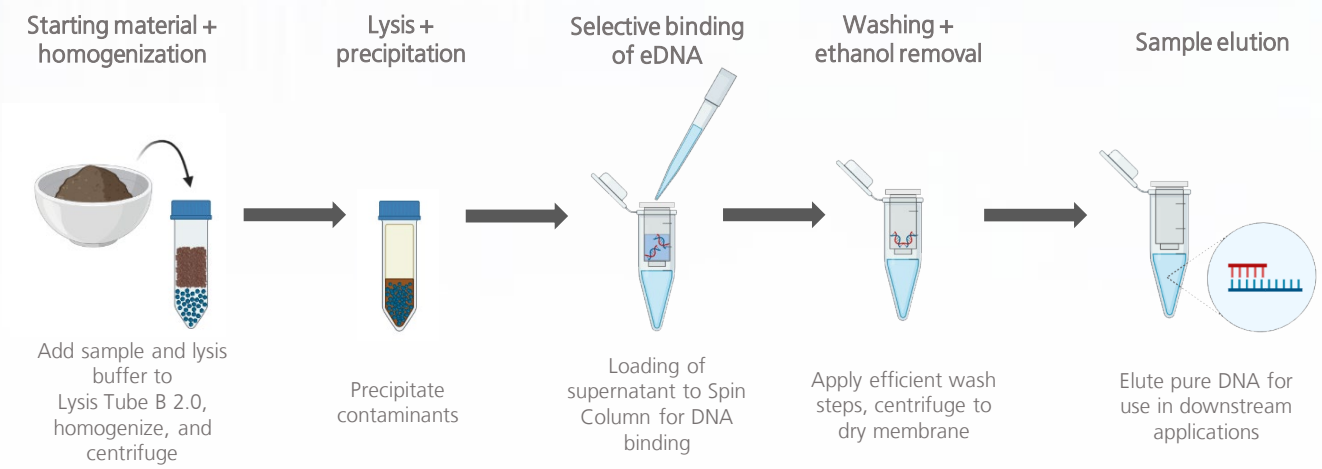
Application Note – innuSPEED Soil DNA Kit 2.0

Excellent yield and purity of eDNA for a variety of downstream applications

Introduction

Understanding the ecology and diversity of microbial communities is challenging due to the difficulty in culturing most microorganisms taken from environmental samples. Consequently, culture-independent molecular analyses of microbial community structures are generally performed. However, the presence of soil compounds such as humic and fulvic acids, which are known to be inhibitory to downstream applications like PCR, has become a major concern in extracting high-quality DNA. Thus, a rapid and robust method of providing higher yield and quality of DNA is important. The innuSPEED Soil Kit 2.0 has been designed as a tool for extracting environmental DNA (eDNA) from soil samples. The kit is based on a patented DNA extraction technology and combines an initial, very efficient homogenization step with the subsequent binding of the DNA on a spin filter. After washing steps, the bound DNA is eluted using a low salt elution buffer. The extracted DNA can be used for different downstream applications like PCR, qPCR, Digital PCR or Nanopore Sequencing.

Workflow: eDNA extraction from soil samples



Materials and Methods

A. Soil DNA Extraction

DNA was extracted from 400 mg of soil sample using the innuSPEED Soil DNA Kit 2.0 (845-KS-1580010/50, IST Innuscreen GmbH) according to the user manual.

B. Spectrophotometry and Genomic DNA Assay

Soil DNA quality and quantity were measured using NanoPhotometer N60® (Implen GmbH). A total of 1 µl was run on Agilent 4150 TapeStation System (Agilent) to determine DNA yield via Genomic DNA Assay.

C. Further analyses via different downstream applications

Purified DNA was used as template for quantitative PCR (qPCR) analysis, for digital droplet PCR (ddPCR) analysis, and for Nanopore sequencing. For qPCR, a total of 1 µl of soil DNA was quantified using innuDETECT Bacteria Quantification Assay (IST Innuscreen GmbH; 845-IDF-0031024/96) following manufacturer's instruction. qPCR reaction was run on CFX Connect Real-Time PCR Detection System (Bio-Rad). To detect possible inhibitory effects in the extracted soil DNA sample, a positive control was added in the sample run.

