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DNA Recovery from Aerosol *F*iltration and *T*ransport

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Abstract

According to an article in *Science Matters*, the newsletter of the Environmental Protection Agency, the average adult breathes in over 3,000 gallons of air every day. For this reason, having good air quality is extremely important. Many allergen particles are present in the atmosphere that interfere with this quality. When HVAC systems are installed to direct airflow through buildings, many of these particles are transferred indoors. To ensure air quality within buildings, tracking the flow patterns of these particles as the air is dispersed through heating and cooling systems is vital. One method to monitor this is through the creation of aerosolized particles with a DNA barcode (about 100 base pairs) that can be released and recaptured. Once released, the particles can be collected on filters of air filtration systems and analyzed using polymerase chain reaction (PCR). Because the PCR is performed on the eluent from a filter, it is of interest to know the DNA barcode recovery from a variety of filter types using a typical extraction medium. This project sought to quantify DNA extraction from three different types of aerosol filters: polyester felt, Teflon, and glass fiber, using 0.1% Triton X-100 in phosphate buffered saline (PBS) buffer as the elution buffer. Percent recoveries were calculated by comparison to no-filter controls. The DNA concentrations tested were 2.4×10^4 , 2.4×10^5 , and 2.4×10^6 copies/ μL , which simulated the DNA present in 1mg, 100 μg , and 10 μg of particles. Through quantitative, real-time PCR analysis, the filter recovery was found to be approximately 100% for all concentrations of DNA and filter types investigated with no significant inter- or intra-day variability. This indicates that DNA barcodes are an excellent method for tracking aerosolized particles.

Introduction

Many buildings use air filtration systems to prevent the spread of small aerosolized particles such as allergens. The most common particles present in the atmosphere include bacteria and archaea, fungal spores and fragments, and pollen.¹ These particles can be transmitted by air currents to buildings. Once the particles enter buildings, they can cause great discomfort among the buildings' inhabitants. To minimize this discomfort, it is important to know the route that air takes through the buildings, both to validate computer models and to optimize the location of air filtration systems. These routes can be additionally complex because they are affected not just by the geometry of the building, but also by the external airflow patterns.² Knowing such routes can also aid in the placement of air ducts and the layout of buildings.

To meet this need, novel aerosol test particles were developed. These particles can be released in simulation of high aerosol contaminant levels and recovered on filters for air filtration systems. Because the particles are made from FDA-approved sugars, their release presents no threat to human health. The manufacture of these particles includes the addition of a DNA barcode that can be used to identify them. When the particles are recaptured, these barcodes are amplified using quantitative real-time polymerase chain reaction (qRT-PCR) assays. The PCR results enable the user to calculate the number of DNA copies present, which can be used to calculate the number of particles collected, assuming a known number of DNA copies/particle. The relative abundance of DNA on filters at various locations throughout the building as well as the time elapsed since release can be used to reconstruct the flow of air. PCR has been used as a method of quantifying amounts of unwanted aerosol particles.³ However with many PCR applications, the particles investigated are cells, resulting in a required cell lysis step. These aerosol test particles, however, are made from maltodextrin, a water soluble sugar, so no cell lysis is required, simplifying the analysis and lowering the amount of noise. Instead, the collected samples can simply be placed in buffer and analyzed. To decrease the likelihood of false positives due to environmental contamination, the DNA barcode in these particles is from *Thermotoga maritima*, a thermophilic bacterium found only in terrestrial hot springs and deep ocean thermal vents.

Before these particles can be useful for airflow analysis, the extraction efficiency from various types of filters must be known. If this efficiency is too small, indicating that the DNA barcodes become stuck in the filters, analysis of the particles collected will be impossible. If, however, the efficiency is high, the DNA extracted from the filters is an accurate representation of the particles collected. This project sought to quantify DNA extraction from three different types of aerosol collection filters: polyester felt, Teflon, and glass fiber.

