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PCR ENZYMES & REAGENTS

G2 DNA/RNA Enhancer improves microbial DNA extraction from active carbon of commercial DNA extraction kits

-This study shows a significant increase of DNA extraction efficiency using the G2 DNA/RNA Enhancer in combination with 7 commercial kits intended for extraction of DNA from soils.

Introduction

G2 DNA/RNA Enhancer (Ampliqon A/S - Odense Denmark) increases the yield of microbial DNA and RNA extracted from difficult matrices, such as clays, subsoils, activated charcoal etc.

G2 DNA/RNA Enhancer is used in combination with either standardized extraction methods or in combination with a commercial kit intended for nucleic acid extraction. The primary function of G2 DNA/RNA Enhancer is to relieve inhibitory nucleic acid-particle complexes. (Bælum et al., 2013).

DNA extraction from low biomass clays and subsoils are often problematic since the samples contain low amounts of DNA and RNA. In addition, clay often contain sorption sinks, which traps the DNA or RNA once released from the microorganisms after lysis. The Nucleic acid backbone contains loads of phosphate groups which bind tightly to clay soils. Two different sorption mechanisms are known; In acidic soils (pH<5) DNA has a positive charge and are thereby able to bind directly to the negatively charged clay particle. In pH>5 soils the sorption process is facilitated by cation bridging. (Levy-Booth et al., 2007)

Activated charcoal is by no doubt one of the most problematic matrices to extract DNA from, as it binds to the DNA with high affinity. Previous study has shown that G2 DNA/RNA improves the DNA extraction yield on samples containing activated charcoal, and this matrix was therefore selected for this study. (Personal communication; Jacobsen CS)

Figure 1. G2 DNA/RNA Enhancer tubes: Ceramic beads covered by G2.

Increased DNA extraction efficiency of 7 commercial extraction kits mediated by the addition of the G2 DNA/RNA Enhancer

The effect of adding G2 DNA/RNA Enhancer to DNA extraction kits, when extracting DNA from activated charcoal was investigated. For this study we selected 7 different commercial kits (named A – G) intended for extraction of microbial DNA and RNA from clay soil.

Experimental setup

Microbial DNA was extracted in triplicates, in either presence or absence of 0.1 mm G2 DNA/RNA Enhancer beads. All samples contained 200 mg pellets of activated charcoal and 50 µl of *E. coli* at a concentration of 3 x 10^7 cfu/ml. DNA extractions were performed strictly according to the manufacturer instructions, except for the addition of 0.1 mm G2 DNA/RNA Enhancer beads. The G2 DNA/RNA Enhancer was in all cases, when included, added before the bead-beating step. The level of extracted DNA from each sample was estimated by quantitative real-time PCR

using RealQ Plus master mix, primers and probes specific for *E. coli*.

A general workflow diagram on how we used the G2 DNA/RNA Enhancer in combination with commercial DNA and RNA extraction kits is found on page 3.

Experimental results

Results are shown in figure 2 and 3.

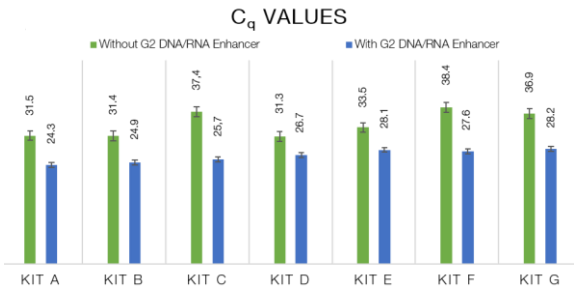

Figure 2. The average C_q values for the purified samples in triplicates. Samples contained 200 mg pellets of activated charcoal and 1.5 x 10^6 cfu of *E. coli*. Green bars represent samples extracted without the addition of G2 DNA/RNA Enhancer beads. Blue bars represent C_q values for samples extracted with addition of G2 DNA/RNA Enhancer beads. (The higher C_q values the lower DNA concentration and vice versa.)