For ddPCR, a 1:100 dilution of soil DNA was prepared by adding 1 µl of purified DNA to 99 µl of ddH_2O. Additionally, a 1:1000 dilution was prepared by adding 1 µl of previously diluted soil DNA to 9 µl of ddH_2O. A total of 1 µl of the 1:100 and 1:1000 diluted soil DNA was then added to separate Crystal Digital PCR™ Mastermixes (Stilla Technologies GmbH) following manufacturer's protocol. The PCR was performed on a Sapphire Chip (Stilla). The ddPCR cycling program was as follows: an initial partitioning step at 25 °C, denaturation at 95 °C for 3 min; followed by 45 cycles of 95 °C for 10 sec, 56 °C for 15 sec; and chip release. ddPCR reaction was performed on naica® system for Crystal Digital PCR™ (Stilla). The fluorescence channel FAM was used to detect positive droplet counts. Data acquisition was done using Crystal Miner v3.1.6.3 software.

For Nanopore sequencing, a total of 10 µl of soil DNA was added to innuDRY qPCR MasterMix probe (IST Innuscreen GmbH; 845-AS-1200100/200) following the manufacturer's protocol. Library preparation was done using the 16S Barcoding Kit (Oxford Nanopore Technologies). Sequencing was performed using SpotON™ Flow Cell on MinION Mk1B (Oxford Nanopore Technologies) and data acquisition was done using MinKNOW v22.03.4 software.

Results and Discussion

A. DNA purity and yield

The quality and quantity of DNA extracted from soil samples were determined via Spectrophotometry (Figure 1) and Genomic DNA Assay (Figure 2), respectively.

Spectrophotometric measurements provide data for assessing DNA purity of the sample. The absorbance ratios $A_{260/280}$ and $A_{260/230}$ both provide information on possible contaminants present in the sample. The resulting $A_{260/280}$ absorbance ratio of 1.84 and $A_{260/230}$ absorbance ratio of 2.15 showed a high DNA purity of the samples.

Results of the Genomic DNA Assay performed showed a DNA size spectrum between 3,000 bp to 19,800 bp and a DNA yield of 3.45 µg. This translates to a DNA concentration of 34.5 ng/µl. These results indicate a high DNA yield.

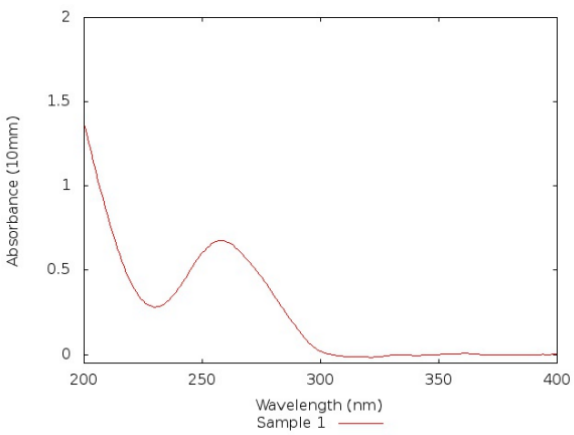


Figure 1. Spectrophotometric measurement of DNA extracted from soil. Total DNA was isolated from 400 mg of soil sample using innuSPEED Soil DNA Kit 2.0. DNA purity was determined using NanoPhotometer N60®. Results showed a high quality of purified DNA.

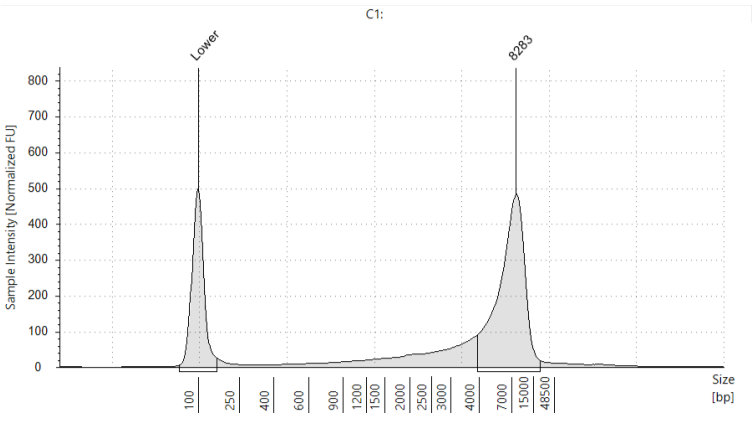


Figure 2. Genomic DNA assay result of DNA extracted from soil. Total DNA was isolated from 400 mg of soil sample using innuSPEED Soil DNA Kit 2.0. Following isolation, a total of 1 µl was loaded on Agilent 4150 TapeStation System to determine DNA yield. Results showed a wide DNA size spectrum and a high DNA yield.

B. Analyses of different downstream applications

A qPCR analysis was performed to determine total bacterial count and to assess a successful amplification of extracted soil DNA (Figure 3). Results showed that DNA isolated from soil samples have a total bacterial count of approximately 1×10^7 per µl sample. The value is high compared to the total bacterial count of the positive control, which is approximately 1×10^5 per µl sample. Nearly identical Cq values were observed from soil DNA. Furthermore, the difference between the DNA extracted from soil samples and the positive control was approximately 4 cycles. This indicated no inhibition took place in the sample run.

