

Modified protocol Wizard® Genomic DNA Purification Kit from Promega

Produce overnight culture of isolated strains in 10mL
Pellet 2mL by centrifugation for 5 min at 13,000 rpm¹
Resuspend the pellet in 540 µL of EDTA at 50 mM, pH 8.5 and 60 µL of lysozyme at 10 mg/mL
Incubate 1 h 30 at 37°C
Centrifuge for 3 min at 13,000 rpm
DISCARD the supernatant
Resuspend the pellet in 600 µL of 'Nucleic Acid Solution' (from kit) and mix
Heat for 5 min at 80°C (allow cooling off until next step)
Add 3 µL of RNase (from kit)
Incubate 1 h 00 at 37°C
Add 200 µL of 'Protein Precipitation Solution' (from kit)
Vortex and place on ice for 5 min
Centrifuge for 3 min at 13,000 rpm
TRANSFER supernatant to a 1.5 mL tube
Add 600 µL isopropanol at ambient temperature
Mix by inverting the tube
Centrifuge for 3 min at 13,000 rpm
DISCARD the supernatant²
Add 600 µL of 70% ethanol at ambient temperature
Centrifuge for 3 min at 13,000 rpm³
DISCARD ethanol
Dry pellet at 37°C
Resuspend gDNA in 50-100 µL of water or elution buffer (from kit)

Notes :

¹Pellet more than one tube per culture if more DNA is necessary. These can be eluted in smaller volumes and pooled at the elution step. Extracting 1 to 4 pellets may be necessary depending on the bacterial strain to achieve desired DNA amounts.

²The DNA pellet may not always be visible. The ethanol wash usually reveals it a bit more

³Centrifuge longer and faster if the white pellet remains loose in order to facilitate collecting clean supernatant.

QC

Assess DNA integrity on 2% gel electrophoresis (1-2 µL DNA + 4 µL loading DYE).

Measure the final concentration on Qubit (broad range kit).

Pool, dilute (with H₂O) and/or concentrate (with magnetic beads) extracts to necessary DNA amount & concentration.