Thomas Scientific

PALL Laboratory

Protocol

Standard Reagent Protocol for Genomic DNA Isolation From Cultured *Escherichia coli* Cells Using Pall Nucleic Acid Binding Nanosep® Device

1. Consumables and Reagents

Table 1

Consumables for gDNA Purification (nuclease-free consumables are recommended)

Supplier	Product Description	Part Number
Pall Laboratory	Nucleic Acid Binding (NAB) Nanosep Centrifugal Device	ODNABC33, ODNABC34
VWR	Ethanol (not denatured)	71001-866
VWR	Spectrophotometer Cuvettes ~700 µL (260/280 nm)	47743-840
VWR	Tubes 15 mL (RNase-DNase free)	89401-574
VWR	Tubes 50 mL (RNase-DNase free)	89401-572
VWR	Microcentrifuge Tubes 1.5 mL (RNase-DNase free)	76005-210

Table 2

Reagents for gDNA Purification (nuclease-free reagents are recommended)

Supplier	Product Description	Part Number
Qiagen	Buffer AL (264 mL)	19075
Qiagen	Buffer ATL (200 mL)	19076
Qiagen	Buffer AW1 (242 mL)	19081
Qiagen	Buffer AW2 (324 mL)	19072
Qiagen	Proteinase K	19131
Qiagen	RNase A (100 mg/mL)	19101
Qiagen	Nuclease-free Water	129115
VWR	Tris Buffer pH 7.0 (1M)	89500-584

2. Instruments

- Microcentrifuge
- Spectrophotometer
- Vortex
- Water bath or heating block

3. Important Points Before Starting

- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution. Before each use, mix Buffer AW1 by inverting several times.
- All centrifugation steps are performed at room temperature at 10,000 – 14,000 x g.
- Use only the collection tubes provided in the NAB Nanosep device box. There is enough to complete the below process in full.
- All buffers should be allowed to equilibrate to room temperature before use.
- Briefly centrifugate the tubes after vortexing to remove drops from inside the lid.
- Increase volumes of lysis buffers and reagents proportionally for samples >200 µL.
- Ensure that any precipitate formed during any of the below steps is not lost.
- Centrifugation at full speed will not affect yield or purity of the DNA. If, after centrifugation the sample has not completely passed through the membrane, centrifuge again until all the solution has passed through.

4. Protocol

1. Centrifuge the appropriate volume of an overnight *E. coli* culture during 10 minutes at 3000 x g. Avoid using cell concentrations greater than 5 x 10^7 for *E. coli*.
2. Resuspend the pellet in 180 µL of Buffer ATL.
3. Add 20 µL of proteinase K to the 180 µL of sample in Buffer ATL.
4. Vortex to mix and then incubate at 56 °C to complete lysis.
5. Vortex occasionally during incubation to homogenise the lysate, or place in a shaking water bath/incubator.
   a. *Lysis is usually complete within 1 – 3 Hrs for E. coli cultures depending on the cell quantity. It is possible to complete lysis overnight. This should not adversely affect the preparation.*
6. Centrifuge the tube for 5 seconds to remove drops from the inside of the lid.
7. *Optional RNase digestion steps: (If you do not wish to perform RNase digestion, move on to step 8)*
   a. Add 4 µL RNase A (100 mg/mL) to the 200 µL sample of Proteinase K in Buffer ATL. Pulse vortex to mix for 15 seconds.
   b. Incubate for 2 minutes at room temperature.
   c. Centrifuge for 5 seconds to remove drops from the inside of the lid.
8. Add 200 µL Buffer AL to the sample and pule vortex for 15 seconds to mix.
9. Incubate at 70 °C for 10 minutes.
10. Centrifuge again for 5 seconds to remove drops from the lid.
11. Add 200 µL of 100% non-denatured Ethanol to the sample and pulse vortex for 15 seconds to mix. Use of alcohols other than ethanol will decrease yields.
12. Centrifuge again for 5 seconds to remove drops from the lid.
    (Be thorough with the mixing at each stage to obtain a homogenous solution. Any precipitate formed in the above steps will not adversely affect the preparation).
13. Apply the mixture in Ethanol, including any precipitate to the NAB Nanosep device insert inside a receiver tube.

14. Close the cap and centrifuge for 60 seconds at 10,000 – 14,000 x g.
15. Discard the flow-through and re-use the receiver tube for the next step.
16. Open the NAB Nanosep device and add 500 µL Buffer AW1.
17. Close the cap and centrifuge for 60 seconds at 10,000 – 14,000 x g.
18. Discard the collection tube and flow-through and transfer the NAB Nanosep device insert into a clean collection tube (provided).
19. Add 500 µL Buffer AW2 to the column insert.
20. Close the cap and centrifuge for 3 minutes at 10,000 – 14,000 x g.
21. Discard the flow-through and re-use the receiver tube for the next step.
22. Centrifuge for 60 seconds at 10,000 – 14,000 x g to remove possible Buffer AW2 carry-over.
23. Discard the receiver tube with the flow-through and transfer the column insert to the final, clean collection tube (provided).
24. To elute the DNA, add 100 µL of DNase-free distilled water (or Buffer AE) and incubate at room temperature for 5 minutes.
25. Centrifuge for 60 seconds at 10,000 – 14,000 x g.
26. *Optional: For maximum DNA yield, repeat the above elution steps with a further 100 µL of DNase-free distilled water.*

Storage of DNA

If you intend to store the purified DNA, we recommend elution with Buffer AE from Qiagen and storage at -30 °C to -15 °C. As high pH or EDTA can affect sensitive downstream applications, water can be used for elution. However, ensure that the pH of the water is a minimum of 7.0 as acid hydrolysis will occur to DNA stored in water which will result in degradation.

Quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see details below). For small quantities of DNA however, it can be difficult to determine these amounts photometrically. Smaller quantities of DNA can be accurately quantified using fluorometric quantification.

Spectrophotometric quantification of DNA

A_{260} readings should be greater than 0.10 and lower than 1.0 to ensure significance. An absorbance reading of 1.0 at 260 nm corresponds to 50 µg of DNA per mL. This is only valid for measurements at neutral pH however. As a result, if it is necessary to dilute the DNA sample, ensure that the dilution buffer is of neutral pH.

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