Efficient DNA Extraction and Purification of Vegetative Cells and Spores from Sterivex™ Filter Units

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INTRODUCTION

Sterivex™ filter units (Millipore) are commonly used for collecting microbes from water samples for subsequent nucleic acid isolation. Protocols developed for the extraction of DNA from these units requires either breaking the plastic casing to remove the filter inside for processing or the incubation of lysis solutions and enzymes inside the unit itself. Breaking open the plastic casing is both time consuming and requires aseptic handling of the unit and membrane. Disadvantages of both protocols include the length of time to complete the extraction and the need for phenol/chloroform to clean the lysates followed by alcohol precipitation for purification and concentration of the DNA. The goal of this project was to develop an in-unit method for Sterivex™ filter unit DNA isolation that was fast and efficient without the use of organic solvents, and then to compare the new method to a commonly used enzymatic protocol.

MATERIALS AND METHODS

Vegetative Cell Cultures: *Bacillus subtilis* subsp. *Spizizenii* ATCC 6633 was plated onto Trypticase Soy Agar (TSA) then grown to mid log phase (OD_{600} = 0.04, NanoDrop®) in Trypticase Soy Broth (TSB). The culture was diluted in sterile 0.85% NaCl at a concentration of 6 x 10^6 CFU/ml and 50 ml was filtered through each Sterivex™ filter unit (3 x 10^8 CFU/unit).

Spore Cell Cultures: Colonies from the plate used to start the vegetative cell culture were inoculated into Difco Sporulation Medium (DSM) following the procedure of Nicholson & Setlow (1990)[1] and grown for 5 days until the culture consisted of 99% spores. Spore formation was confirmed using the Schaeffer Fulton Method[2]. The culture was diluted and filtered as described for the vegetative cells with a final concentration of 8.3 x 10^7 CFU/unit.

Sterivex™ GP filter units (Millipore catalog # SVGPL10RC) were used exclusively because they have a male Luer-Lok ™ outlet that can be easily capped.

Sterivex™ GP Filter Unit

DNA Extraction and Purification: DNA was extracted and purified in triplicate from both vegetative cells and spores using an in-unit enzyme extraction and phenol/chloroform purification protocol[3] or a new in-unit extraction protocol that was based on the PowerWater® DNA Isolation Kit (MO BIO catalog #14700).

The following changes were made to adapt and optimize the PowerWater® chemistry for use in the Sterivex™ filter units. (Figure 1):

1) Use of a Sterivex™ filter unit (Millipore catalog # SVGPL10RC) that had an easily sealed outlet to prevent leakage.

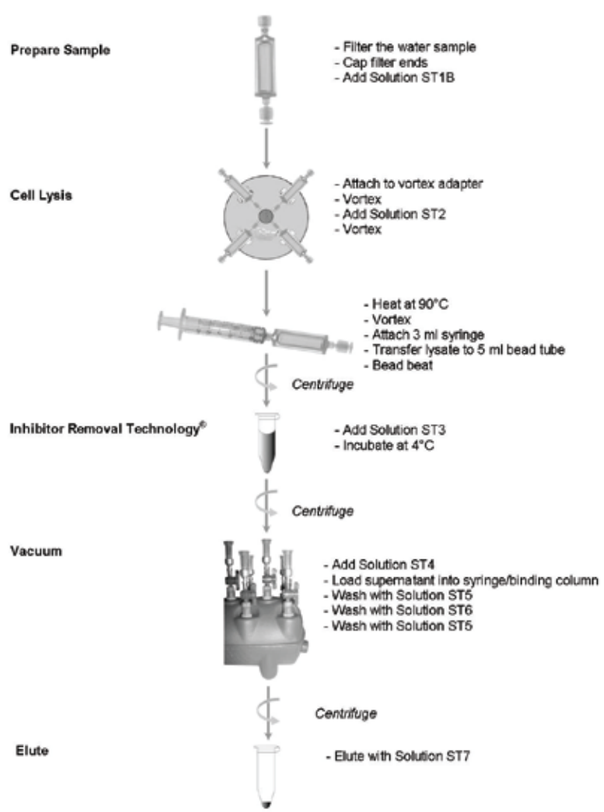
2) Development of a novel cell release solution (ST1B) to improve microbial extraction from the filter membrane without the need for enzymes.

3) 90 C heating step to improve microbial lysis.

