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UNI University of Northern Iowa Biology

Evaluation of Pre-lysis Rinses To Improve DNA Yield and Purity

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Abstract

Currently, there are a variety of published protocols for environmental DNA extraction. Most protocols use similar, but not identical buffers, incubation times and temperatures, and vary in the amount of starting material, which makes it difficult to compare results from different sources. For our research, we sought a standardized protocol that would work with a variety of environmental samples that are found in Wind Cave National Park. We found that the addition of a pre-lysis rinse to our standard DNA extraction protocol was beneficial. The two rinse solutions we tested, 100 mM sodium phosphate pH 7.2 (Na_3PO_4) and 100mM Tris pH 8.0, 5mM EDTA, 200 mM sodium chloride (TEN), resulted in darker bands on our electrophoresis gels that were of the expected size (greater than 10 kilobases) and showed less degraded DNA. In the future, the addition of a pre-lysis rinse will improve our limit of detection for microbial life in environmental samples such as paleofill sediments in Wind Cave National Park and soil samples above ground near the cave entrance.

Background

DNA extraction kits are commonly used for soils and other environmental samples which contain large amounts of inhibitors against DNA-testing because of their relative speed and ease of use. However, even the best commercial kits lose 83% of the starting DNA and thus can only isolate about 17% of the available sample (Hershey, Kallmeyer, and Barton 2019). This decreases the limit of detection for commercial kits. Instead of commercial kits, some researchers have published a variety of protocols designed for their particular environmental samples. For example, Zhou, Bruns, and Tiedje (1996) compared the effect of CTAB and PVPP on humic acid contamination, using 5 g of starting material in their extraction buffer. A study by Högfors-Rönnholma et al. (2018) used 8 g of soil as the starting material and used a sodium phosphate buffer to rinse the soil before running a DNA extraction kit on the product. Starting with 50 or 200 mg of soil, Guerra et al. (2020) conducted a study comparing SDS to CTAB as detergents in a phosphate lysis buffer. Each of these studies used different amounts of starting material with different buffers for protocols specific to the samples they obtained.

Pre-lysis rinsing of soil samples was used in an early study by Tsai and Olson (1991). Their protocol included a sodium phosphate pre-lysis rinse step as part of the DNA extraction protocol and yielded bright bands on their agarose gel. Later studies (Tarnovetskii et al. 2018; Yamaguchi et al. 2012; Rainer W. Erb and Irene Wagner-Döbler 1993) followed the same methods as Tsai and Olson. He, Zu, and Hughes (2005) tested the effect of including a pre-lysis rinse to their DNA extraction protocol. They found that including a phosphate rinse before lysis of cells decreased humic acids and increased DNA yield when compared to the absence of a pre-lysis rinse. These limited results suggest sodium phosphate is a useful buffer for pre-lysis rinsing of environmental samples.

In this study, we compared pre-lysis rinses to test if they increase DNA yield and purity from our environmental samples. In addition to a sodium phosphate rinse, we tested a TEN rinse since TEN is the base of our lysis buffer.

