Protocol for Plasmid DNA Purification from Cultured *Escherichia coli* Cells Using Pall Nucleic Acid Binding Nanosep® Centrifugal Device

1. Consumables and Reagents

Table 1

Consumables for pDNA Purification

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 ~100 µL (260/280 nm)	47743-840
VWR	Tubes 15 mL (RNase-DNase free)	89401-574
VWR	Microcentrifuge Tubes 1.5 mL (RNase-DNase free)	76005-210

Table 2

Reagents for pDNA Purification

Supplier	Product Description	Part Number
Qiagen	Buffer PE	19065
Qiagen	Buffer P1	19051
Qiagen	Buffer P2	19052
Qiagen	Buffer N3	19064
Qiagen	Buffer PB	19066
VWR	Tris Buffer pH 7.0 (1M)	89500-584
Qiagen	RNase A (100 mg/mL)	19101
Qiagen	Nuclease-free Water	129115

2. Instruments

- Microcentrifuge
- Spectrophotometer
- Vortex

3. Important Points Before Starting

- Add the provided RNase A solution to Buffer P1 prior to use to a final concentration of 100 µg/mL. Mix and store at 2 – 8 °C
- Add Ethanol (not denatured) to buffer PE prior to use. See bottle label for instructions.
- Check Buffers P2 and N3 before use. If precipitate is observed, heat to 37 °C and gently agitate – Do not vigorously shake Buffer P2 bottle.
- Ensure that the lid of Buffer P2 is closed immediately after use to avoid acidification of buffer from CO_2 in the air.
- All centrifugation steps are performed at room temperature at 10,000 – 14,000 x g.
- For each NAB Nanosep device insert there are three receiver tubes. This is enough to complete the below process. Use only the receiver tubes provided with the NAB Nanosep device.
- All buffers should be allowed to equilibrate to room temperature before use.
- Briefly centrifuge tubes after vortexing to remove drops from inside the lid.
- 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

This protocol is designed for purification of plasmid DNA from 1 – 5 mL (overnight) cultures of E. coli in rich-medium

1. Harvest the bacterial cells by centrifugation at 3,000 x g in a table-top microcentrifuge for 10 minutes at room temperature.
2. Discard the supernatant and resuspend pelleted bacterial cells in 250 µL of Buffer P1 (with added RNase A) without creating cell clumps. Transfer to a microcentrifuge tube.
3. Add 250 µL Buffer P2 and invert the tube 4 – 6 times to mix. Avoid vortexing as this can shear the DNA. If necessary, continue inverting until the solution becomes viscous and slightly clear. Do not allow the lysis to continue for more than 5 minutes.
4. Add 350 µL Buffer N3 and mix immediately by inverting 4-6 times. Avoid localized precipitation by mixing thoroughly, immediately after the addition of Buffer N3.
5. Centrifuge for 5 – 10 minutes at 10,000 – 14,000 x g.
6. Transfer 500 µL of the supernatant to the NAB Nanosep device at a time and centrifuge for 45-60 seconds at 10,000 – 14,000 x g discarding the flow through. Retain the receiver tube and repeat this step with the remaining supernatant.
7. Add 500 µL of Buffer PB to wash the NAB Nanosep device, centrifuging for 60 seconds at 10,000 – 14,000 x g. Discard the flow-through and retain the receiver tube for the next step.
8. Perform a second washing step by adding 500 µL of Buffer PE and centrifuging for 60 seconds at 10,000 – 14,000 x g.
9. Discard the flow-through and centrifuge again at full speed for an additional 1 minute to remove any residual buffer.

10. Transfer the NAB Nanosep device to a clean receiver tube (provided) and add 50 µL of nuclease-free water (or Buffer EB) to the center of the membrane of each NAB Nanosep device. Leave to stand for 1 minute.
11. Centrifuge for 60 seconds at 10,000 – 14,000 x g and recover the purified plasmid DNA in the receiver tube.

Storage of DNA

Purified plasmid DNA can be stored in nuclease-free water at -20 °C for several months.

Quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see details below). For lower concentrations 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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