



# METAGENOMICS (ALMOST) OF GREENLAND GLACIAL ICE



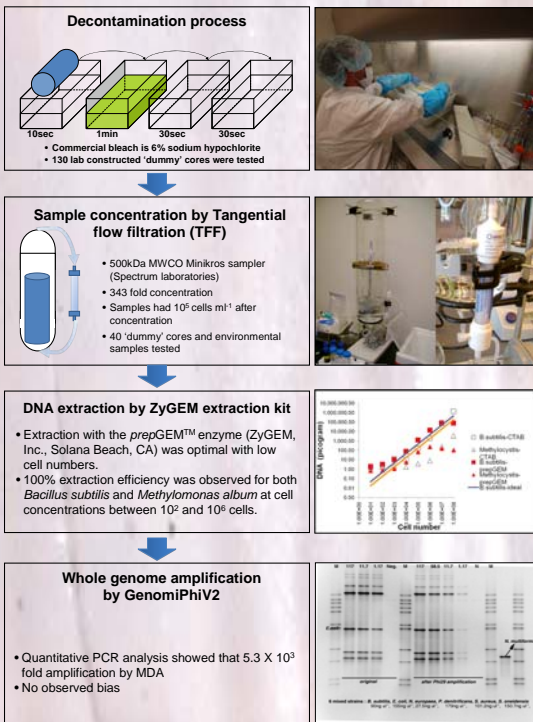
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## Abstract

A variety of recent studies of ice cores indicate the presence of a microbial assemblage that may be active and adapted to life in the ice. To understand the mechanisms of adaptation to the extreme cold, dark and isolated (hence oligotrophic) and potentially high salt and/or low pH conditions found in ice core environments, we have undertaken a metagenomic approach. Due to the extremely low abundance of cells and paucity of sample, we developed an extensive hypochlorite bleach-based decontamination protocol and DNA cell concentration and extraction protocols. As a test of our protocols, we decontaminated, melted, and concentrated particles from a sample of ice from the Greenland Ice Sheet Project 2 core (GISP2f). After decontamination and melting the cell count using DAPI was determined to be  $3.2 \times 10^3$  cells  $ml^{-1}$ . Following a 343-fold concentration via tangential flow filtration, DNA was extracted using the prepGEM™ enzyme. Subsequent multi-strand displacement amplification (MDA) provided sufficient genomic DNA for PCR amplification and/or cloning. Sequencing results indicated the presence of relatives of *Bacillus simplex*, *Paenibacillus chondronitinus*, *Bradyrhizobium* spp. and members of the Gemmatimonadales. We are currently characterizing functional genes from this sample as well. These protocols are broadly useful for all ice core samples, and the resulting metagenomic and single cell genomic sequences will provide insights into adaptations to life in ice.

## Introduction

- More than 70% of Earth's fresh water occurs as ice (1) and recent studies indicate the presence of microorganisms, some of which may be active, in glacial ice (2, 3).
- Little is known about the adaptations necessary to survive and/or metabolize in the permanently cold, dark, and oligotrophic environment found in ice.
- Due to extremely low cell abundances, issues with possible contamination and obtaining sufficient biomass have precluded the use of advanced molecular biological methods to examine adaptation in such environments.
- We developed an integrated protocol that decontaminates ice cores, concentrates cells in a sealed, sterile environment, extracts DNA from cells in a single tube reaction, and provides sufficient DNA amplification for subsequent PCR-based or cloning steps.
- We tested these protocols extensively on lab-constructed ice cores and samples from the GISP2f core from Greenland. Here we report the composition of the microbial assemblage in the GISP2f core section.



## Results

Age	1660-1670 AD
depth (m)	102.00-103.00
Location	72° 36' N, 38° 30' W; 3200 masl
Major Cations (ppb)	Na <sup>+</sup> (2.1), Mg <sup>2+</sup> (0.73), K (0.64), Ca <sup>2+</sup> (3.6), NH <sub>4</sub> <sup>+</sup> (4.2)
Major Anions (ppb)	Cl <sup>-</sup> (6.3), SO <sub>4</sub> <sup>2-</sup> (31.6), NO <sub>3</sub> <sup>-</sup> (78.6)
Cell Abundance	$3.2 \times 10^3$ cells $ml^{-1}$

Table 2. Characteristics of GISP2f Ice core

- Major cations and major anions are averages of the 3 depths.
- Cell abundance determined in this study.