Methods

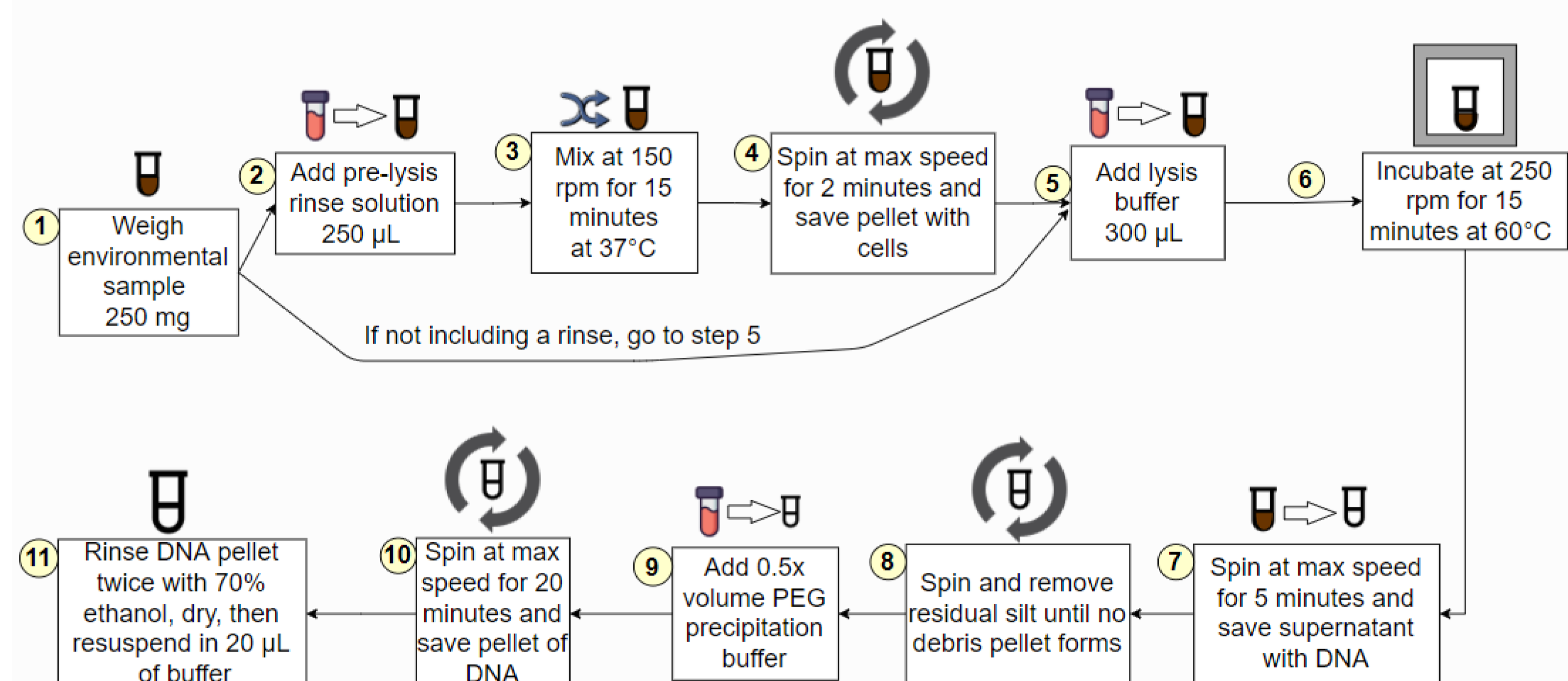

Fig. 1. Visual Description of Methods
DNA was extracted using this step-wise protocol. Centrifugation was performed at 19090 rcf. The DNA pellet was resuspended in 10 mM Tris pH 8.0. RNaseA was added to remove RNA before running samples on agarose gel electrophoresis.

