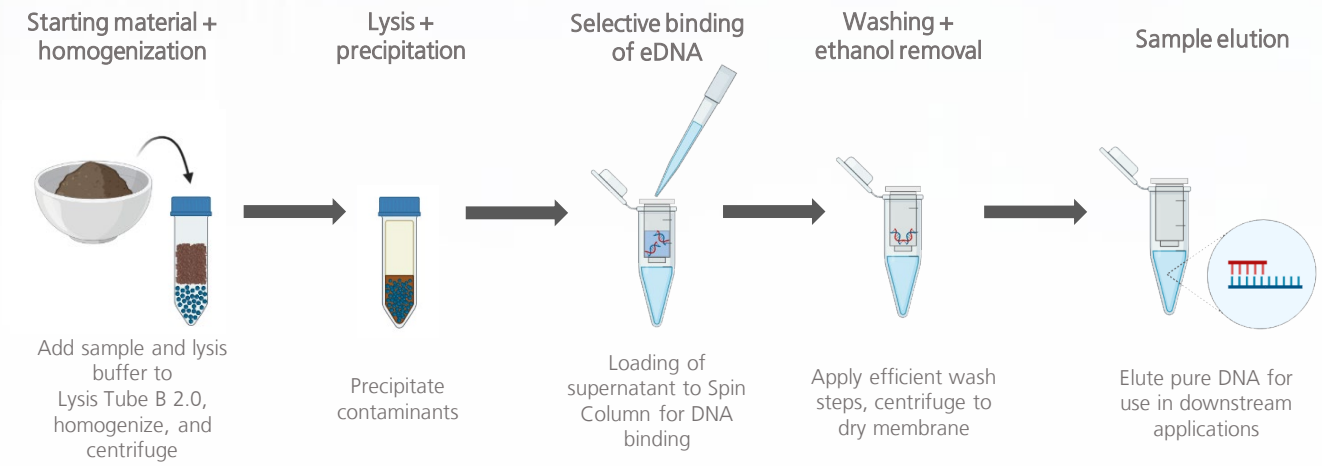
Application Note – innuSPEED Soil DNA Kit 2.0

Superior performance in direct comparison with a common competitor

Introduction

Microbial communities play essential and unique roles in the sustainability and functioning of diverse ecosystems. The advancement and implementation of various nucleic acid-based techniques in ecological research greatly contributed to our understanding of the dynamics and activities of microbial communities in natural environments, especially since these analyses surpass the limitations of culture-dependent methodological approaches. These molecular and genomic techniques, however, depend heavily on the yield and purity of extracted nucleic acids from environmental samples. Co-purification of soil organic compounds, particularly the known PCR inhibitors fulvic and humic acids, often results in poor DNA quality. Therefore, a quick, cost-effective method of environmental DNA extraction from soil samples is essential. The innuSPEED Soil DNA 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 quality and quantity of purified soil DNA was compared to a competitor kit to certify the kit's performance.

Workflow: eDNA extraction from soil samples



Materials and Methods

A. Soil DNA Extraction

DNA was extracted from 400 mg of soil samples taken from 3 different sources using the innuSPEED Soil DNA Kit 2.0 (845-KS-1580010/50, IST Innuscreen GmbH) and a main competitor kit according to their manufacturer's protocol.

B. Spectrophotometry and Genomic DNA Assay

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

C. Quantitative real-time (qPCR) Analysis

Purified DNA from both kits was used as template for qPCR analysis. In brief, a total of 1 µl of soil DNA for each of the three soil samples 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).

Results and Discussion

A. DNA purity and yield

The quality and quantity of DNA extracted from soil samples using both the IST kit and the main competitor kit 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. For the three soil DNA samples extracted using the IST kit, the resulting average $A_{260/280}$ absorbance ratio of 1.90 and average $A_{260/230}$ absorbance ratio of 2.10 showed a high DNA purity of the samples. For the soil DNA samples extracted using the main competitor kit, the average $A_{260/230}$ absorbance ratio of 0.06 showed a carryover of contaminants in the samples (Table 1). Co-purification of contaminants would affect several downstream applications such as PCR.

