The Effect of Sample Preparation on Humic Acid Removal from Peat Samples: A Comparative Study

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INTRODUCTION

Despite numerous methods of microbial detection employed today, we have only scratched the surface of the true prokaryotic diversity present in environmental samples[1]. Only a small proportion of soil microorganisms can be cultured using standard media, representing a large obstacle in the identification of microbes in soil samples[2,3]. Alternatively, microorganisms residing in soil samples can be identified rapidly through the isolation of total DNA from these samples, and through polymerase chain reaction (PCR).

Direct extraction of DNA from soil samples inevitably results in the extraction of other contaminating substances naturally found in soil, such as humic acids, which often interfere with the success of many detection systems[2]. Humic acids are high-molecular-weight polymers[4] resulting from the decomposition of dead plants and other organic matter[4]. Humic acids are characteristically brown in colour[3], and are often co-purified with DNA because they both have a similar size and charge[3]. For this reason, they are the most notorious PCR inhibitors, leading to false negative results, or underestimated quantifications[5]. Humic acids have been found to inhibit restriction endonucleases, Taq polymerase, and DNA-DNA hybridizations[2]. In fact, as little as 1 µL of humic acid extracts has been found to inhibit DNA polymerases, even with high amounts of DNA in the sample[3]. Therefore, it is extremely important for DNA isolation techniques to effectively eliminate humic acids from environmental samples, while maximizing DNA yields. Many methods that are available today elute DNA that requires further purification steps (leading to a loss of DNA yield), or dilution steps prior to qPCR analysis[3].

In this study, we compared two commercially available kits for total DNA extraction from peat samples: 1) Norgen's Soil DNA Isolation Kit (High Humic Acid) and 2) a competitor's Soil DNA Isolation Kit. Both kits were used to isolate total DNA from a typical peat sample, and a high humic acid peat sample.

The objective of this study was to compare the aforementioned soil DNA isolation kits on their ability to remove humic acids from peat samples. The comparisons were based on:

a) **Visual analysis**
b) **Quantity of DNA Isolated**
c) **Quality of DNA Isolated**

MATERIALS AND METHODS

Peat DNA Purification

Total DNA was extracted from 250 mg of either high or regular humic acid level peat samples using the **Soil DNA Isolation Kit (High Humic Acid; Norgen Biotek; Cat #49300**) and the competitor's **Soil DNA Isolation Kit**. DNA isolations were performed as per manufacturer's instruction. All elutions took place in 50 µL of each kit's elution solution.

Agarose Gel Electrophoresis and qPCR

Purified DNA (10 µL from 50 µL) was loaded on a 1X TAE, 1.2% agarose gel. The gel was then run for 30 minutes at 150V. Samples were quantified using gel densitometry, using the AlphaEase™ software supplied by AlphaImager™ 2200. Quantifications were used to adjust DNA concentrations to 500ng, which was used in a 2-step qPCR reaction involving Norgen's 2x PCR Mastermix spiked with SYBR-green using 16S rDNA primers.

RESULTS AND DISCUSSION

Humic acids are notoriously known by peat and soil researchers as the most common PCR inhibitor[5]. They are commonly co-extracted with DNA during purification procedures, requiring extensive clean up procedures or serial dilutions of samples in order to be used in qPCR analyses[3]. Two commercially available soil DNA extraction kits were therefore used in this study to compare and contrast the two kits for their ability to a) remove humic

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acids from peat samples b) isolate high DNA yields and c) isolate high quality DNA from peat samples for subsequent qPCR analyses.

A) Visual Analysis. Humic acids are brown in colour, making them visible during purification. **Figure 1** shows the differences found after sample homogenization between Norgen's and Mo Bio's kits. **Figure 1A** shows a regular peat sample with typical humic acid levels, while **Figure 1B** shows the high humic acid sample. Norgen's kit effectively removed humic acids (clear supernatant) in the lysis step, while Mo Bio's samples still appear brown, indicating high levels of humic acids remaining after homogenization. Mo Bio's kit requires further purification steps to remove humic acids from the peat samples, which could result in lower DNA yields.

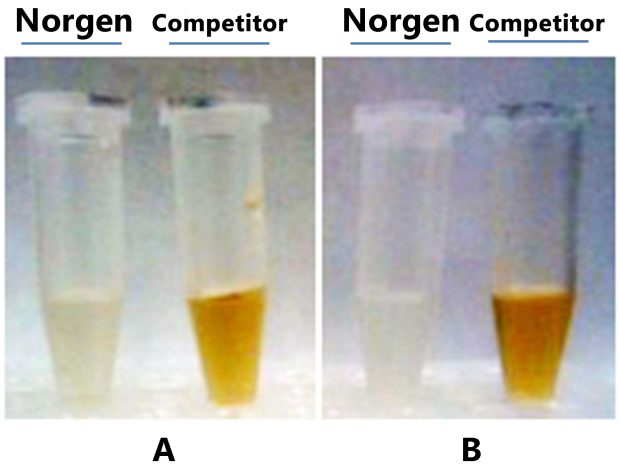

Figure 1. Humic Acid Removal Efficiency from Typical and High Humic Acid Peat Samples. Image A: supernatants from a regular peat sample with typical humic acid levels, after homogenization. Image B: supernatants from a high humic acid peat sample, after homogenization.

