LABORATORY

Application Note

Pall NAB Nanosep® Centrifugal Device Offers Flexibility and High-Quality Extraction of Genomic DNA and RNA from Yeast, *Saccharomyces Cerevisiae*

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Introduction

Yeast belongs to the kingdom of fungi, but they share common cellular architecture and rudimentary life cycle with multicellular eukaryotes such as plants and animals. Yeast has a high endogenous rate of homologous recombination, and a host of extrachromosomal DNA elements that can result in stably transformed yeast cells[1].

The budding yeast *S. cerevisiae* has emerged as a versatile and robust model system of eukaryotic genetics. The genome sequence of the budding yeast *S. cerevisiae*, released in 1996, was the first completely sequenced genome from a eukaryote. Its compact size and low genetic redundancy due to short intergenic regions and fewer introns make it an ideal model in the analysis of gene function studies[1]. In order to harness the full potential of yeast as a model, high quality DNA and RNA need to be extracted and purified as a starting point for further studies.

Pall's Nucleic Acid Binding (NAB) Nanosep centrifugal device, is capable of binding various nucleic acid types like genomic DNA (gDNA), RNA and plasmid DNA (pDNA) from various sources, when used with the appropriate buffers.

In this report, we analyze the quality and quantity of gDNA as well as RNA from *S. cerevisiae* with Pall NAB Nanosep centrifugal devices in comparison with a renowned commercial brand. The commercial brand has separate kits comprising of buffers and reagents for the extraction of both DNA as well as RNA. The appropriate buffers from the other commercial brand have been used with the Pall NAB devices to purify gDNA and RNA. The nucleic acid extraction data generated in this study is a comparative analysis of ten centrifugal devices from each brand (n=10) and they were processed in parallel with the same pool of cells to reduce sample variability. This study shows that the quality and quantity of the gDNA and RNA extracted and purified with Pall's NAB Nanosep centrifugal devices is on par with the devices from the other commercial brand.

Materials and Methods

Materials

The materials used in this report were Pall NAB Nanosep centrifugal devices, DNA and RNA extraction kits from a commercial brand which will be subsequently denoted as Brand A. The DNA extraction kit brand A will be denoted as kit "AD" and the RNA extraction kit will be denoted as kit "AR". Both the kits are supplied with buffers and reagents in addition to centrifugal devices. The respective buffers from kit AD and AR were used with Pall NAB Nanosep centrifugal devices for gDNA and RNA extraction respectively.

In addition to the kits, all the media, buffers and reagents used were prepared as follows: YPD broth: 50 g/L in deionized water and autoclaved at 120 °C for 15 minutes. Buffer Y1: 1M sorbitol, 0.1M EDTA pH 7.4-8.0. 20 µL of 2M DTT was added just before use. Zymolase solution (VWR; 20U): 2 mg powder added to 1 mL of Y1 buffer.

Cell culture

The strain of yeast used was *S. cerevisiae*; ATCC◆ 7754◆(2). The strain was grown under sterile conditions in yeast extract peptone dextrose (YPD) broth at 30 °C with constant shaking at 200 rpm. The culture was harvested at OD_{600} of 2.9 for RNA and 11.3 for DNA extractions respectively. The number of colony forming units (CFU) at an OD_{600} of 1 are 10^7 cells/mL.

Lysis and spheroplast generation

The *S. cerevisiae* cells are covered by a polysaccharide rich cell wall, which needs to be broken down. This procedure can be performed enzymatically with zymolase resulting in spheroplasts.

The cells were lysed from the same pool and loaded into Pall NAB Nanosep centrifugal devices as well as the centrifugal devices from Brand A. The cells were pelleted by centrifugation at 5000 x g for 5 minutes. Spheroplasts were formed with zymolase in 500 µL of Buffer Y1 in each device by incubation at 35 °C for 30 minutes with constant shaking in a thermomixer at 300 rpm. Spheroplasts were obtained in the pellet by centrifugation at 5,000 x g for 5 minutes.

gDNA extraction with Pall NAB Nanosep centrifugal devices and kit AD from Brand A