Results of the Genomic DNA Assay performed showed that, despite good DNA yield extracted using both kits, the DNA yield of soil samples extracted using the IST kit was higher compared to the main competitor kit. The average DNA yield of soil samples extracted using the IST kit is 3.1 µg, which corresponds to a DNA concentration of 31 ng/µl. The average DNA yield of soil samples extracted using the competitor MP kit is 1.77 µg, which corresponds to a DNA concentration of 17.7 ng/µl (Table 2).

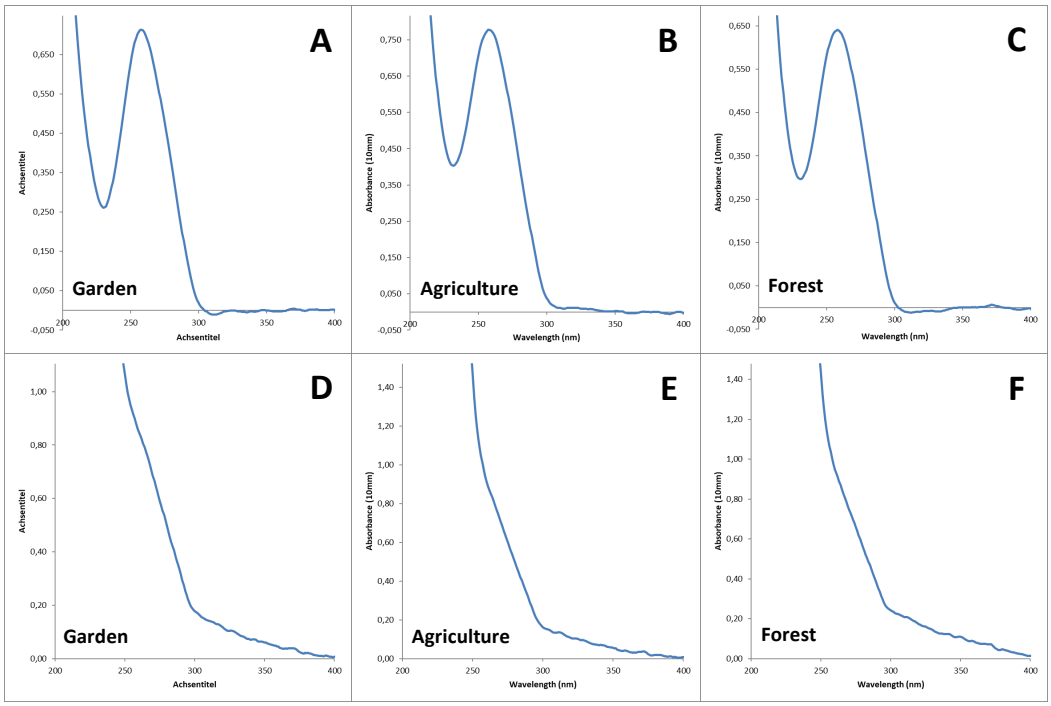


Figure 1. High purity of DNA samples isolated from different soil samples. Total DNA was isolated from 400 mg of soil samples taken from three different sources using innuSPEED Soil DNA Kit 2.0 (A – C; IST Innuscreen GmbH) and a main competitor kit (D – F). DNA purity was determined using NanoPhotometer N60®. Results showed a high quality of purified DNA using the IST kit. DNA purified by the main competitor kit showed a low $A_{260/230}$ absorbance ratio, indicating copurification of impurities or contaminants. Overall, results indicate that the eDNA extracted using the IST kit has higher DNA quality compared to the main competitor kit.

Table 1. Spectrophotometric measurements of eDNA extracted using IST Kit and the main competitor kit.