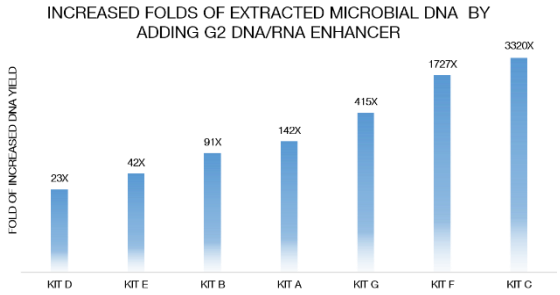

Figure 3. Relative amounts of purified DNA are calculated in this way: $2^{(\Delta Cq)}$. ΔC_q = (C_q values without G2 – C_q values with G2). C_q values can be found in figure 2A. Example kit A: Increased yield - $2^{(31.5-24.3)}$ = 142 fold

The increase in DNA extraction efficiency, by adding G2 DNA/RNA Enhancer is significant for all kits tested here. (23 – 3320 fold)

The best extraction result was obtained using kit A in combination with G2. (C_q = 24.3)

Conclusion and discussion

The effect of adding the G2 DNA/RNA Enhancer before the bead-beating step of the DNA extraction protocol was investigated. 7 different commercial kits intended for extraction of DNA from soil were tested.

The obtained results presented here, clearly demonstrate that the addition of G2 DNA/RNA Enhancer to samples containing activated charcoal improves the DNA yield significantly, by 20 – 3000 fold.

Since the role of the G2 DNA/RNA Enhancer is to relieve inhibitory nucleic acid-particle complexes, it is conceivable that addition of the G2 DNA/RNA Enhancer to the extraction procedure of other difficult matrices than soil, soil clays and activated charcoal might be beneficial.

References

1. Bælum J, Chambon JC, Scheutz C, Binning PJ, Laier T, Bjerg PL, Jacobsen CS. 2013. A conceptual model linking functional gene expression and reductive dechlorination rates of chlorinated ethenes in clay rich groundwater sediment. Water Research 47:2467-2478.

2. Levy-Booth D, Campbell R, Gulden R, Hart M, Powell J, Klironomos J, Pauls K, Swanton C, Trevors J and Dunfield K. 2007. Cycling of extracellular DNA in the soil environment. Soil Biology & Biochemistry 39: 2977–2991

3. Jacobsen CS. 2013. Personal communication

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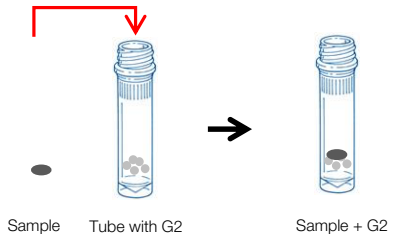
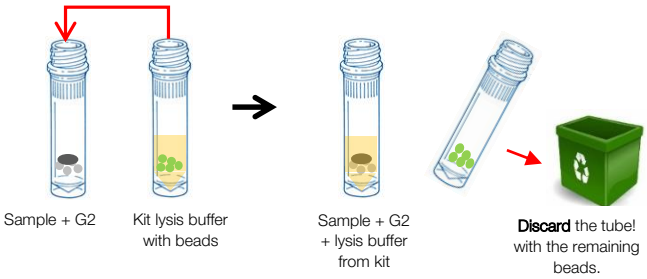
How to use G2 DNA/RNA Enhancer in combination with commercial extraction kits:

This protocol serves as a guideline for extraction of DNA and RNA from difficult matrices, such as soil clays, soils, activated coal etc. when using the G2 DNA/RNA Enhancer (G2) in combination with a commercial DNA or RNA extraction kit intended for soil.

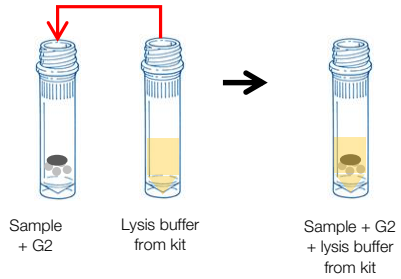
1. Add 0.25 gram of your sample to the G2 DNA/RNA Enhancer tube



2. Prepare the lysis – using the lysis buffer of your DNA or RNA extraction kit
   I. If the bead-beating tube of your extraction kit contains the lysis buffer, transfer the lysis buffer to the G2 tube and discard the emptied bead-beating tube from the kit



   II. If your extraction kit offers separate bead-beating tubes and lysis buffers, transfer lysis buffer to the G2 tube.



3. Gently vortex the G2 tubes – containing sample and lysis buffer from your extraction kit

4. Proceed the DNA or RNA extraction by following the manufacturer´s kit instruction