B) Quantity of DNA Isolated. Purified DNA samples were then run on an agarose gel to assess differences in yields (**Figure 2**). For both the regular peat sample (**Figure 2A**) and the high humic acid peat (**Figure 2B**), Norgen isolated higher yields of DNA than the competitor (nearly double the amount.

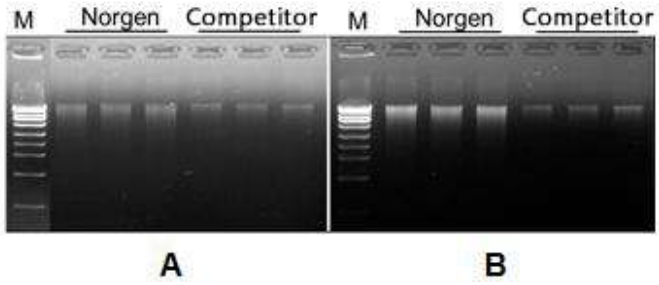

Figure 2. Differences in DNA Yields from Two Peat Samples Using Norgen and the Competitor's Kits. Image A: DNA eluted from a regular peat sample with typical humic acid levels. Image B: DNA eluted from a high humic acid peat sample.

C) Quality of DNA Isolated. The quality of the DNA for each sample was determined based on Ct values generated from a 2-step qPCR reaction using 500ng of DNA and 16S rDNA primers. **Figure 3** shows the qPCR amplifications from Norgen-isolated and competitor-isolated DNA from a typical peat sample. Norgen (blue lines) consistently amplified sooner (had lower Ct values) than the competitor's samples (red lines). This is due to Norgen's kit being more efficient than the competitor's kit at removing humic acids from peat samples.

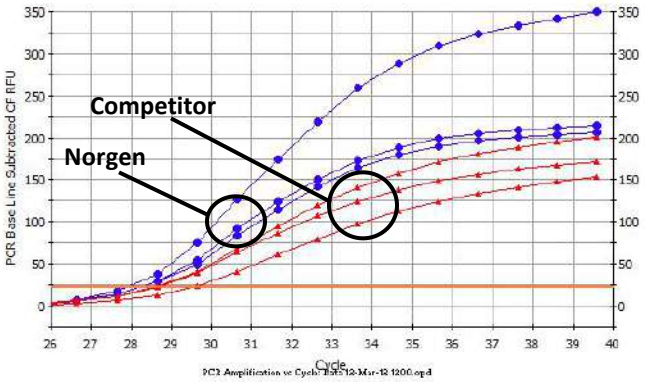

Figure 3. Typical Peat Sample: Comparison of Ct Values Generated from a qPCR using Norgen-Isolated and the Competitor-Isolated DNA. Five hundred nanograms of DNA was used in a qPCR reaction using 16S primers. Norgen samples= blue dots ● ; Competitor samples= red triangles ▲ .

The qPCR amplifications from the DNA isolated from the high humic acid peat samples can be seen in **Figure 4**. This time, competitor samples (red dots) were found to be completely inhibited by humic acids. Norgen samples, on

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the other hand, were found to be unaffected by the humic acid content of the sample, as indicated by the similar Ct values generated from both peat samples.

Therefore, Norgen's kit isolates high quality DNA, compatible with many downstream applications such as qPCR, regardless of the level of humic acids in the peat sample.

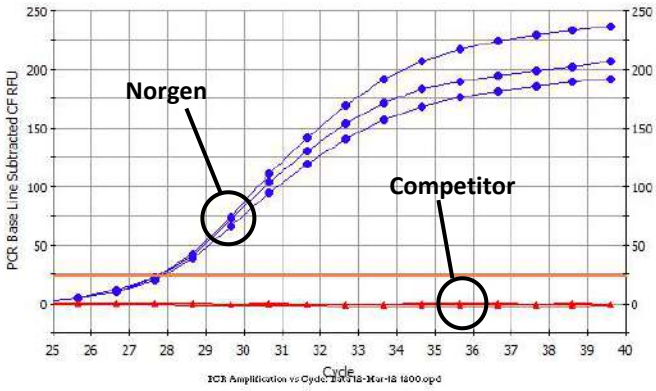

Figure 4. High Humic Acid Peat Sample: Comparison of Ct Values Generated from a qPCR using Norgen-Isolated and Competitor-Isolated DNA. Five hundred nanograms of DNA was used in a qPCR reaction using 16S primers. Norgen samples= blue dots ● ; Competitor samples= red triangles ▲ .

CONCLUSIONS

1. **DNA Yields.** Norgen's kit isolated higher yields of DNA from peat samples compared to the competitor's kit, which is ideal for detecting microbes in soil.
2. **Unbiased Humic Acid Removal.** Norgen's Soil DNA Isolation Kit (High Humic Acid) effectively removes humic acids from typical and high humic acid peat samples, making it the kit of choice when humic acid levels are unknown or high.
3. **Quality of DNA.** Norgen's kit eluted higher quality DNA (as shown by lower Ct values), due to lower humic acid levels in the purified samples.
4. **Ease of Use.** Norgen's kit requires fewer steps and reagents compared to the the competitor's kit, making it faster and more customer-friendly.

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