Pall's NAB Nanosep centrifugal devices were used along with the DNA extraction buffers from kit AD. The extraction of gDNA was performed along with the centrifugal devices and buffers/reagents supplied by AD. The spheroplast pellet was suspended in the resuspension buffer from AD and homogenization was performed with a combination of metal beads with Star-Beater mixer mill (VWR) for 15 seconds at 30 Hz followed by pulse-vortexing glass beads (100 mg/sample) for 60 seconds. 20 µL of proteinase K was added to each sample and incubated at 56 °C in a thermomixer with constant shaking at 300 rpm. The sample was further treated to digest contaminating RNA by the addition of 4 µL of RNase solution per sample with an incubation time of 5 minutes. 200 µL of lysis buffer was then added to the sample and incubated at 70 °C for 10 minutes followed by the addition of 200 µL of 70% ethanol and pulse-vortexed for 15 seconds. The final mixture, approximately 600 µL, was transferred to the NAB Nanosep centrifugal devices and the centrifugal devices from kit AD in two steps, 300 µL at each time followed by centrifugation at 12,000 x g for 1 minute. The subsequent steps were followed according to the manufacturer's instructions for both brands. The elution of gDNA was performed by the addition of 100 µL of nuclease-free water with an incubation time of 5 minutes at room temperature before centrifugation at 12,000 x g.

The concentration of gDNA was determined by measuring the absorbance at 260 nm (A_{260}) after dilution of the samples in 10 mM Tris buffer, pH 7.0, applying the following conversion:

Concentration of DNA (µg/mL) = 50 x A_{260} x dilution factor. The absorbance at 280 nm (A_{280}) was recorded to calculate the A_{260}/A_{280} ratio to assess the purity of the extracted DNA. The total amount of gDNA (µg) was calculated as: Concentration (µL/mL) x final sample volume (mL)

RNA extraction with Pall NAB Nanosep centrifugal devices and kit AR from Brand A

RNA extraction was performed with NAB Nanosep centrifugal devices and RNA extraction kit, AR. The buffers from the kit AR are used with the centrifugal devices from Pall and AR.

The spheroplast pellets were resuspended in 350 µL of resuspension buffer with 20 µL of 2M DTT and homogenized by pulse-vortexing for 1 minute using glass beads (100 mg/sample). Subsequently, 350 µL of 70% Ethanol was added per sample. The sample was mixed by pipetting and 700 µL was transferred to NAB Nanosep centrifugal devices and centrifugal devices of AR Kit in two stages, 350 µL each time to accommodate the reservoir of the NAB Nanosep centrifugal device. The devices containing the sample mixtures were centrifuged at 12,000 x g for 1 minute. The filtrates were discarded. All the subsequent steps were followed according to the instructions given by the manufacturer of the AR Kit. The final elution was performed by adding 100 µL of nuclease-free water with an incubation time of 1 minute before centrifugation for 2 minutes at 12,000 x g.

The concentration of the filtrate containing RNA was determined by measuring the absorbance at 260 nm (A_{260}) by performing a dilution of the RNA samples in 10 mM Tris buffer, pH 7.0 and applying the conversion: Concentration of RNA (µL/mL) = 44 x A_{260} x dilution factor. The total amount of RNA (µg) was calculated as: Concentration ((µL/mL x final sample volume (mL)

The absorbance at 280 nm (A_{280}) was recorded to calculate the A_{260}/A_{280} ratio to assess the purity of RNA.

Results and Discussion

gDNA extraction from *S. cerevisiae*

As shown in Figure 1, both Pall NAB Nanosep centrifugal devices and the centrifugal devices from the DNA extraction kit AD from brand A showed similar amounts of gDNA in all the 10 devices tested from each brand. The quality of gDNA was determined by assessing the A_{260}/A_{280} ratio and was found to be 1.7 for both the Pall and Brand A devices, which is an acceptable standard for DNA.