Results

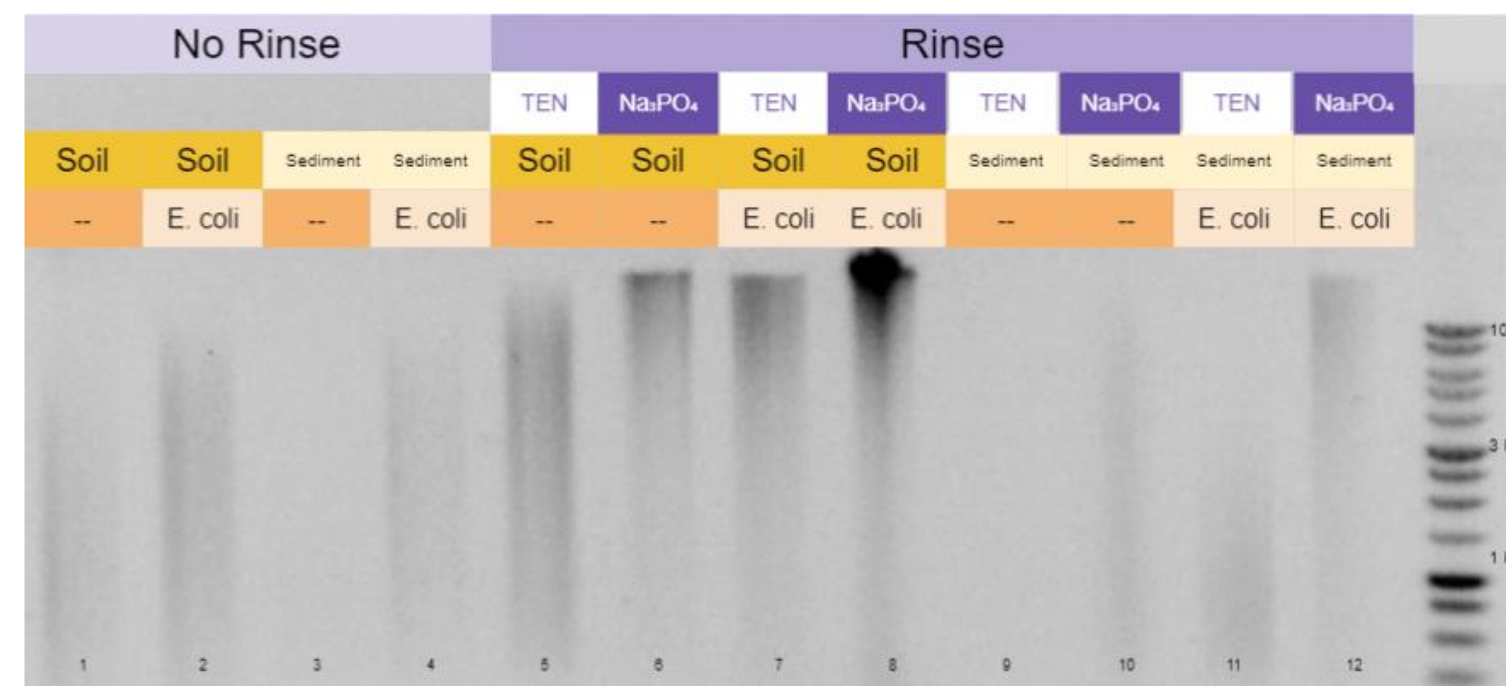

Fig 2. Pre-lysis Rinsing of Soil and Stream Sediment Improved Yield.
DNA was extracted from prairie soil (S) and stream sediment (SS). The rinses yielded more DNA with less degradation as shown by comparing gel lanes 1-4 versus 5-12. Sediment on its own needed a rinse to detect DNA using gel electrophoresis. See Table 1 for additional experimental details.

Table 1. Experimental Design for Soil and Stream Sediment Pre-lysis Rinse Trials

			Lysis Buffer								
Tube	Sample	Pre-lysis Rinse	Detergent	Buffer	Chelator	Osmolarity	Lysis Enzyme	Incubation	Precipitation	Resuspension Buffer	Final Enzyme
1	Soil	n/a	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
2	Soil + E. coli (S+E)	n/a	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
3	Stream Sediment (SS)	n/a	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
4	SS + E. coli (SS+E)	n/a	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
5	Soil	TEN*	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
6	Soil	Na_3PO_4**	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
7	S+E	TEN*	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
8	S+E	Na_3PO_4**	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
9	SS	TEN*	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
10	SS	Na_3PO_4**	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
11	SS+E	TEN*	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A
12	SS+E	Na_3PO_4**	0.2% SDS	100 mM Tris***	5 mM EDTA	200 mM NaCl	1.5 µL proK****	60C @ 15 min	0.5x volume 30% PEG : 1.6 M NaCl	20 µL Tris	1 µL RNase A

*100mM Tris, 5mM EDTA, 200mM NaCl **100 mM Sodium Phosphate pH 7.2 ***pH 8.0 ****Proteinase K Qiagen