Materials

The PCR kits were purchased from Invitrogen (Grand Island, NY) and were assembled according to the provided instructions. These kits used the enzyme Platinum® Taq DNA Polymerase. Primers and probes from Integrated DNA Technologies (Coralville, IA) were ordered specific to the DNA templates purchased from Biosearch Technologies (Novato, CA). 0.1% Triton X-100 in PBS buffer was compiled from phosphate buffered saline buffer purchased from Amresco (Solon, OH) and Triton X-100 purchased from Sigma-Aldrich (St. Louis, MO) in a 0.1% solution by volume. All filters used were 4.7 cm in diameter, with a pore size of 1 μm .

Both Teflon and glass fiber filters were purchased from Pall Life Sciences (Port Washington, NY). The polyester felt filters were purchased from Lockheed Martin (Bethesda, MD).

Methods

This project tested three different concentrations of DNA in triplicate on three different types of filters. The concentrations tested were 2.4×10^4 , 2.4×10^5 , and 2.4×10^6 copies/ μL in PCR-grade water. These were rough approximations of the DNA present in 1 mg, 100 μg , and 10 μg of the test particles. To test the recovery, 100 μL of a given concentration of DNA was pipetted onto the filter. The filter was then submerged into a 50 mL falcon tube containing 10 mL of 0.1% Triton X-100 in PBS buffer. Triton X-100 is a surfactant that assists in the removal of the DNA from the filter. The falcon tube was then shaken for two minutes and vortexed for thirty seconds. Once the vortexing step was completed, 1 mL of liquid was pipetted from the tube and placed into a labeled Eppendorf tube. The solution was then serially diluted by factors of 2 down to a 1:10 dilution, and the undiluted, 1:2, 1:4, and 1:10 dilutions were tested by quantitative real-time polymerase chain reaction (qRT-PCR). A control solution without a filter was tested alongside each experiment. This control contained 100 μL of the same DNA solution, but the solution was spiked directly into a Falcon tube with 10 mL of 0.1% Triton in PBS buffer. The PCR results for the filter sample were then compared to the PCR results from the control tube, and the percent recovery was calculated. Calibration curves were also

constructed and were used to calculate the actual number of copies of DNA in each solution, which was compared to the theoretical number of copies.

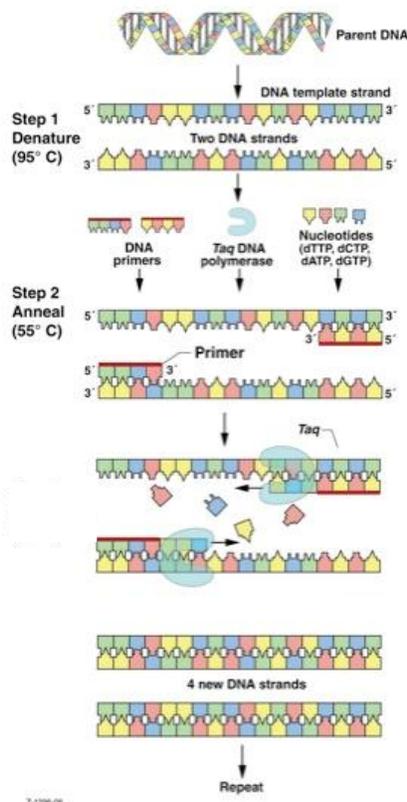


Figure 1: The qRT-PCR Reaction

Master mix for the PCR reaction was made with kits from Invitrogen, according to the manufacturer's instructions. This master mix was then aliquotted into Cepheid SmartCycler® tubes (20 μL per tube). 5 μL of template sample were added to each tube and the reaction was run in a Cepheid SmartCycler® using the following protocol: the sample was first heated to 50 °C for 120 seconds, and then heated to 95 °C for 600 s. The sample then remained at 95 °C for 15 s and then was cooled to 55 °C for 60 s. This cycle was repeated 40 times. Each sample was run in triplicate alongside negative controls.

The PCR results were then analyzed using calibration curves. Real-time PCR uses a fluorescent probe that is attached to a quencher molecule. The process is illustrated in Figure 1. When the probe is in close proximity to the quencher, there is no fluorescence. However, if the probe is separated from the quencher, the probe begins to fluoresce. This probe is

specific to a target DNA sequence. During qRT-PCR, the DNA is first annealed, and the probe binds to the single stranded DNA that is created. A set of primers also bind to specific sequences of DNA, one on each half of the annealed strand. After the probe and primers bind to the sequence, the enzyme begins at the primers to convert the one-stranded DNA into two-stranded by adding complimentary base pairs. As the strand is replicated, any molecules bonded to the other side of the DNA (that are in the way of the enzyme) are disassociated. This step separates the probe from the quencher and the probe begins to fluoresce. These steps are then repeated, increasing the fluorescence as the DNA continues to replicate.