Kit	Sample	$A_{260/280}$	Mean	$A_{260/230}$	Mean
Competitor Kit	Sample 1	1.9	2.0	0.09	0.06
	Sample 2	2.1		0.04	
	Sample 3	2.0		0.05	
IST Kit	Sample 1	1.9	1.9	2.2	2.1
	Sample 2	1.9		2.0	
	Sample 3	1.9		2.1	

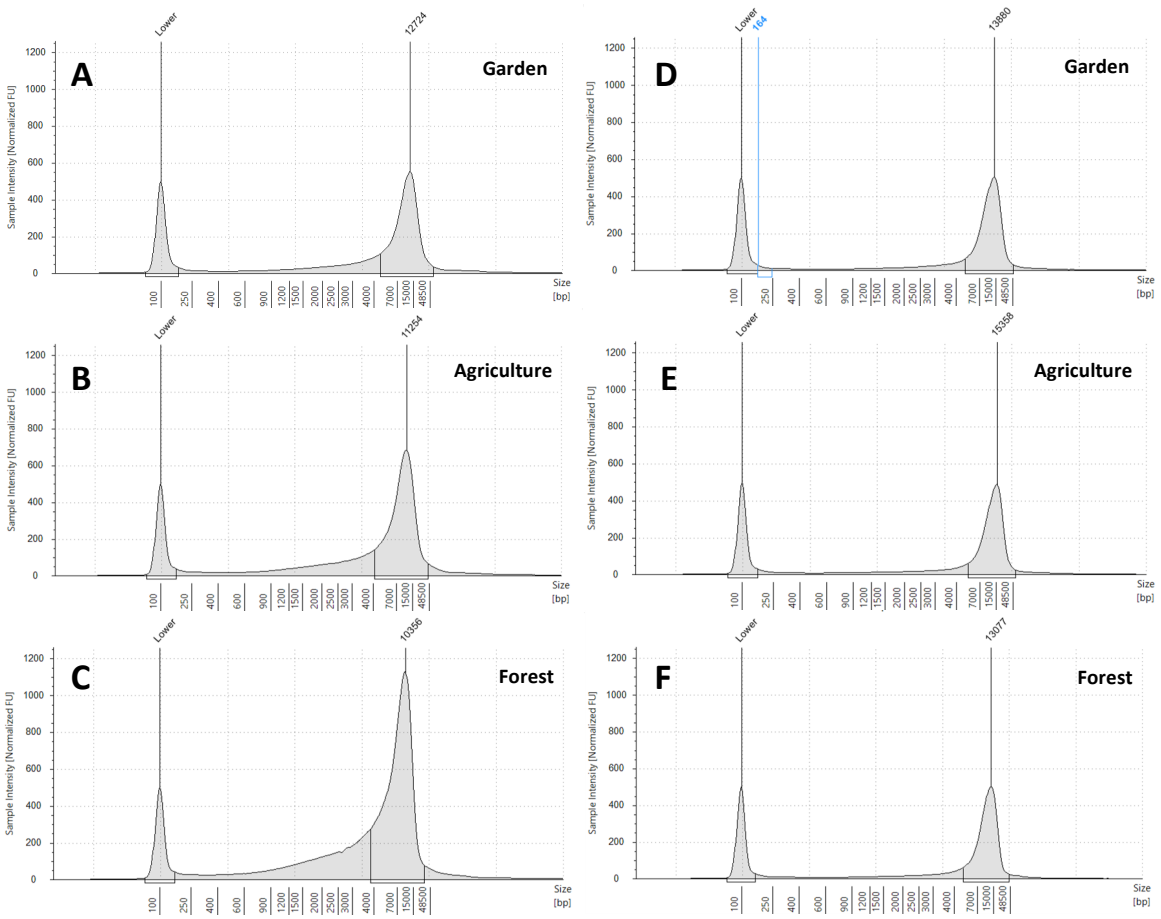


Figure 2. High yields of eDNA extracted using IST Kit compared to the main competitor kit. Total DNA was isolated from 400 mg of soil samples taken from three different sources using innuSPEED Soil DNA Kit 2.0 (A – C; IST Innuscreen GmbH) and the main competitor kit (D – F). Following isolation, a total of 1 µl was loaded on Agilent 4150 TapeStation System to determine DNA yield. Results showed the eDNA extracted using the IST kit has generally higher DNA yield compared to the main competitor kit.

