

Microbial barcodes

The future of object provenance

- Object provenance – determining an object's origin and history – is a fundamental challenge for human health and commerce.
- To address this problem, Nathan McDonald at the US Army DEVCOM Chemical Biological Center has optimised bio-engineered microbes containing DNA 'barcodes' paired with a sensitive CRISPR/Cas detection assay called SHERLOCK.
- His team demonstrated that barcoded microbes can be detected after one month on surfaces including carpet, sand, tile, linoleum and concrete, even after vacuuming or mopping.
- This system has multiple real-world applications in human health, commerce, food safety, and forensics.

A surprising amount of time and energy goes into answering the deceptively simple question of 'where did this come from?' – known as object provenance. From tracking agricultural and manufactured goods in our complex, globalised supply chains to determining who, or what, passed through particular locations, object provenance is a fundamental challenge for human health and commerce.

One example of this is in combating foodborne illness – a global problem, with an estimated 48 million cases a year in the USA alone. The first step in dealing with foodborne illness is to determine what the contaminants are and where they were introduced into the supply chain. Due to the complexity of modern markets, however, this can take weeks, causing massive costs and potentially leading to further cases of illness.

Object provenance is also a key consideration in forensic investigations and law enforcement. Alongside video surveillance and fingerprinting, technologies that enable the determination of whether an object or person was at a particular place are highly valuable.

Microbial fingerprints

One method for determining object provenance in forensic investigations is to analyse the microbes present on an object. Microbes are found in all environments, and any object will gradually adopt the naturally occurring microbes present in the environments it is exposed to. In principle, sequencing these microbial samples can provide a cheap and sensitive way to determine where the object has been.

However, there are a number of hurdles with this approach, including the requirement for expensive and time-consuming surveys of natural microbial communities in locations of interest, the fact that many environments share very similar microbial populations, and that microbial communities in one location can vary over time. This is where a new study, led by Nathan McDonald at the US Army DEVCOM Chemical Biological Center, comes in.

Building on previous work by Professor Michael Springer at Harvard, McDonald and colleagues tested the use of synthetic, non-viable microbial spores containing 'DNA barcodes' to determine object provenance. Using modern molecular biology techniques, the researchers were able to take advantage of the natural information-encoding

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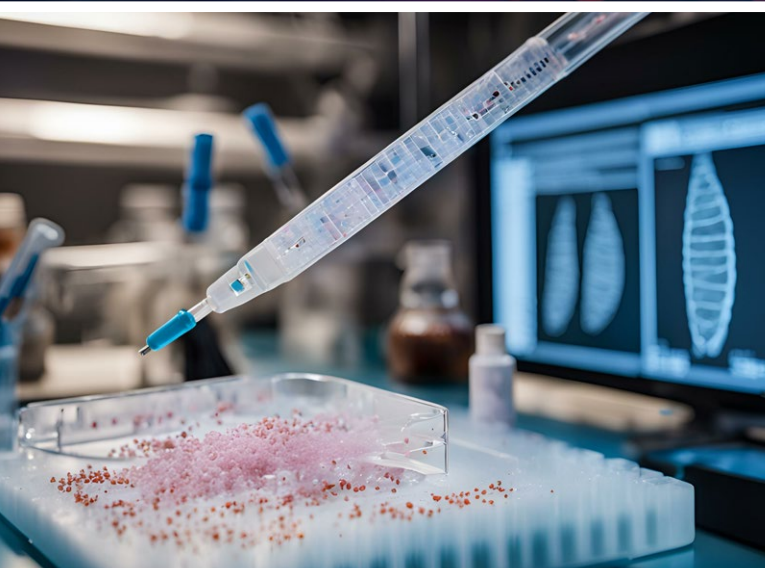
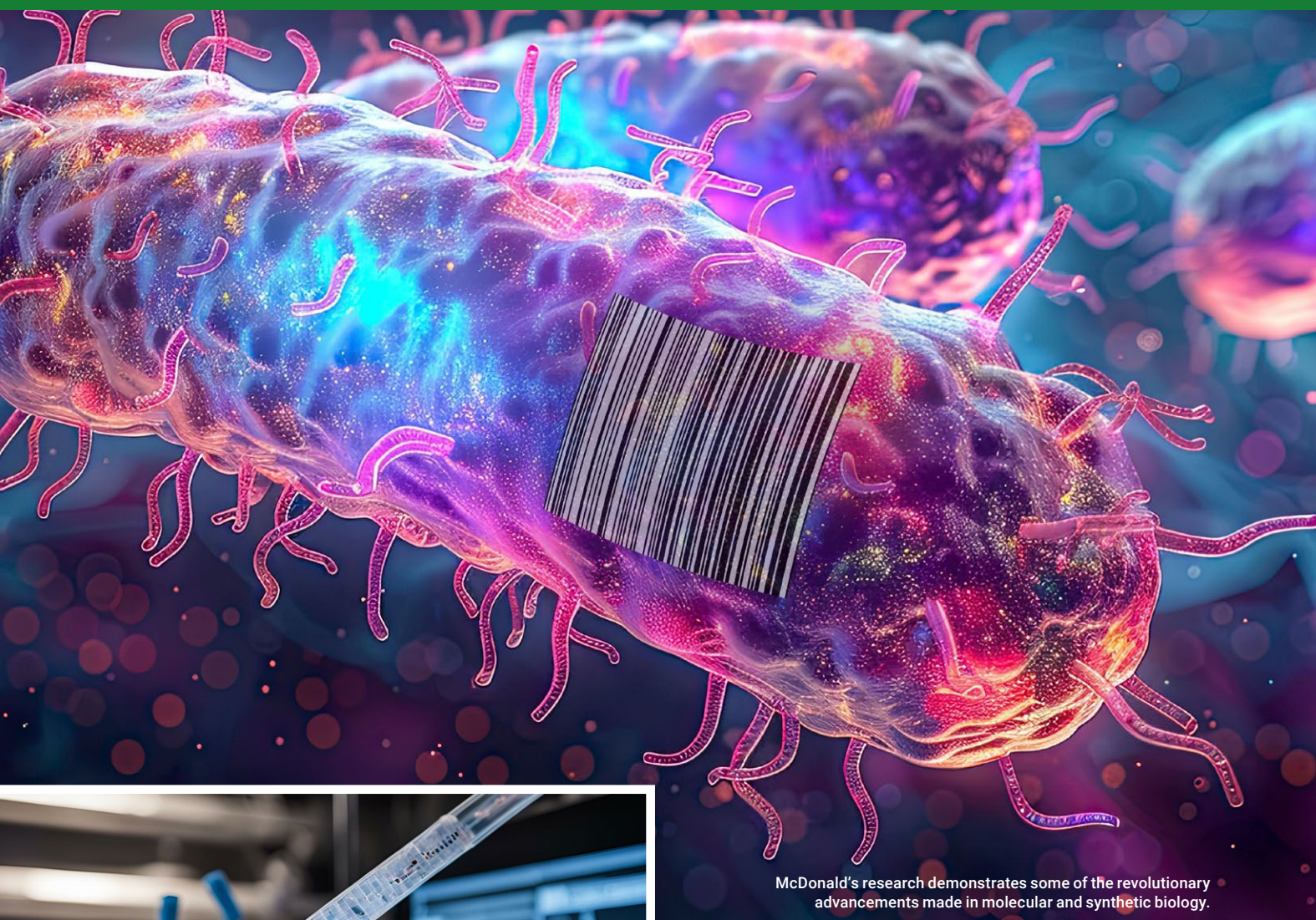
property of DNA to include DNA barcodes in microbial genomes – short artificial DNA sequences that can be read and identified by standard sequencing technologies. Barcoded microbial spores (BMS) with specific barcodes can then be introduced to locations or objects of interest, creating a

