

The Effect of Different Light Wavelengths on the Dust Microbiome



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Introduction

- Goal:** to investigate the impact of UV vs. visible light on the viability of the dust microbiome.
- Purpose:** findings could inform future decisions about lighting schemes in hospitals and other healthcare facilities where biological insight is crucial.
- Requirements:** develop a method to distinguish live dust cells from dead dust cells and develop an effective killing method to establish a negative control.

Results

- Autoclaving samples significantly lowers the concentration of amplifiable DNA and was therefore selected as the method to provide negative controls.
- Addition of PMA to dust samples prior to extraction appears to decrease the amount of DNA that is amplified.
- Approximate C_T difference for live and dead samples is 10.
- Since the DNA concentration doubles with each cycle, this implies that the initial DNA concentration is approximately 2^{10} (~1000) times lower in the autoclaved samples compared to live samples.

Future Directions

- Treat samples with broad-spectrum light to determine the appropriate dosage for killing dust cells.
- Investigate the relationship between different wavelengths of light and bacterial viability by subjecting dust samples to sunlight with UV wavelengths removed, sunlight with visible and infrared wavelengths removed, and dark conditions.

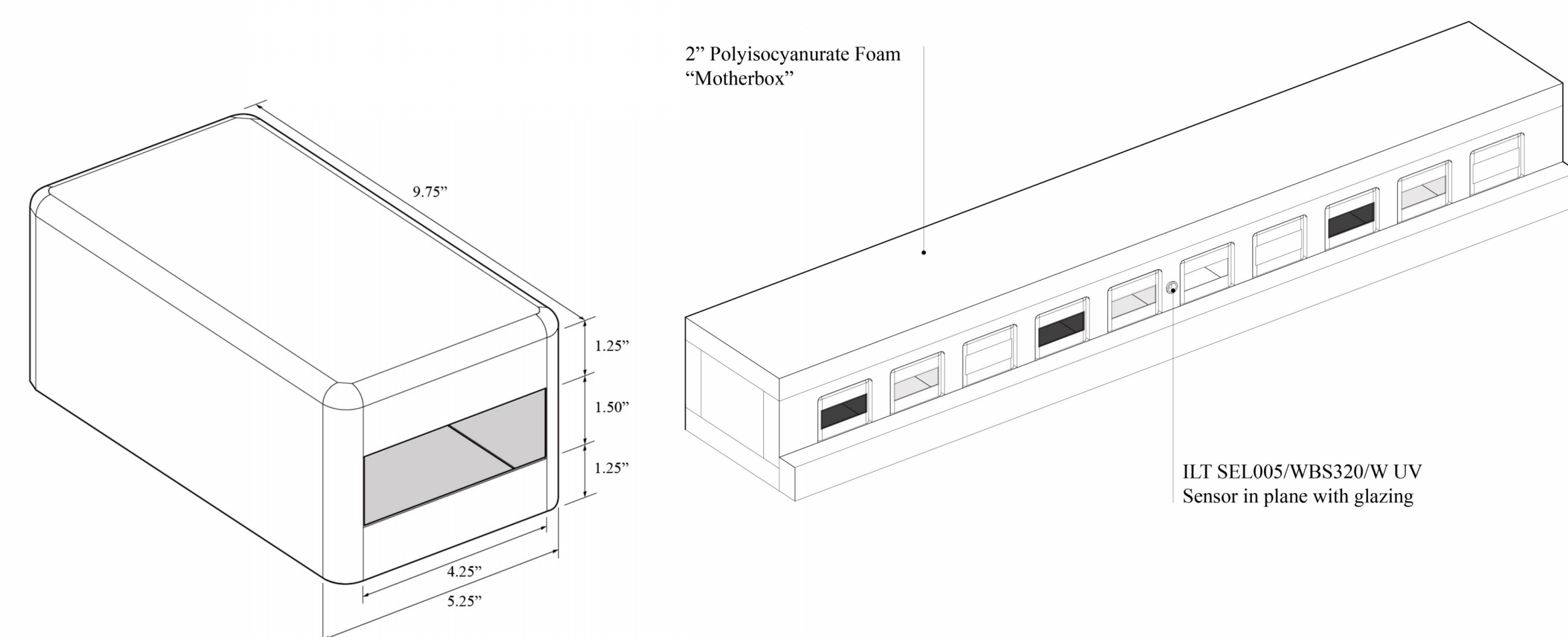


Figure 1: Model of boxes that simulate the outdoor environment. The image on the left represents a model of each box used in this experiment. Each box is a 1:32 scale model of a 14' x 26' x 10'-8" classroom with a 4' view window and a 3'-4" sill. The image on the right represents the arrangement of the boxes when placed on the roof of Pacific Hall.⁶