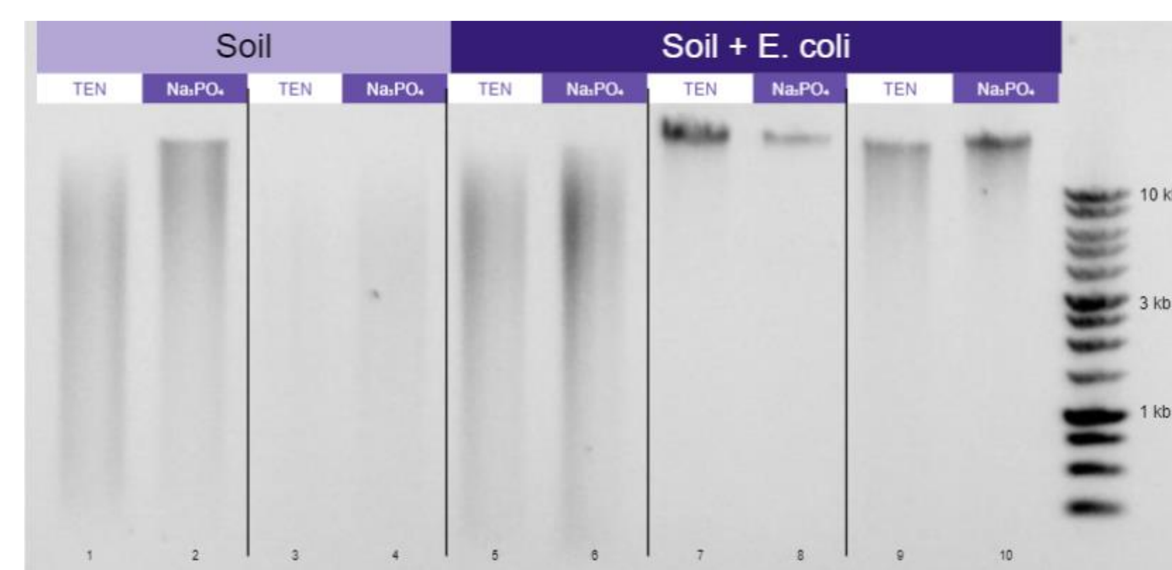

Fig. 3. Variability in the Effect of TEN Versus Na_3PO_4 Between Independent Trials.
The effectiveness of increasing DNA yield between TEN and sodium phosphate was compared. Neither rinse performed better than the other in every trial, so we cannot say with certainty which rinse works best without further testing. Replicate experiments are separated by lines.

Table 2. DNA Concentration and Purity

Tube	Sample	Pre-lysis Rinse	Concentration (ng/µL)	Purity (A260/A280)	Purity (A260/A230)
1	Soil	n/a	56.30	1.908	1.005**
2	Soil + E. coli (S+E)	n/a	90.20	1.942	1.470**
3	Stream Sediment (SS)	n/a	8.35*	1.758*	0.337**
4	Stream Sediment + E. coli (SS+E)	n/a	199.75	2.053	1.923
5	Soil	TEN (100mM Tris, 5mM EDTA, 200mM NaCl)	80.15	1.847	1.180**
6	Soil	100 mM Na phosphate (pH 7.2)	98.10	1.858	1.159**
7	S+E	TEN (100mM Tris, 5mM EDTA, 200mM NaCl)	136.65	1.969	1.472**
8	S+E	100 mM Na phosphate (pH 7.2)	276.65	2.002	1.747**
9	SS	TEN (100mM Tris, 5mM EDTA, 200mM NaCl)	15.45*	1.618*	0.401**
10	SS	100 mM Na phosphate (pH 7.2)	55.05	1.686	0.813**
11	SS+E	TEN (100mM Tris, 5mM EDTA, 200mM NaCl)	92.85	1.934	1.655**
12	SS+E	100 mM Na phosphate (pH 7.2)	181.4	2.028	1.866

*Absorbance level was < 0.4, so numbers may not be accurate
**Bad purity ratio due to contaminants absorbing at 230 nm

Conclusions

The addition of pre-lysis rinses yielded more DNA with less degradation (Fig. 2). It is unclear whether rinse solutions of TEN or sodium phosphate perform better as variation was found between separate trials (Fig 3). The rinses had inconsistent effects on the purity of DNA (Table 2). Now, with a protocol that consistently gives us higher DNA yields in both soil and stream sediment, we can use this method for further study of the soils within and around the caves at Wind Cave National Park.

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