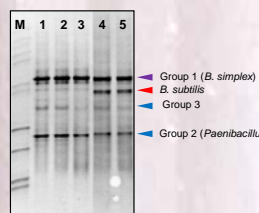


Figure 1. DGGE of GISP2f core from 102.00-102.40 meters.

- Lanes result from replicate extractions performed on separate dates
- No *B. subtilis* observed
- Three dominant bands related to *Bacillus simplex*, *Paenibacillus chondronitinus*, and Gemmatimonadales were present.
- Lanes 4 and 5 are from failed decontamination trials

Major group	Number of clones	Proportion of clone library (%)	Nearest Neighbor	Accession number	Similarity (query / subject)
group1	114	70.8	<i>Bacillus simplex</i> isolate Ph18.16S ribosomal RNA gene, partial sequence	AY833099	98 (960/980)
group2	12	7.5	<i>Paenibacillus alginolyticus</i> gene for 16S rRNA, partial sequence	AB073362	97 (959/987)
group3	6	3.7	Uncultured Gemmatimonadetes bacterium clone AKYH968.16S ribosomal RNA gene, partial sequence	AY922177	97 (865/892)
group4	4	2.5	<i>Bacillus</i> sp. BAM295 gene for 16S ribosomal RNA, partial sequence	AB330406	98 (767/783)
group5	4	2.5	<i>Bacillus</i> sp. MHS035.16S ribosomal RNA gene, partial sequence	DQ993300	99 (753/757)
group6	3	1.9	<i>Bradyrhizobium</i> sp. BTA1, complete genome	CP000494	98 (897/914)
group7	3	1.9	Uncultured bacterium clone FCP694.16S ribosomal RNA gene, complete sequence	EF516124	97 (793/818)
group8	2	1.2	<i>Bacillus</i> sp. MHS035.16S ribosomal RNA gene, partial sequence	DQ993300	98 (894/909)
group9	2	1.2	<i>Bacillus</i> sp. BAM520 gene for 16S ribosomal RNA, partial sequence	AB300599	96 (859/891)
Single	11	6.8	various		
Total	161	100%			

Table 3. DOTUR analysis of the 16S rDNA clone library on GISP2f ice core sample

- DOTUR analysis was performed and the sequence identification was grouped by 3% tolerance level (sequence dissimilarity).

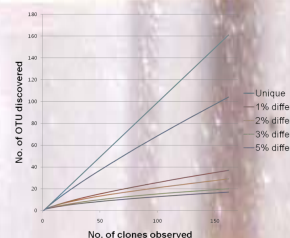


Figure 4. Rarefaction curve based on 16S rRNA BLAST results.

- 1:1 line represents the number of unique OTU if every clone were unique
- Unique line represents the number of OTU in GISP2f using 0% difference; 3% difference line represents the number of unique OTU in GISP2f using 3% dissimilarity

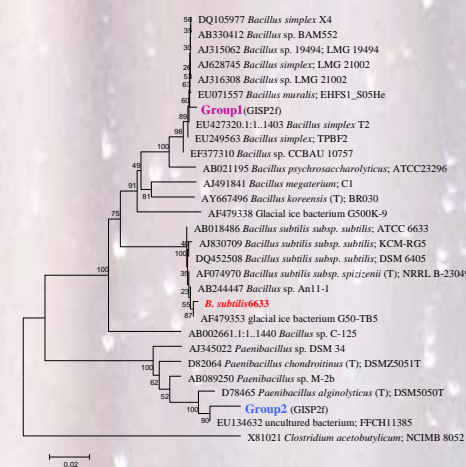


Figure 3. Phylogenetic tree of *Bacillus subtilis* and relatives.

- Bacillus*-related 16S rRNA gene clones from the GISP2f core were <95% similar to the *Bacillus subtilis* strain used to contaminate the outside of the ice core. Furthermore, ITS sequences were different lengths and did not align.

- Thus, we conclude that the GISP2f *Bacillus* clones are derived from in situ DNA.
- The green represents sequences from GISP2f; red represents *B. subtilis* ATCC 6633

## Summary

- Our integrated protocol thoroughly decontaminated, concentrated, and amplified DNA from low-abundance microbial assemblages in ice cores.
- Through intentional contamination of the ice core surface, we have demonstrated complete decontamination of the GISP2f ice core.
- 16S rRNA gene sequences found in the GISP2f samples included *Bacillus simplex*, *Paenibacillus chondronitinus*, *Bradyrhizobium* species and members of the Gemmatimonadales.
- Currently we are characterizing functional genes in the samples.

## References

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