 2.7 **Step 7.** An aliquot of the sample is then removed from the main stock and analysed spectrophotometrically using a NanoDrop system. It is important to ensure that the Nanodrop reading plate is cleaned before use and calibrated using grade 1 molecular water; the DNA samples must then have a minimum quantity of 1.7 and a ratio reading similar to that of the quantity.

Bead beating methodology for enhanced extraction

- 3 After the previous study, eight initial samples were sent to NoVo Gene, two came back as failing to meet the parameters set by their quality control check of needing to have ≥ 200 ng of nucleic acid, and a purity (A260/A280) of 1.8-2.0 with no degradation and no contamination (Table 6), despite meeting the requirements when tested using the Nanodrop. The previous study used a variety of techniques to extract as much high-quality DNA as possible from samples 7 and 5, which had previously failed quality control, to address this problem and get a complete set of data. Following an assessment of the literature, the Fujimoto et al. (2004) technique of bead-beating was applied.
- 3.1 **Step 1.** All tools and reagents, including glass beads, tweezers, and Eppendorf tubes, must be autoclaved before use. This sterilisation step is critical for maintaining the integrity of the extracted DNA and avoiding external contamination during the procedure (Fujimoto et al., 2004). Additionally, prepare the lysis solution and ensure the magnetic shaker is fitted with a multi-Eppendorf attachment.
- 3.2 **Step 2.** Add 200 μ l of the sample to an autoclaved Eppendorf tube. To this, add 400 μ l of the prepared lysis solution. The lysis solution facilitates the breakdown of cellular structures, aiding the release of DNA from the sample. Using autoclaved tweezers, carefully place two sterilized glass beads into the Eppendorf tube containing the sample (Broothaerts et al., 2000). Ensure that the beads are handled carefully to avoid contamination.
- 3.3 **Step 3.** Secure the Eppendorf tubes containing the samples onto the magnetic shaker with the multi-Eppendorf attachment. Set the shaker to operate at 2000 revolutions per minute (rpm) and agitate the samples for 10 minutes. The collisions between the glass beads and the sample cause mechanical disruption of the lysosomes, effectively releasing DNA and other cellular contents (Bolano et al., 2001; Bielawski et al., 2001).
- 3.4 **Step 4.** Once the bead-beating process is complete, use a second pair of autoclaved tweezers to remove the glass beads from the Eppendorf tube. The use of a separate pair of tweezers for this step ensures that the risk of cross-contamination is minimized. Proper disposal or cleaning of the beads after extraction is necessary to maintain laboratory safety and hygiene
- 3.5 **Step 5.** After the bead-beating step, incubate the sample for an extended period to ensure complete DNA release. For this protocol, the incubation time is increased to 20



minutes, compared to the typical 10 minutes used in standard procedures. This adjustment accommodates samples requiring additional time for the lysosomes to fully break down and release DNA (Fujimoto et al., 2004).

- 3.6 **Step 6.** Following the incubation step, continue with the standard protocol for DNA extraction as given in Section 2 (See above).

Protocol references

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Acknowledgements

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