Methods

- Collect, homogenize, and distribute dust samples
- Autoclave dust samples to kill the dust
- Place live and dead dust samples in light boxes on the roof to simulate daylight through windows (Figures 1 & 2)
- Subdivide each sample, treat half with propidium monoazide (PMA), and extract DNA from all samples⁷
- Use qPCR to determine threshold cycle (C_T) and relative 16S gene copy numbers from each sample⁸

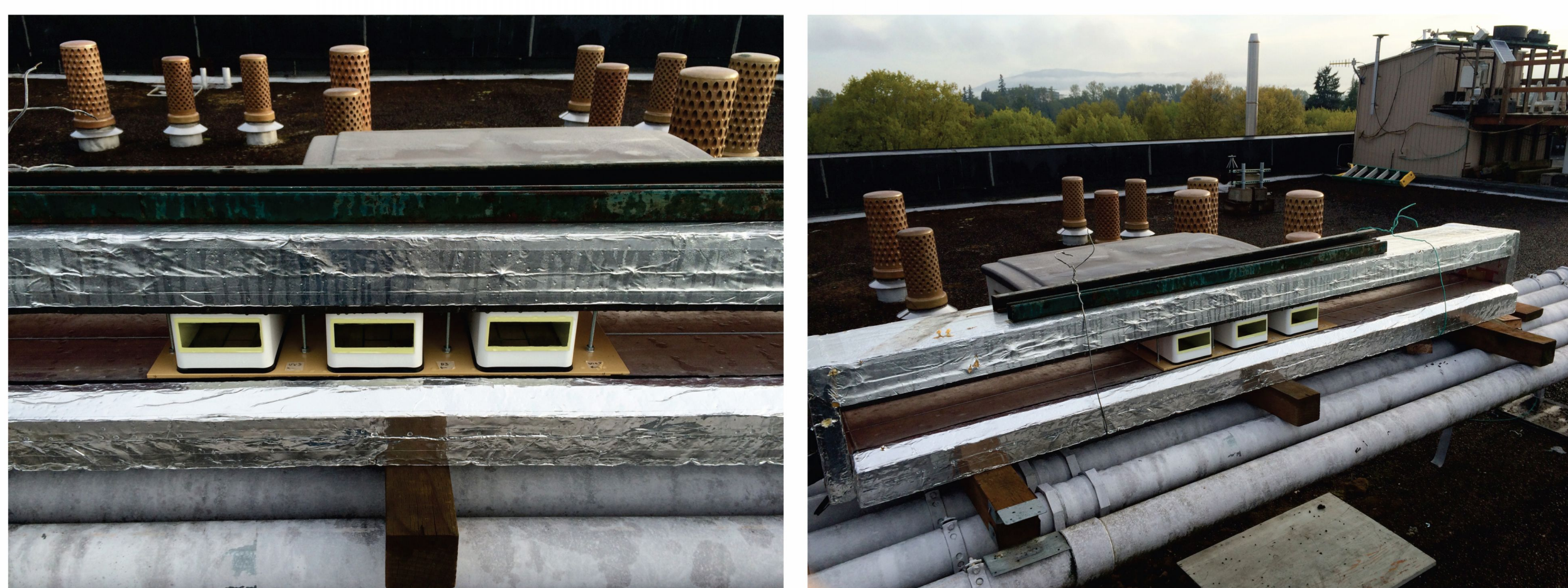


Figure 2: Experimental setup on the roof of Pacific Hall. Each box is has the potential to be equipped with a temperature probe and be insulated to reduce external solar heating. Surface temperature can be regulated to approximately normal indoor levels by heating or cooling a water-filled chamber below the boxes.

