DNA Extraction using FastDNA Spinkit for Soil with PolyA Modifictaion

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dx.doi.org/10.17504/protocols.io.ur8ev9w

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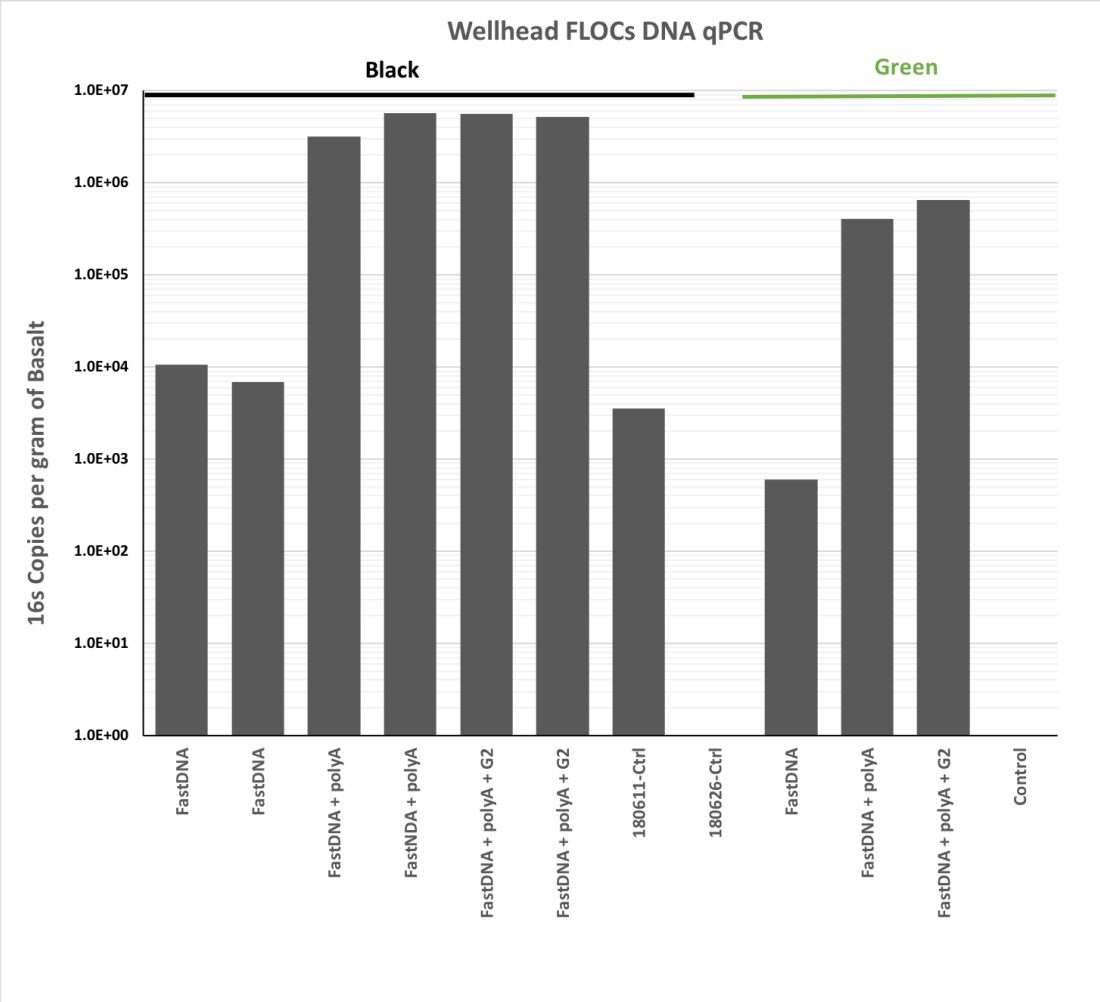
ABSTRACT

This protocol describes a modification to the MP Biomedicals FastDNA SpinKit for Soil. It has been changed to use a Retsch MM400 Mixer Mill instead of the MP Biomedicals bead beating machine. The addition of polyadenylic acid (poly(A)) is also used, which we have emperically determined increases DNA yeild from basalt substrates. This protocol is a modification from the protocol described in Jorgensen and Zhao (2016). Here we do not use the Amplicon G2 DNA/RNA Enhancer Tube because we found that it did not markedly increase DNA yeilds. See figure below.



qPCR analysis from DNA extracts of two incubated Basalt samples. Results show that using the Amplicon G2 DNA/RNA Enhancer tube does not markedy increase DNA yeild on Basalt samples.
FastDNA = Following the MP Biomedicals Protocol, FastDNA + PolyA = As described in this protocol, FastDNA + PolyA + G2 = As described in this protocol but using the G2 Tube instead of the MP Biomedicals bead tube.

NOTE: polyA sometimes creates false-positive readings for no-template control extractions when measuring DNA concentration with the Qbit Fluorometric Assay. Quantitive-PCR will allow you to determine if NTC samples are actually contaminated.

A description of a similar protocol can be found here:
Jorgensen, SL. And Zhao, R. (2016).Microbial Inventory of Deeply Buried Oceanic Crust from a Young Ridge Flank. Front. Microbio. 7

PROTOCOL STATUS

Working
We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
FastDNA Spin Kit for Soil111212		MP Biomedicals
Polyadenylic acid (poly-A_111212	27-4110-01	Ge Life Sciences

SAFETY WARNINGS

Prep PolyA dilution

1 Following the manufacturers protocol, create a stock solution of polyA in filter sterilized molecular grade water. We have used 4 ug/ul this may be modified depending on the circumstances.

FastDNA Spinkit Protocol with modifications

2 thaw out samples and polyA solution (4 ug/ul), get reagents from Fast DNA kit, turn on refrigerated centrifuge

3 Carefully transfer sample and 978 ul of SPB,122 uL MT buffer and 64 ul PolyA solution into tube (if stock is 4 ug/ ul)

4 Put tube in Retsch M400 shaker holder, shake 30 r/s for 5 minutes to homogenize

FastDNA SpinKit Protocol with modifications

5 Under a hood, transfer homogenate into new tube, add 250 uL PPS, invert 10 times and centrifuge for 5 minutes at 14,000 g

6 Transfer precipitate to 15 ml tube, add 1 ml binding solution, invert for 2 minutes, let sit for 3 minutes

7 Dispose of 500 ul of supernatant. Mix remaining supernatant and binding matrix by pipetting. Transfer 600 uL of DNA solution to a Spin Filter Tube. Discard waste. Repeat until all binding matrix has been run through the column.

8 Add 500 uL of SEWS-M Solution, centrifuge. Dispose of waste

9 Air dry fillter column for 5 minutes then centrifuge for 2 minutes at 14,000g. Transfer filter column into fresh elution tube.

10 Elute DNA using 100 ul of DES for 1 minute at 14,000g