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Measuring the Microbial Limit of Detection for a Modified DNA Extraction Protocol

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Biology

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Abstract

A typical kit-based DNA extraction only isolates ~17% of the total starting DNA. When analyzing environmental samples with few microbial cells, this would decrease the limit of detection. In this study, we developed a modified DNA extraction protocol and tested the limit of detection using known amounts of *Escherichia coli (E. coli)* cells. A dilution series was conducted using *E. coli* over a range of one million cells (1 x 10^6) down to ten cells. DNA was isolated from each of the aliquots using the modified protocol. Polymerase chain reactions (PCR) followed by ethidium bromide staining of agarose gels was used to test the presence of template DNA. The limit of detection by this method was found to be 1 x 10^5 cells.

Background

In microbiology, molecular DNA techniques are frequently used to detect microbial life in environmental samples. Molecular analyses of soil microbial communities depend on the extraction and isolation of DNA from soil, and its amplification using PCR. When working with samples that contain small amounts of DNA, typical kit-based extraction protocols are ineffective; the most efficient of these approaches are only able to isolate ~17% of total DNA from a sample (Claassen, du Toit, Kaba et al. 2013). Instead of kits, some researchers use their own modified DNA extraction protocols to improve the recovery of DNA from environments with low numbers of microbes, such as our sample sites in Wind Cave National Park, South Dakota.

Microbes are typically detected from environmental samples through the use of PCR to amplify the 16S small subunit ribosomal RNA gene (16S rRNA), which requires only small amounts of DNA as template in the amplification reaction. It is recommended to use a minimum of 300 ng of template for 16S rRNA analysis, and if the average bacterium contains ~3 femtograms of DNA, this would be a minimum of 6.6 x 10^8 cells required in the environmental sample (Hershey, Kallmeyer, Barton 2019). In the isolated environment of Wind Cave, Hershey et al. found that samples typically contained far fewer cells.

In this study, we measured the microbial limit of detection using our modified DNA extraction protocol to determine its utility for analyzing paleofill samples from Wind Cave and soil samples surrounding the cave system.

Methods

detergent	buffer	chelator	osmolarity	Enzyme	Incubation	Carrier	Precipitation	Enzyme
0.2% SDS	100 mM Tris (pH 8.0)	5 mM EDTA	200 mM NaCl	2.5 uL proK	60℃ 15 min.	4 uL LPA	0.5x volume 30% PEG : 1.6 M NaCl	3.75 uL RNAse A

Figure 1. Graphical representation of the modified DNA extraction protocol used in this study

The modified DNA extraction protocol included a lysis incubation that was conducted in a shaking incubator at 150 rpm. To visualize the location of the DNA pellet, 5 uL of 50% silica resin was added during the DNA precipitation step. A second DNA precipitation was conducted after the addition of
RNAse A.

The dilution series began with an overnight culture of *E. coli* that was grown in tryptic soy broth (TSB), and 1 mL of cells was pelleted for dilutions, as shown in Fig. 2. The initial culture concentration was 7.8 x 10^8 cells / mL. The broth was then removed and the pellet was resuspended in 1 mL of sterile saline (0.9% NaCl). 127 uL were transferred from that initial tube to 873 uL of saline, to create a suspension of 1 x 10^8 cells / mL. From there, each resulting dilution was by a factor of 10.

To prepare samples for limit of detection testing, 100 uL were taken from the 1 x 10^7 cells / mL dilution tube and transferred to a clean tube, resulting in 1 x 10^6 cells to undergo DNA extraction. The same was done for the 1 x 10^6 cells / mL and 1 x 10^5 cells / mL dilution tubes. To confirm dilutions were correct, cells from the most dilute samples were grown on petri dishes. 100 uL from the tube containing 1 x 10^3 cells / mL and the tube containing 1 x 10^2 cells / mL were placed onto separate TSA petri plates and grown overnight.

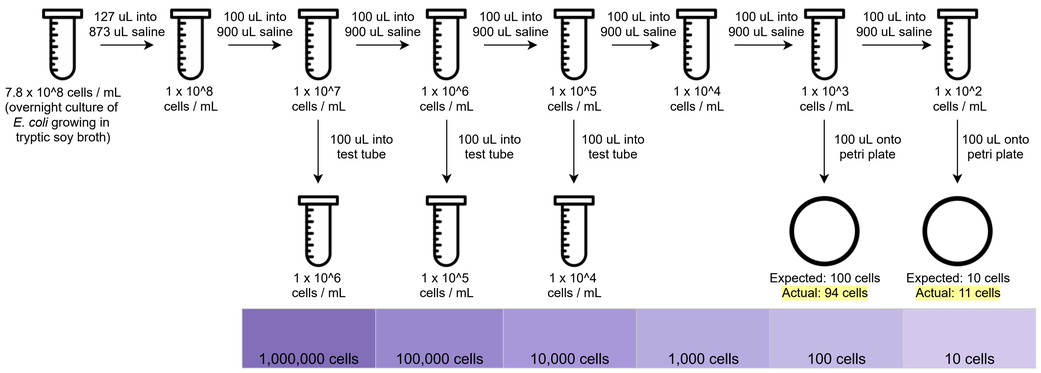

Figure 2. Graphical representation of dilution series for DNA extraction of small numbers of *E. coli* cells.