Table 2. Higher yields of eDNA extracted using IST Kit compared to the main competitor kit.

Kit	Sample	DNA Yield (µg)	Mean
Competitor Kit	Sample 1	1.8	1.77
	Sample 2	1.7	
	Sample 3	1.8	
IST Kit	Sample 1	2.0	3.1
	Sample 2	2.8	
	Sample 3	4.5	

B. Analyses via qPCR application

A qPCR analysis was performed to determine total bacterial count and to assess a successful amplification of extracted soil DNA (Figure 3) between the IST kit (shown in green lines) and the main competitor kit (shown in red lines). Results showed that amplification of DNA isolated using the main competitor kit is, in average, delayed by 2 Ct compared to amplification of DNA isolated using the IST kit. This delay could be explained by amplification of unspecific PCR products generated during the PCR run. These, in turn may affect the amplification of the target DNA via inhibition. This theory can be supported by the generally low $A_{260/230}$ absorbance ratio previously measured (Figure 1; Table 1).These results, in turn, showed that soil DNA extracted using the IST kit amplified no inhibiting PCR products. This further showed that while spectrophotometric reading is important to determine DNA quality, qPCR analysis is also important as a means to validate the quality of extracted DNA from soil for downstream applications.

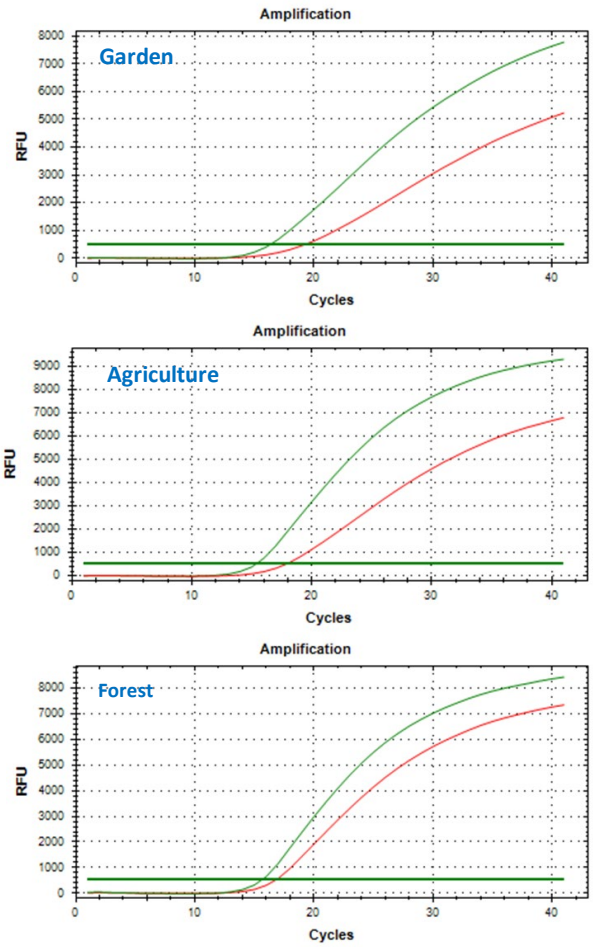


Figure 3. Successful amplification of eDNA using IST kit compared to main competitor kit. Total DNA was isolated from 400 mg of soil samples taken from three different sources using innuSPEED Soil DNA Kit 2.0 (IST Innuscreen GmbH) and the main competitor kit. qPCR was performed using primers on 1µl of DNA isolated from soil using the IST kit (green line) and the main competitor kit (red line). Results showed that delayed amplification of extracted DNA using the main competitor kit could be due to amplification of unspecific PCR products that interfered with amplification of the target DNA. These showed that DNA extracted using the IST kit amplified no inhibiting PCR products.

Conclusion

The results show that the innuSPEED Soil DNA Kit 2.0 outperforms the main competitor kit in terms of not only in DNA quality and yield but also in a successful downstream applications such as PCR. DNA extracted using the kit was able to successfully amplify the target sample without any inhibition during the qPCR run.