DNA sorption blocker “G2” increase DNA recovery from subsoil clay sediment >1.000 times

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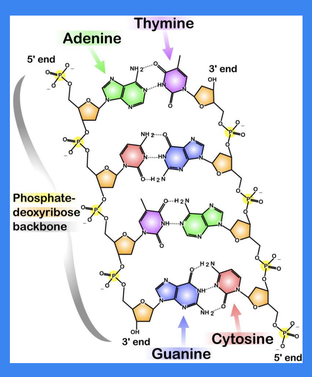
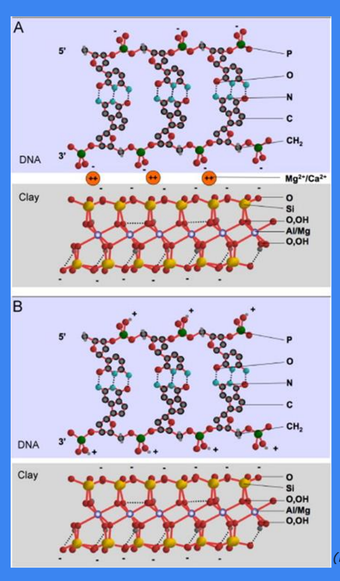
Abstract

One of the obstacles when doing molecular microbial ecology studies in low biomass clayey till sediments is a very low recovery of nucleic acids. The aim of the present study was to develop a suitable extraction procedure for DNA and mRNA from clayey sediments, and to apply this in order to investigate microbial dynamics based on mRNA as well as DNA. With the development of the G2 blocking reagent we were able to optimize a suitable nucleic acid extraction protocol for these difficult sediments with a >thousand-fold increase in extraction yields. Using this extraction protocol we obtained high resolution expression profiles of the functional genes vcrA, bvcA, and tceA. The blocking reagent is being commercial developed and is currently available at the cost price from GEUS formulated in 2 ml beadbeating tubes with either 0.1 mm glassbeads or 1.4 ml ceramic beads as lysing matrix.

Phosphate and DNA sorption





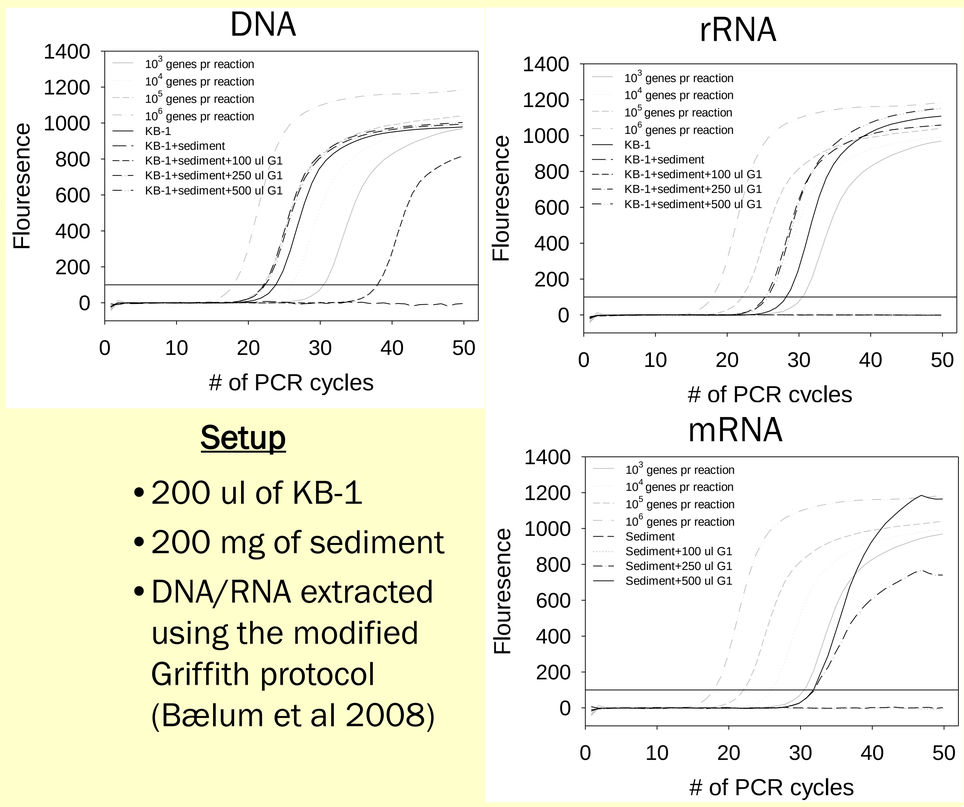
(Levy-Booth et al. 2007)

Phosphate is known to be strongly bound especially in clay soil. As the DNA backbone contain loads of phosphate groups, it is reasonable to believe that DNA binds 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, while in pH>5 soils the sorption process is facilitated by cation bridging

DNA/RNA extraction optimization

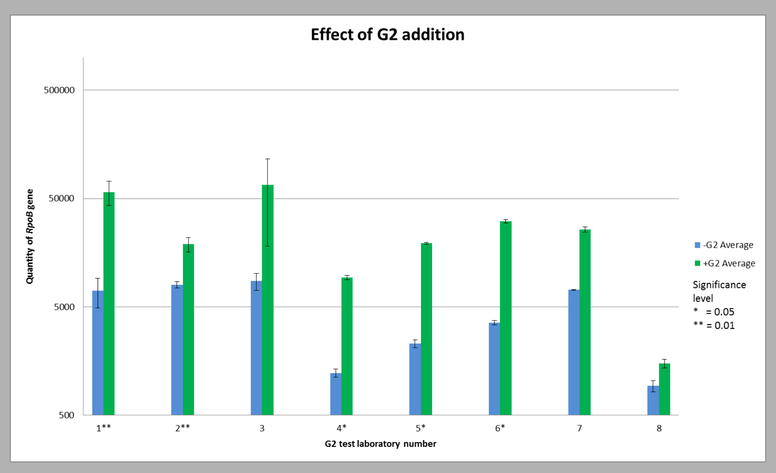
Optimization of DNA/RNA extraction efficiency from clayey sediments. The method was optimized with the addition of different amount of the blocker. In some cases we were able to increase yields more than 10.000 fold.



Setup

- 200 ul of KB-1
- 200 mg of sediment
- DNA/RNA extracted using the modified Griffith protocol (Bælum et al 2008)

Conclusion and multi-laboratory test of G2 effect in clay subsoil



Some details

A clay subsoil (0.8 meters depth) from Kolding, Denmark was freeze-dried and send to >10 laboratories together with a MoBio PowerLyzer PowerSoil kit with 25 normal and 25 G2 amended bead tubes. Out of these 8 laboratories has yet returned samples. The samples are analyzed using r*po*B gene copy number.

The triplicate DNA extracts are tested for significance using T-test .

CONCLUSION:

- ✓ In low biomass clay sub-soils addition of the DNA based blocker (G2) has large effect.
- ✓ Using a homebrewed (phenol/chloroform/PEG) based extraction protocol the increase in yield was more than 1000 times
- ✓ Using a well buffered commercial kit (Mobio PowerLyzer PowerSoil) the increase in yield with G2 was more modest (between 2 and 10 times yield

References

D J Levy-Booth, R G Campbell, R H Gulden, M M Hart, J R Powell, J N Klironomos, K P Pauls, C J Swanton, J T Trevors, K E Dunfield (2007).Cycling of extracellular DNA in the soil environment. Soil Biol. Biochem. 39:2977–2991

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