Results

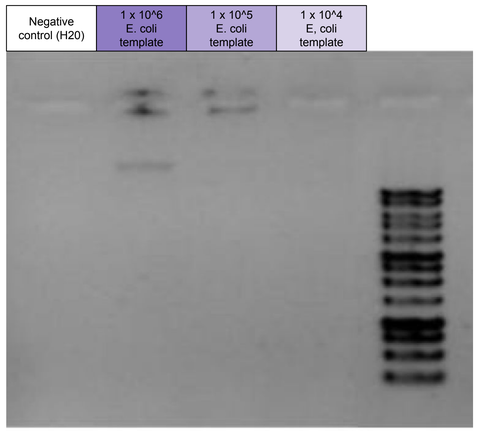

Figure 3. DNA extracted from *E. coli*

The *E. coli* DNA templates were checked via gel electrophoresis; no DNA was visible in the negative control, and 1 x 10^6 cells produced a faint band. The templates for 1 x 10^5 cells and 1 x 10^4 cells were below the limit of detection on our ethidium bromide stained agarose gel.

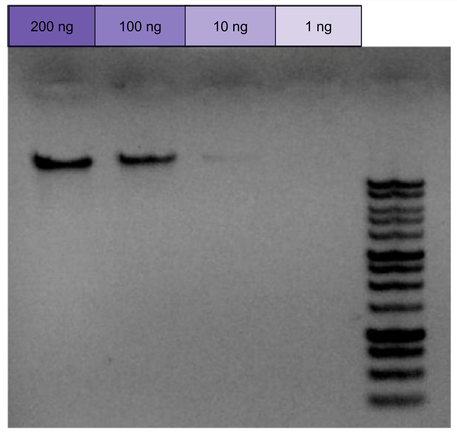

Figure 4. Standard curve of known DNA

Known quantities of DNA were checked via gel electrophoresis. Based on band intensity, 1 x 10^6 cells show a band between the darker well 2 and the fainter well 3; thus, we can conclude that the DNA band from 1 x 10^6 cells (Fig. 3) is between 100 ng and 10 ng.

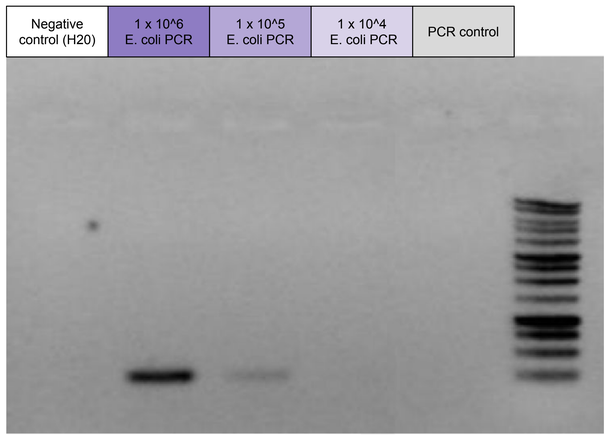

Figure 5. Limit of detection by PCR

The DNA extracted from *E. coli* samples (Fig. 3) was used in DNA testing via 16S rRNA PCR. The amplified DNA was checked via gel electrophoresis. Each negative control was uncontaminated, and both
1 x 10^6 cells and 1 x 10^5 cells were detected, as shown by the presence of bands.
1 x 10^6 cells showed a much darker band than 1 x 10^5 cells.
1 x 10^4 cells did not produce a band.

Our limit of detection was 1 x 10^5 cells.

#	Number of Cells	Expected DNA**	DNA Testing Result
1	1,000,000	18.3 ng	Detected
2	100,000	1.83 ng	Detected
3	10,000	0.183 ng	-
4	1,000	0.0183 ng	-
5	100*	0.00183 ng	-
6	10*	0.000183 ng	-

Table 1: Aliquots of *E. coli* cells used to measure limit of detection
*The 100 cell sample petri dish test contained 94 cells, and the 10 cell sample petri dish test contained 11 cells.
**Calculations based on 18.3 femtograms of DNA per *E. coli* cell (https://bionumbers.hms.harvard.edu/bionumber.aspx?s=n&v=3&id=104908).

Conclusions

Our modified DNA extraction protocol successfully isolated DNA from our *E. coli* aliquots.

Using PCR followed by ethidium bromide staining of agarose gels, the limit of detection was found to be 1 x 10^5 cells.

In future trials to improve DNA yields, samples will be centrifuged at higher speeds up to 100,000 g to pellet DNA.

Citations

So A, Pel J, Rajan S, Marziali A (2010) Efficient genomic DNA extraction from low target concentration bacterial cultures using SCODA DNA extraction technology. Cold Spring Harb Protoc 5(10):1150–1198.

Claassen S, du Toit E, Kaba M et al (2013) A comparison of the efficiency of five different commercial DNA extraction kits for extraction of DNA from faecal samples. J Microbiol Methods 94:103–110.

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