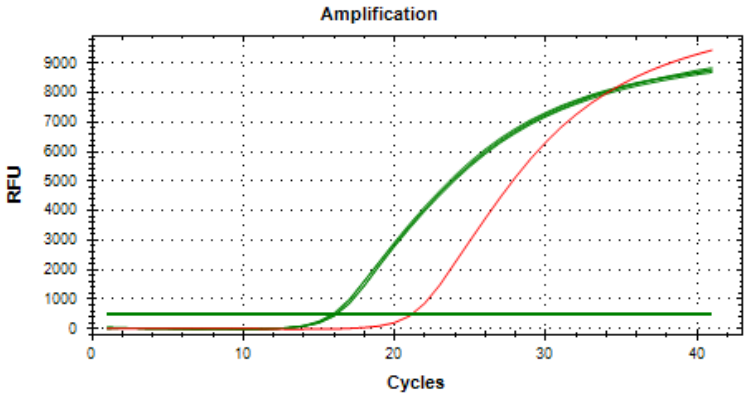


Figure 3. Successful amplification of eDNA extracted from soil. Total DNA was isolated from 400 mg of soil sample using innuSPEED Soil DNA Kit 2.0. qPCR was performed using primers on 1µl of DNA isolated from soil (green line) compared to a positive control (red line). Results showed a high total bacterial count and no inhibitory reaction occurred during the analysis.

A ddPCR analysis was performed to determine total DNA copies per µl samples and to assess a successful amplification of extracted soil DNA (Figure 4). Results of the analysis calculated a total of $1 \times 10^6 - 1 \times 10^7$ per µl sample for both the 1:100 and 1:1000 diluted soil DNA samples. This number indicated a high total DNA detected in the analysis.

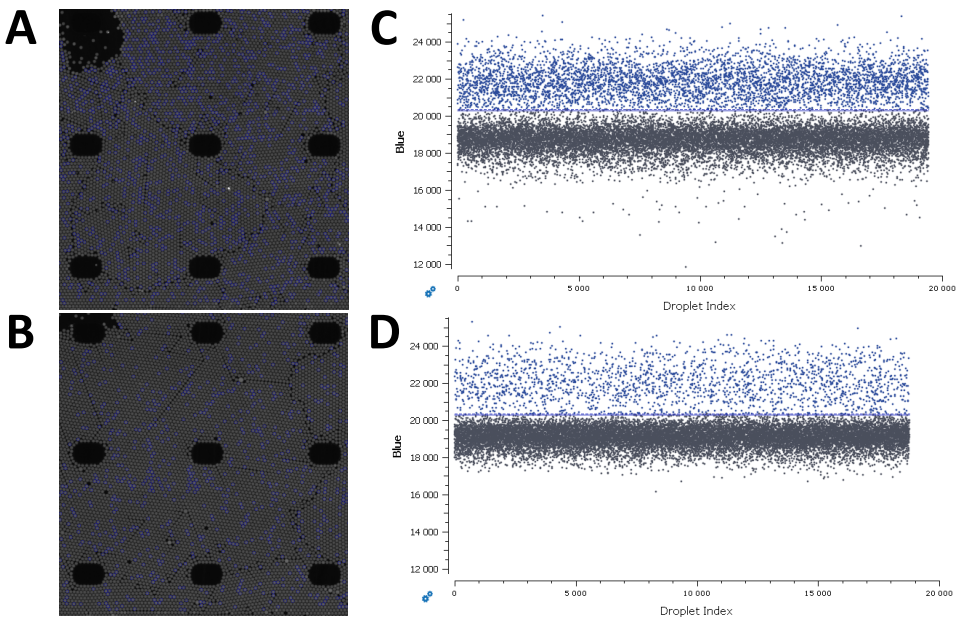


Figure 4. High number of total DNA copies detected from soil sample. Total DNA was isolated from 400 mg of soil sample using innuSPEED Soil DNA Kit 2.0. ddPCR was performed using 1 µl soil DNA using naica® system for Crystal Digital PCR™. Microscopic image of the droplets for the 1:100 diluted (A) and 1:1000 diluted (B) soil DNA samples are shown, with the positively detected droplets marked in blue. The clustering scatter plot of the 1:100 diluted (C) and 1:1000 diluted (D) soil DNA samples, showing the separation of positive (in blue) and negative (in gray) droplets based on their fluorescence intensity. Overall, results showed a high number of total DANN copies detected in the analysis.