4) A new 5 ml, 0.1 mm glass bead tube for crude lysate bead beating

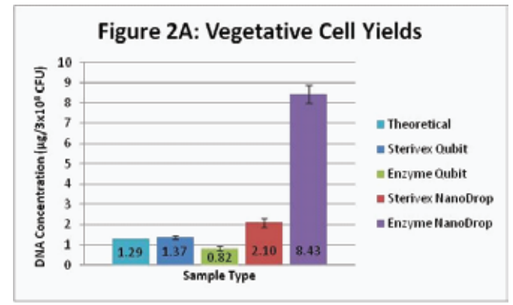
5) A new binding column with a column extender for large volume (4.5 ml) binding under vacuum and elution in 100 µl.

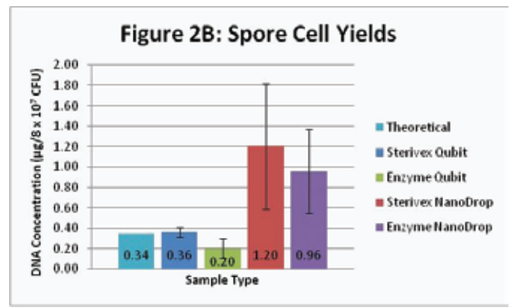
Figure 1
PowerWater® Sterivex™ DNA Isolation Kit



RESULTS

The highest DNA yields achieved from both vegetative cells and spores was with the new PowerWater® Sterivex™ (PWS) method (Figure 2A,B). PWS yields were also more consistent with theoretical yields based on the *B. subltilis spizizenii* genome size[4] and number of cells filtered through each Sterivex™ unit.



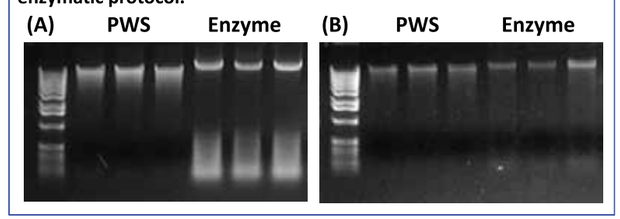


DNA extraction efficiencies were better and more consistent with the PWS protocol than the enzyme based protocol (Table 1) but only when the Qubit was used for analysis. Efficiencies were greatly overestimated when using the NanoDrop® regardless of protocol. NanoDrop® overestimation was a result of genomic DNA shearing and RNA carrryover (Figure 3A,B).

Table 1. Extraction Efficiencies Based on Theoretical Yield

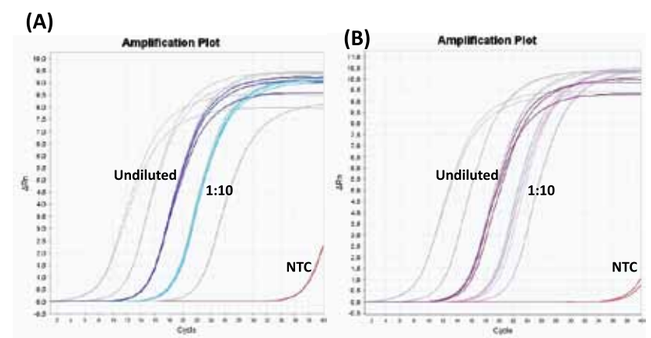
	Qubit	NanoDrop®
Vegetative Cells:		
PWS	106%	163%
Enzyme	63%	653%
Spores:		
PWS	102%	338%
Enzyme	57%	269%

Figure 3 . Genomic DNA isolated from *B. subtilis* vegetative cells (A) and spores (B) using the PowerWater® Sterivex™ protocol or the enzymatic protocol.



The DNA isolated using the PWS protocol was free of PCR inhibitors and suitable for quantitative PCR (Figure 4A, B)

Figure 4. Quantitative PCR results from *B. subtilis* vegetative cells (A) and spores (B). Primers were specific to *B. subtilis* 16S rDNA. Assay efficiencies were 91% and 93% respectively.



CONCLUSIONS

- The new protocol was performed both rapidly and easily without loss of critical microbial DNA and without the use of organic solvents (*i.e.* phenol/chloroform).
- Theoretical yields were more consistent with PWS than the enzyme method.
- The novel extraction method using the cell release solution required only 0.7 hours (42 minutes) from start to finish while the enzymatic method required 20 hours.
- Adaptation of the PowerWater® chemistry for use in Sterivex™ filter units has resulted in the creation of a new kit, PowerWater® Sterivex™ DNA Isolation Kit (MO BIO catalog #14600-50).

REFERENCES

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3. M. Celussi & B. Cataletto. Gene. 2007 Dec 30;406(1-2):113-23. Epub 2007 Jul 21.
4. L. Fan *et al.* J Bacteriol. 2011 Mar;193(5):1276-7. Epub 2010 Dec 23.