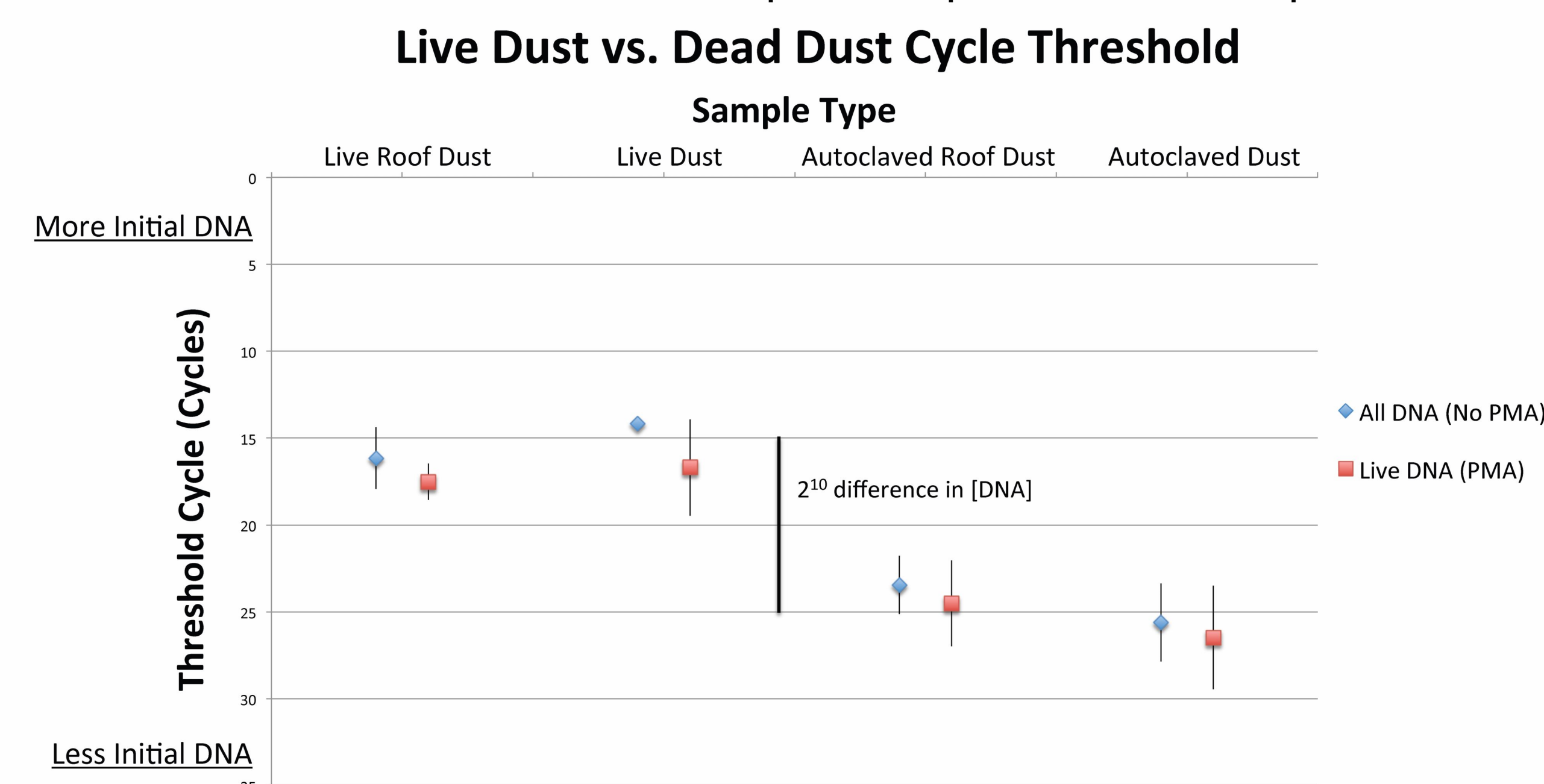


Figure 3: Threshold Cycle (C_T) results for dust samples that were on the roof compared to samples not on the roof. During each cycle of a qPCR reaction, the concentration of DNA in each well doubles. The threshold cycle represents the number of qPCR cycles required before the DNA concentration is high enough that its fluorescence can be recognized. Consequently samples with higher initial DNA concentrations will require fewer cycles to reach threshold.