Figure 1
gDNA extracted from the same pool of lysed *S. cerevisiae* with Pall NAB Nanosep centrifugal devices and the centrifugal devices from DNA extraction kit AD from brand A depicting similar amounts of gDNA in all the 10 devices tested from both brands

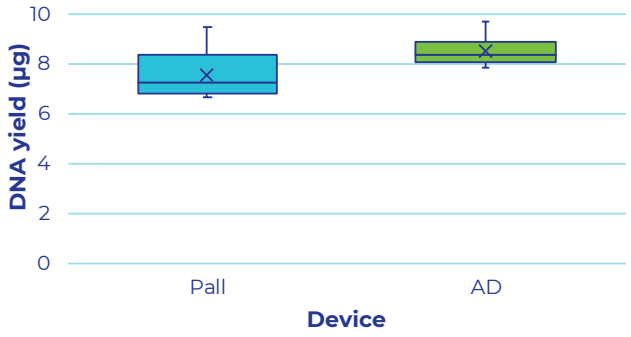

Table 1
Average quantity of gDNA extracted from *S. cerevisiae* using Pall NAB Nanosep centrifugal devices and centrifugal devices from kit AD of brand A

OD_{600}	Volume of Culture/ Sample (mL)	Cells/Sample	Pall NAB Nanosep Centrifugal Devices gDNA (µg)	A_{260}/A_{280}	Centrifugal Devices from Kit AD gDNA (µg)	A_{260}/A_{280}
11.3	3	3×10^8	7.6 ± 0.9	1.7 ± 0.1	8.5 ± 0.6	1.7 ± 0.1

RNA extraction from *S. cerevisiae*

Pall NAB Nanosep centrifugal devices delivered marginally higher yields of RNA in comparison to RNA extraction kit AR from brand A in all the 10 devices tested from each brand as shown in Table 2 and Figure 2. Similar to DNA, the quality of RNA is also determined by assessing the A_{260}/A_{280} ratio. The purity ratio for both the brands shown in Table 1 is 2.2, which is indicative of highly pure RNA[2] (Table 2).

Figure 2
RNA extracted from the same pool of lysed *S. cerevisiae* with Pall NAB Nanosep centrifugal devices and the RNA extraction kit AR from brand A, depicting similar amounts of RNA in all the 10 devices tested from both brands

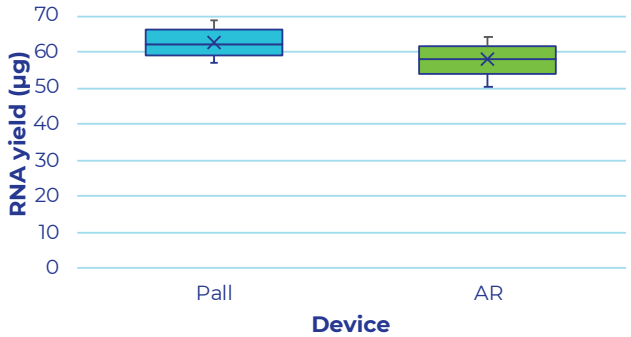

Table 2
Average quantity of RNA extracted from *S. cerevisiae* using Pall NAB Nanosep centrifugal device and centrifugal devices from kit AR of brand A

OD_{600}	Volume of Culture/ Sample (mL)	Cells/Sample	Pall NAB Nanosep Centrifugal Devices RNA (µg)	A_{260}/A_{280}	Centrifugal Devices from Kit AR RNA (µg)	A_{260}/A_{280}
2.9	1.5	4.5×10^7	62.7 ± 4.0	2.2 ± 0.0	57.9 ± 4.6	2.2 ± 0.0

Conclusions

Pall's NAB Nanosep centrifugal device serves as a universal device, offering the flexibility of binding to both gDNA and RNA from *S. cerevisiae* as shown in the current study. The NAB Nanosep centrifugal device gave a similar yield of gDNA and slightly higher yield of RNA with repeatability across all the devices tested in comparison to leading nucleic acid extraction kit manufacturer without compromising on the quality of extracted DNA and RNA.

References

1. Burgess, S.M., Powers, T. and Mell, J.C. (2017). Budding Yeast *Saccharomyces Cerevisiae* as a Model Genetic Organism. In eLS, John Wiley & Sons, Ltd (Ed.). https://doi.org/10.1002/9780470015902.a0000821.pub2

2. Saccharomyces cerevisiae Meyen ex E.C. Hansen (ATCC 7754) product sheet: https://www.atcc.org/en/Products/Quality_Control_Strains/By_Identifier/7754.aspx#documentation

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