Low-cost High-throughput DNA Extraction Protocol

Foster-Nyarko *et al* 2021

Overview

This protocol describes a cost-effective, high-throughput method for DNA extraction from Gram-negative bacterial cultures in a 96-well format, using Solid-Phase Reversible Immobilization (SPRI) bead-based clean-ups for purification.

Materials and Equipment

Reagents & Buffers

- MacConkey agar plates
- Luria Bertani (LB) broth
- TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0)
- Lysozyme (10 mg/ml)
- RNAse A (10 mg/ml)
- 10% Sodium dodecyl sulfate (SDS) solution
- Proteinase K (20 mg/ml)
- SPRI magnetic beads (Beckman Coulter Inc., Brea, CA, USA)
- 80% ethanol (freshly prepared)
- 10 mM Tris-Cl, pH 8.0

Equipment

- 96-well deep-well plates
- Adhesive plate seals
- ThermoMixer (with adjustable speed & temperature)
- Centrifuge with 96-well plate rotor (3500 rpm capability)
- Magnetic 96-well plate holder
- 1000 µl & 200 µl multichannel pipettes
- Pipette tips (filtered, sterile, low-retention)
- Foil or blue roll for covering plates
- Lo-bind 96-well PCR plates
- -20°C freezer for DNA storage

Protocol

Day 1

- Plate out 96 samples on MacConkey agar, **being careful to change streak for single colonies**.
- Incubate overnight at 37°C overnight.

Day 2

Pick a single colony from each sub-culture to 1ml of LB broth and incubate overnight at 37°C overnight **in a deep-well plate. Do not seal the plate tight** (simply cover with a foil or blue roll).

Day 3

1. Following overnight incubation, spin the broth cultures at **3500rpm for 2 min** in a large centrifuge to pellet the bacteria growth.
2. Remove the culture supernatant by placing a clean 1000 µl tip box over the plate and rapidly inverting the plate upside down.
3. Next, tap the plate gently to break surface tension in some wells.
4. Pulse-spin the plate in a large centrifuge to sediment the bacterial growth.

DNA extraction

1. Prepare the lysing buffer using:
    1. 10 ml of TE buffer
    2. 100 µl of lysozyme
    3. 10µl of RNAse A
2. To each well, add 100 µl of the lysing buffer and resuspend the sediment by careful pipetting.
3. Seal firmly using an adhesive seal and place on a thermomixer **at 37°C and 1600 rpm for 25 min**.
4. While the plate is incubating, prepare a fresh lysing additive as follows:
   a. 528 µl of TE buffer
   b. 600 µl 10% of SDS buffer
   c. 60 µl of Proteinase K
   d. 12µl of RNAse A
5. Remove the plate from the thermomixer and add 10 µl of the lysing additive to each well.
6. Firmly seal the plate with a new adhesive tape and place on a thermomixer, this time at **- 65°C 1600 rpm for 15 min**.
7. Spin the plate briefly in a large centrifuge

Solid-Phase Reversible Immobilisation (SPRI) clean-ups

1. To each well, add 50 µl of SPRI magnetic beads (Becter Coulter Inc., Brea, CA, USA) and carefully mix by pipetting.
2. Incubate the SPRI beads-extractions mixture **at room temperature for 5 min**.
3. Place the plate on a magnetic 96-well plate holder for 2-5 min (**Keep the plate on the magnetic apparatus till step 8**).
4. Remove the liquid and discard.

5. Add 100 µl of **freshly prepared 80 % ethanol** added to all wells, carefully running the liquid over the magnetic beads.
6. Remove the ethanol and discard.
7. Repeat this washing step two more times.
8. Remove the final ethanol and allow the plate to dry for 2 min.
9. Take the plate off the magnetic plate holder, and to each well, add 50 of 10mM Tris-Cl, mixing by pipetting to resuspend all the magnetic beads.
10. Incubate at room temperature for 5 min.
11. Place the plate back on the magnetic 96-well plate holder and leave to stand for 2 min.
12. Finally, transfer the 50 µl genomic extraction from each well into a new lo-bind 96 well PCR plate.
13. Store at -20 °C until further analysis.

Reference

Foster-Nyarko E, Nabil-Fareed A, Anuradha R et al. *Genomic diversity of Escherichia coli isolates from non-human primates in the Gambia*. *Microb Genom* 2023; **9**: mgen000428.

Notes & Troubleshooting

- Ensure pipette tips are low-retention to minimise DNA loss.
- Avoid drying the beads for too long, as overdrying can reduce DNA yield.
- Ensure all reagents are prepared freshly, particularly SDS and ethanol washes.
- If the DNA yield is low, confirm pelleting efficiency during centrifugation steps.