Propidium Monoazide (PMA) Treatment

- Chemical binding agent that prevents DNA amplification during PCR
- Use PMA to distinguish live cells (with intact membranes) from dead cells (with fractured membranes)
- Without PMA, all DNA should amplify regardless of whether it came from a live cell or a dead cell.
- With PMA, only DNA from live cells will amplify. Consequently more cycles will be required to reach threshold because there is less amplifiable DNA

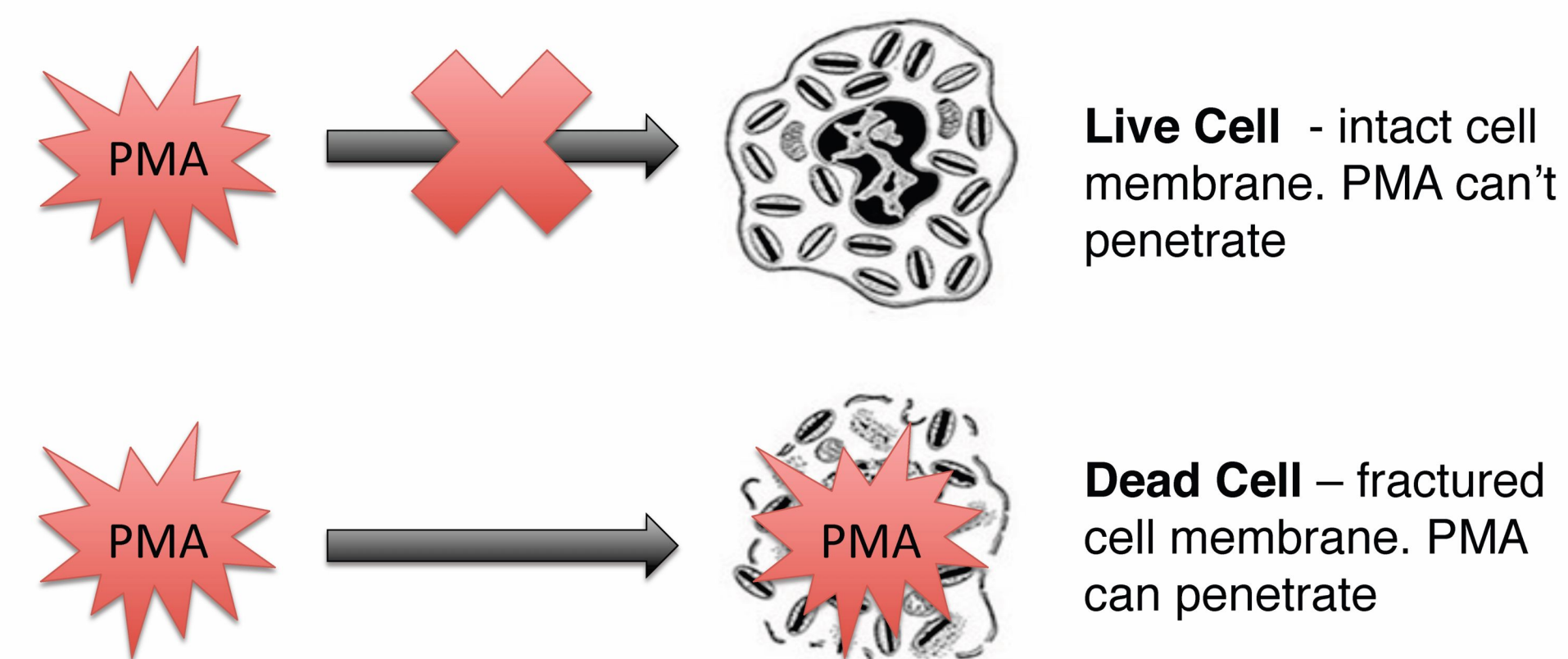


Figure 4: DNA amplification inhibition by PMA. Model of PMA interacting with dead cells but not with live cells.