A calibration curve plots average Ct values, that is, the average number of cycles required for the fluorescence to reach a certain threshold, versus the log of the starting number of copies in the PCR reaction. Once a calibration curve is constructed, it can be used in future experiments to determine the number of copies originally present in the solution, given a Ct value, as illustrated in Figure 2. A more detailed discussion of the analysis methods used for these experiments is included in the results and discussion section.

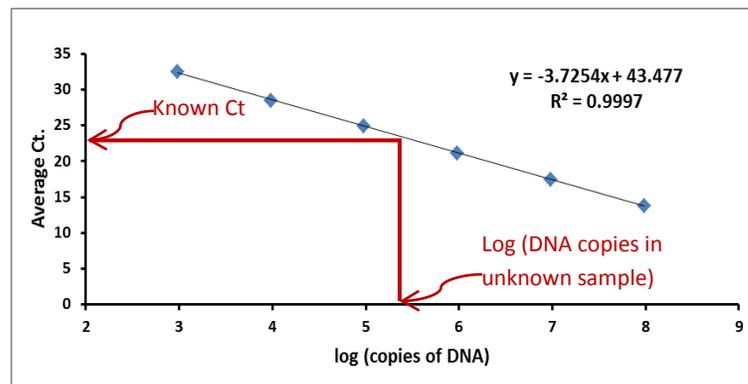


Figure 2: A typical calibration curve in Tris-EDTA buffer. $n=3$, error bars represent one standard deviation. A known Ct can be used to find the number of copies originally present in the solution.

Results and Discussion

The first step of analysis was to compare calibration curves constructed in Tris-EDTA (TE) buffer (DNA storage solvent) to those constructed in 0.1% Triton in PBS buffer, to determine if the curves differed between solvents. Average calibration curves are shown in Figure 3. Although these data points appear to be quite similar, a small difference in slope can have a large effect. The analysis showed a difference in PCR reaction efficiency between the two solvents. The efficiency is represented by Equation 1, where C is the number of copies present in the solution, C_0 is the initial number of copies, E is the efficiency of the PCR reaction, and n is the number of temperature cycles. In the ideal case, the amount of DNA should double with each thermal cycle. Equation 1 can be modified through logarithms and converted to a percentage to yield Equation 2 for calculation of the percent efficiency of the PCR reaction under certain conditions. This percent efficiency compares the

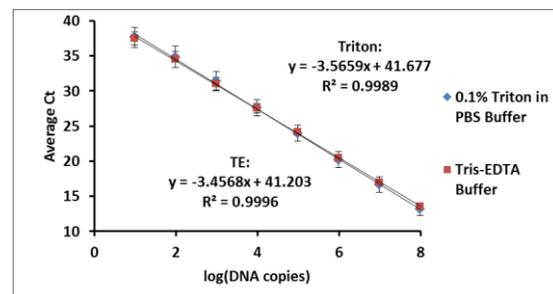


Figure 3: Average calibration curves in 0.1% Triton in PBS buffer ($n=8$) and in TE buffer ($n=4$). Error bars represent the standard deviation

efficiency of a given reaction with the ideal case, $E=2$, where the number of copies of DNA doubles during each cycle.

$$C = C_0 * E^n \quad (1)$$

$$\%Efficiency = \left(-1 + 10^{\frac{-1}{slope}} \right) * 100\% \quad (2)$$

When Equation 2 is used with the slopes in Figure 3, it results in a 94.7% efficiency in TE buffer and a 90.7% efficiency in 0.1% Triton in PBS buffer, indicating a difference of efficiency of 4% between the two solvents and suggesting that either Triton or PBS has an inhibitory effect. This result indicates that calibration curves run in the Triton solution should be used to interpret the results, to ensure correct calculation of the copies of DNA.