A Nanopore sequencing run was performed to determine data and sequence output of DNA/RNA bases and to assess a successful amplification of extracted soil DNA (Figure 5). DNA sample extracted from soil and sequenced using Oxford Nanopore sequencing technology generated high-quality read data and very good throughput (Figure 5A; Table 1). Sequence parameters were also determined to detect possible issues in the sequence run. Results of the sequence parameters showed a sucessful sequence run (Figures 5B – D).

Table 1. Ocford Nanopore sequencing metrics generated using DNA extracted from soil samples.

Sample	Run Summary				Run Duration (h)	Basecalling		
	Estimated bases (Gb)	Reads generated (M)	Data Produced (Gb)	Estimated read length N50 (kb)		Reads called (%)	Passed Base calling (Gb)	Failed base calling (Gb)
Soil DNA	7.92	5.21	103.08	1.58	26.06	100	6.4	1.09

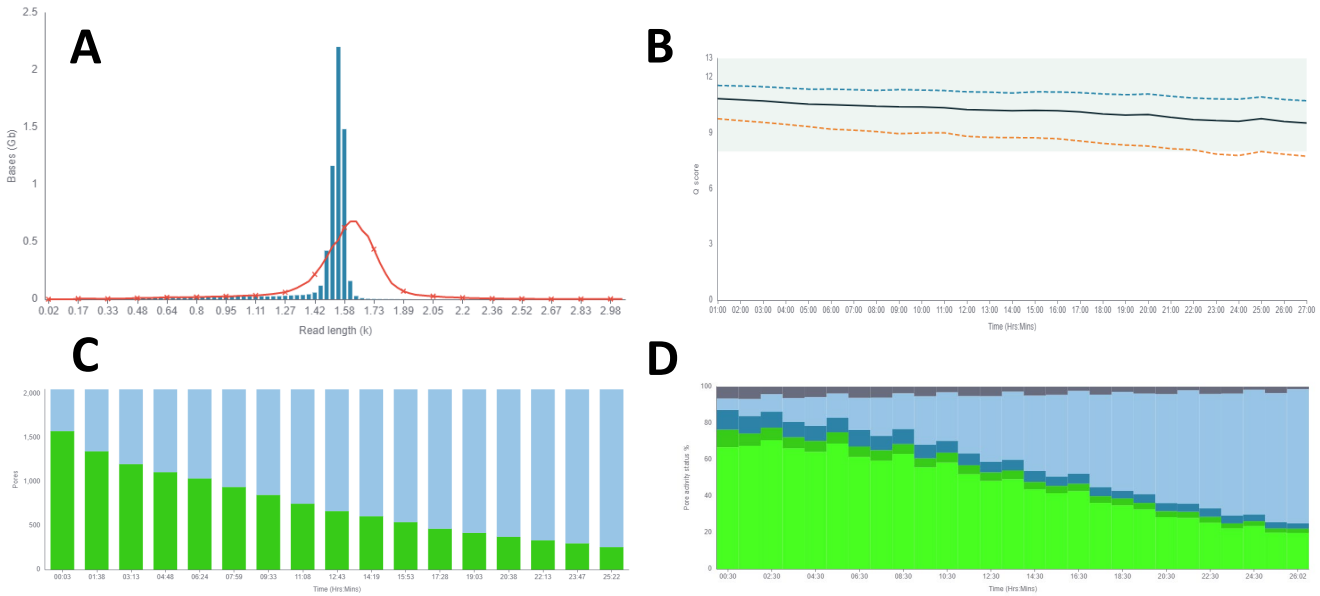


Figure 5. High data and sequence output generated from extracted DNA soil sample. Total DNA was isolated from 400 mg of soil sample using innuSPEED Soil DNA Kit 2.0. Nanopore sequencing was performed using 10 µl soil DNA on SpotON™ Flow Cell run on MinION Mk1B. The sequence output (A) showed the total number of successful bases compared to read length. Read line indicates the estimated basecall. The quality score (B) was calculated as basecalling in the device and showed a high-quality score (Min. Q score > 8). Black line indicate median, orange dotted line indicated 25% quartile, and blue dotted line indicates 75% quartile. The pore activity (C) showed sample performance during sequence run while pore scan (D) showed current status of pores within channels on a flow cell. For both parameters, results showed a high proportion of available and active pores at the start of the run. Green bars indicate active pores for sequencing while blue bars indicate inactive pores for sequencing. Overall, results showed a very high quality read data and sequence throughput.

Conclusion

The results show that the innuSPEED Soil DNA Kit 2.0 is successfully able to extract DNA from environmental soil samples. The DNA quality and quantity are sufficient for several downstream application such as qPCR, ddPCR, and Nanopore sequencing as depicted in Figures 3, 4 and 5, respectively.