Molecular Microbiology 2024

Protocol 1

Genomic DNA Extraction

This protocol describes how to extract abundant, high-molecular weight genomic DNA from bacterial cultures for sequencing on the Oxford Nanopore Technologies platform.

Materials

- 15 mL liquid bacterial culture grown to approx. optical density 2.0 in a 50 mL Falcon tube
- Qiagen Buffer B1 with RNase A solution added (3.5 mL)
- Qiagen Buffer B2 (1.2 mL)
- Qiagen Buffer QBT (4 mL)
- Qiagen Buffer QC (30 mL) (Pre-warmed to 50°C)
- Qiagen Buffer QF (15 mL)
- 10 mM Tris-HCl, pH 8.5 (100 µL)
- Isopropanol (10.5 mL)
- 70% ethanol (4 mL)
- RNase A stock solution (22 µL)
- Lysozyme stock solution (100 mg/mL) (300 µL)
- Proteinase K stock solution (500 µL)

Equipment

- Genomic-tip 500/G
- Single-use serological pipettes (20 mL)
- Electric serological pipette controller
- 50 mL Falcon tubes
- 1000 µL micropipettor
- 1000 µL pipette tips
- Heating block with 50 mL falcon tube holder set to 37°C and 50°C

Procedure

1. Centrifuge 15-20 mL of liquid bacterial culture at 5000 x g for 10 minutes. Discard the supernatant into the waste beaker and retain the pellet, ensuring that all liquid is completely removed. The volume, centrifuge speed, and centrifuge time may need to be adjusted for certain cultures, speak to your instructor for advice.

2. Resuspend the bacterial pellet with 11 mL of Buffer B1 by vortexing at top speed.

3. Add 300 µL of lysozyme stock solution and 500 µL of Proteinase K solution. Vortex briefly to mix and incubate in a heating block set to 37°C for at least 30 minutes. *Lysozyme breaks down the cell wall, while detergents in buffer B1 ensure complete lysis of the bacteria.*

4. Add 4 mL of Buffer B2, and mix by inverting the tube several times or by vortexing for a few seconds. Incubate in a heating block set to 50°C for 30 min. *Buffer B2 denatures proteins such as nucleases and DNA-binding proteins. The excess QIAGEN Protease digests the denatured proteins into smaller fragments, facilitating efficient removal during purification. The sample must be as clear as possible following this step.*

5. Equilibrate the the Genomic-tip 100/G by transferring 10 mL of Buffer QBT to the tip and allowing it to drain completely into a 50 mL falcon tube by gravity flow. *The flow of buffer will stop when the meniscus reaches the upper frit. The frit prevents the QIAGEN Genomic-tip from running dry, allowing it to be left unattended. Do not force out the remaining buffer, as this will necessitate restarting the flow with a syringe and adapter.*

6. Vortex the bacterial lysate for 10 s at maximum speed, then transfer it to the Genomic-tip. Allow it to enter the resin by gravity flow and collect the flow-through in a 50 mL falcon tube. *Genomic DNA will bind to the resin.*

7. Wash the Genomic-tip twice with 15 mL Buffer QC, collecting the flow-through in an empty 50 mL falcon tube. *Buffer QC cleans the DNA sample, removing impurities.*

8. Place the Qiagen Genomic-tip over a new, clean 50 mL Falcon tube. Elute the genomic DNA with 15 mL buffer QF, allowing the flow-through to flow into the tube. *Buffer QF will release the DNA from the resin.*

9. Add 10.5 mL room-temperature isopropanol to the eluted DNA solution, a milky precipitate should appear. Mix the precipitate by inverting the tube several times and centrifuge immediately at 10,000 x g for 15 minutes at 4°C. Carefully remove the supernatant. *The precipitated DNA will form a solid, glassy pellet at the base of the tube which may be difficult to see.*

10. Add 4 mL of cold 70% ethanol. Vortex briefly and centrifuge at 10000 x g for 10 min. Carefully remove the supernatant without disturbing the pellet. Air-dry for 5-10 minutes and resuspend gently in 100 µL of 10 mM Tris-HCl buffer, pH 8.5. Dissolve the DNA overnight at room temperature. *DNA will in the buffer, ready for sequencing library preparation.*