When beginning to calculate copies of DNA, the first decision to be made is the method of analyzing the PCR data. Many programs that analyze PCR use a constant Ct method of analysis

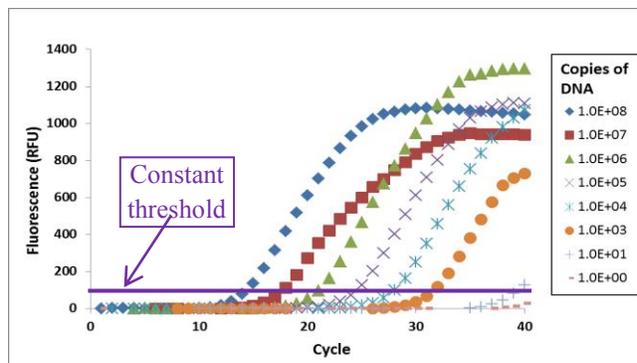


Figure 4: Fluorescence curves for different DNA concentrations. Higher DNA concentrations begin increasing in fluorescence at a lower cycle number. Threshold is shown in purple.

by default. The constant Ct method constructs a user-set threshold value that is constant for all samples and determines the fractional number of cycles required for a sample to pass that fluorescence mark.

Figure 4 illustrates the constant Ct method. In a plot of fluorescence intensity vs. cycle number, the higher concentrations of DNA will cross the threshold first, while lower concentrations do not cross the threshold until the far right side of the graph, as shown in Figure 4.

While this method is widely used, analyses have shown that the constant Ct method is not the most accurate because it assumes uniform reaction efficiency.⁴ If this assumption is incorrect, it can negatively impact the accuracy of the data. Authors have suggested that a decrease in PCR efficiency of 4% could result in an error of 400%.⁴ To avoid these errors, Luu-The, *et al.* suggest that the second derivative method is a better choice because it does not involve any decision by the user.⁵ The second derivative method of analysis finds the point where the second derivative of fluorescence with respect to cycle number is at a maximum. This point does not depend on a user set threshold limit but instead defines the point where the curve transitions to a log-linear phase, which is not affected by the magnitude of the fluorescence. A set of second derivative points for known DNA concentrations can be used to construct a calibration curve. Through use of this curve, second derivative results can be interpreted in a manner analogous to the constant

threshold method. The second derivative analysis was used for the experiments described in this paper.

The recovery was approximately 100% for all filter types and DNA concentrations investigated. While some of the results were above 100%, all results were within one standard deviation of 100%, as shown in Table 1. This indicates that the DNA is easily removed from the filters. The data was analyzed for intra-day outliers using Dixon's test (Q-test) with $p=0.05$ and 0.970 as a critical value.⁶ There were no outliers, indicating that there was no significant intra-day variability and the results are reproducible. Grubb's test was used to check for inter-day outliers with $p=0.05$ and 2.215 as a critical value.⁷ Both the highest and lowest percent recovery values from each dataset were tested and yielded no outliers. To further investigate the inter-day variation, One-way ANOVA was used. With $p=0.05$, there was no significant inter-day variation for any filter type or DNA concentration indicating the method is repeatable.

DNA Recovery (% , mean \pm one standard deviation)			
Simulated Particles Collected onto Filter	Teflon Filter	Glass Fiber Filter	Polyester Felt Filter
10 μg	104 \pm 11	109 \pm 21	111 \pm 13
100 μg	106 \pm 8	95 \pm 9	97 \pm 10
1 mg	104 \pm 9	105 \pm 9	101 \pm 10

Table 1: DNA filter recovery using second derivative analysis.

Conclusions

As the PCR reaction efficiency was determined to decrease in 0.1% Triton in PBS buffer, calibration curves constructed in 0.1% Triton X-100 Buffer were used to analyze the sample results. The percent recovery of DNA was approximately 100% for all filter types and concentrations investigated, indicating the DNA was easily extracted from the filters using the described method. This indicates that DNA barcodes provide an excellent method for tracking aerosolized particles. Future studies should examine the recovery of particles collected as air is drawn through filters, to more accurately simulate real-life conditions.

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