Hypothetical Results

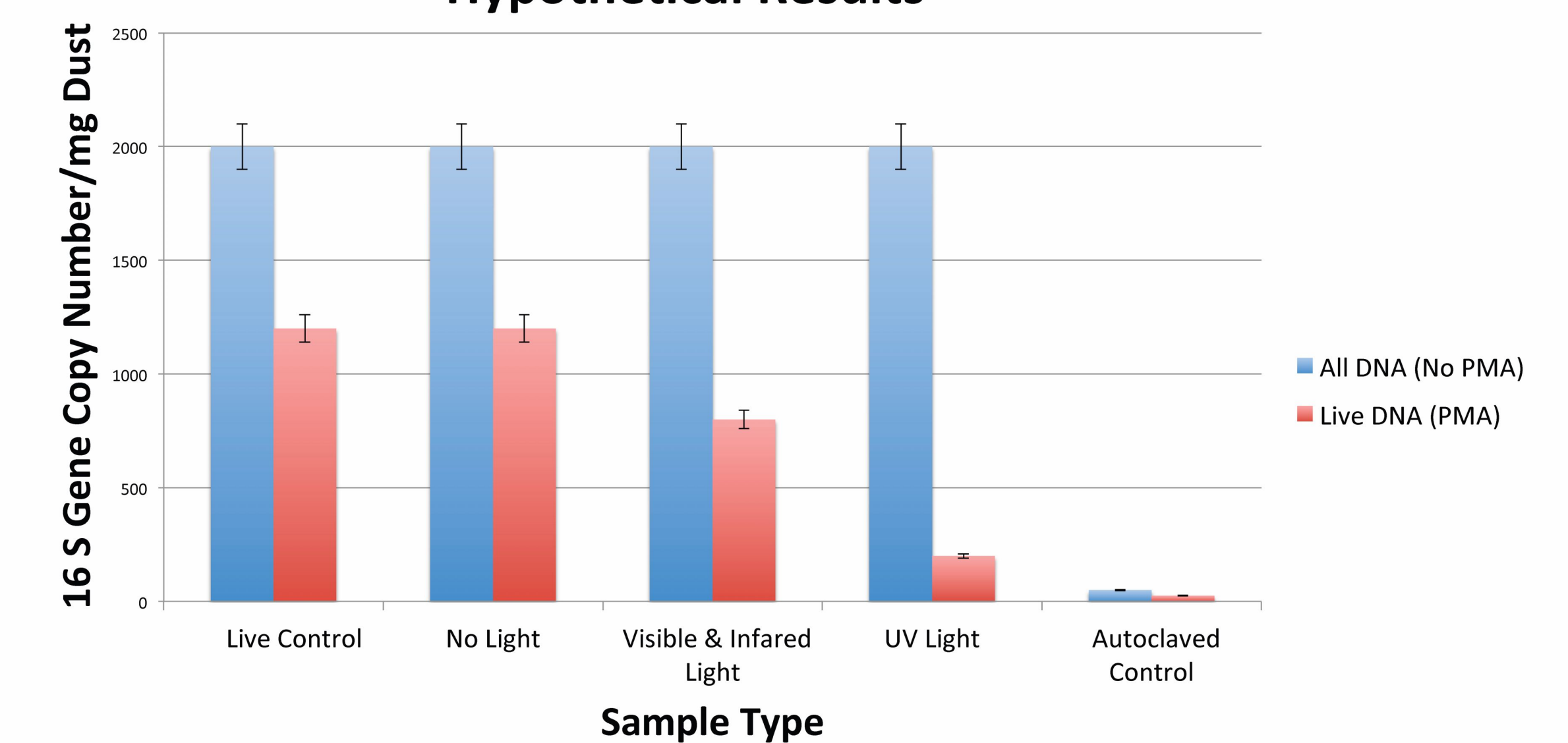


Figure 5: Projected results from the experiment investigating the impact of different wavelengths of light on the dust microbiome. Using plasmid DNA that contain known quantities of 16S gene copies as our standards, we will be able to determine the number of bacterial cells in each of the dust samples. In this figure the blue bars represent the total 16S gene copy number in each of the samples while the red bars represent the 16S genes from live dust cells.

Expected Results:

- Similar total DNA concentrations for all samples except the autoclaved samples. (Autoclave destroys DNA)
- Different wavelengths of light will "kill" dust to various extents. As more dust is killed, more cell membranes will fracture allowing for the penetration of PMA which will prevent amplification.

Conclusion

- Preliminary evidence suggests that the viability of microorganisms in the built environment is impacted by light exposure.
- PMA is an effective method for distinguishing live samples from dead samples
- Integrating biological knowledge into architectural decisions can create a bioinformed perspective on buildings that promotes human health.

References & Acknowledgements

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⁸ Maeda, H., C. Fukimoto, Y. Haruki, T. Maeda, S. Kokeguchi, M. Petelin, H. Arai, I. Tanimoto, F. Nishimura, & S. Takashiba. (2003). Quantitative real-time PCR using TaqMan and SYBR Green for Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, tetQ gene and total bacteria. FEMS Immunology and Medical Microbiology 39, 2003.

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