PALL Laboratory

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Protocol

Protocol for Genomic DNA Purification from Cultured Yeast Cells Using Pall Nucleic Acid Binding Nanosep® Centrifugal Device

1. Consumables and Reagents

Table 1

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

Supplier	Product Description	VWR Cat. No.
Pall Laboratory	Nucleic Acid Binding (NAB) Nanosep Centrifugal Device	76360-454, 76360-456
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	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	VWR Cat. No.
Various	Lysis Buffer	N/A
Various	Cell Debris Removal Buffer	N/A
Various	Wash Buffer 1	N/A
Various	Wash Buffer 2	N/A
Various	RNase A (100 mg/mL)	N/A
Various	Nuclease-free water	N/A
Various	Proteinase K	N/A
VWR	Zymolase	IC320921
VWR	Tris Buffer pH 7.0 (1 M)	89500-584
VWR	DTT (25 g)	97063-758
VWR	D-(+)-Sorbitol	97062-202
VWR	EDTA, pH 8 (0.5 M)	BDH7830-1

2. Instruments

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

3. Important Points Before Starting

- For the Lysis Buffer, combine 1 M Sorbitol, 0.1 M EDTA, pH 7.4 – 8 and before use, add 20 mM DTT + Zymolase (refer to supplier information for quantity to use).
- Some commercially supplied buffers 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 these buffers 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 this 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 number of cells for 5 - 10 minutes at 1,000 x g. Cell concentrations up to 10^7 may be used. Remove the supernatant and discard.
2. Resuspend the pellet in 500 µL of the prepared Lysis buffer with the appropriate quantity of Zymolase (refer to supplier guidelines) and incubate at 30 °C for 2 hours.
3. Centrifuge for 5 minutes at 5,000 x g, discard the supernatant and resuspend the pellet in 180 µL of lysis buffer.
4. To each 180 µL sample of resuspended cells in lysis buffer, add 20 µL of proteinase K and vortex to mix.
5. Incubate at 56 °C until the tissue is completely lysed, vortexing occasionally during incubation.
6. *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 each sample of cells in lysis buffer and Proteinase K. 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.
7. Add 200 µL of cell debris removal buffer and pulse vortex thoroughly for 15 seconds.
8. Incubate at 70 °C for 10 minutes to complete lysis.
9. Add 200 µL of 100% non-denatured ethanol to the sample and pulse vortex for 15 seconds to mix.

10. Centrifuge again for 5 seconds to remove drops from the lid.
    (Thoroughly mix at each stage to obtain a homogenous solution).
11. Apply the mixture, including any precipitate, to the NAB Nanosep device insert inside a receiver tube. If the sample volume is greater than 500 µL, apply half the sample to the NAB Nanosep device insert, perform steps 11 and 12 and then repeat with the second half of the sample.
12. Close the cap and centrifuge for 60 seconds at 10,000 – 14,000 x g.
13. Discard the flow-through and re-use the receiver tube for the next step.
14. Open the NAB Nanosep device and add 500 µL wash buffer 1.
15. Close the cap and centrifuge for 60 seconds at 10,000 – 14,000 x g.
16. Discard the collection tube and flow-through and transfer the NAB Nanosep device insert into a clean collection tube (provided).
17. Add 500 µL wash buffer 2 to the device insert.
18. Close the cap and centrifuge for 3 minutes at 10,000 – 14,000 x g.
19. Discard the flow-through and re-use the receiver tube for the next step.
20. Centrifuge for 60 seconds at 10,000 – 14,000 x g to remove possible buffer carry-over.
21. Discard the receiver tube with the flow-through and transfer the device insert to the final, clean collection tube (provided).
22. To elute the DNA, add 100 µL of DNase-free distilled water (or elution buffer) and incubate at room temperature for 5 minutes.
23. Centrifuge for 60 seconds at 10,000 – 14,000 x g.
24. 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 a commercially available elution buffer 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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