microbial 'fingerprint' for cheap and sensitive object provenance.

A case for SHERLOCK

The team identified several key criteria for BMS to be viable in the real world: the microbes must be bio-contained (not viable in the wild), they must persist in the target environment and label objects passing through, and the decoding of the barcodes should be fast, sensitive, and specific. To address persistence, the researchers cultured metabolically dormant microbial spores, a natural part of some microbes' life cycle, which allows them to persist in harsh environments for long periods without growth. The spores were made non-viable by removing key genes required for cell division and germination. Finally, for rapid detection, the researchers combined the BMS with SHERLOCK – a sensitive detection assay that uses cutting-edge CRISPR/Cas technology to produce a visible signal if a specific DNA sequence (or barcode) is present in a sample. SHERLOCK can be thought of as the scanner for the barcoded spores.

With the engineered BMS in hand and SHERLOCK on the case, McDonald and his team performed rigorous testing of the system in simulated home environments designed to mimic real-world scenarios. They started by spraying mixtures of BMS on carpet and linoleum. They monitored the detection of the spores over four weeks, both before and after vacuuming or mopping. Some of the BMS strains were easily detectable on both surfaces, even after four weeks and four rounds of vacuuming or mopping – these strains were selected for further testing. For the second round of testing, the best-



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cardboard, Styrofoam, and plastic – were placed on the squares for five minutes. The objects were moved to fresh carpet or concrete for another five minutes, and then again for 30 days; samples were taken following each transfer. BMS were readily detected even after 30 days on both cardboard and Styrofoam when using swabs and wipes. While BMS were not easily detected on the plastic object itself, it did transfer BMS to fresh carpet or concrete squares. Together, these results demonstrate the utility of BMS and SHERLOCK in addressing object provenance.

Case closed?

McDonald's research demonstrates some of the revolutionary advancements made in molecular and synthetic biology utilising CRISPR/Cas systems, and how they can be applied in the fields of detection and diagnostics. This work shows that barcoded spores can be easily transferred and detected across multiple surface types over a period of at least one month, enabling use cases in object provenance. In addition, the optimisations developed in the study to both sample collection and preparation provide increased sensitivity for SHERLOCK and improve the transferability of this CRISPR/Cas technology from the lab to real-world applications. It looks like the cases of the future may again be solved by Sherlock, but this one won't be smoking a pipe!

performing BMS were released on sand, concrete, loose tiles, and carpet, and once again monitored over four weeks. Spores were still detectable after this period on all surfaces except concrete.

Inconsistencies in the BMS detection data led the team to hypothesise that variability was coming not from the spores themselves but from the sampling or sample processing methods. To address this, three sampling methods (swabs, wipes, or tape) were tested, alongside three sample processing protocols. Over the course of four weeks, they determined that both swabs and wipes worked well, and a commercial DNA extraction kit provided more consistent and sensitive detection of BMS.

Finally, the researchers wanted to test the applicability of the system to object provenance. Spores were sprayed on squares of carpet and concrete, then objects made of three common materials –

Personal response

What inspired you to conduct this research?

As a scientist for the US Army Department of Defense we have a mission to provide solutions that help protect our nation and allies. We believe there are critical steps in science that are necessary to transition cutting-edge research out of the lab and into applications. Our group serves as a critical step that can test research solutions in the real world. As a molecular biologist and synthetic biologist, this work was particularly interesting to utilise information carrying DNA as a tool for detection applications.

What further research or developments would you like to see to improve the reliability of this technology for real-world use cases?

Overall the process needs to be further refined and streamlined before it is ready for real-world use cases. The SHERLOCK technology works as intended with high sensitivity and specificity but requires trained technicians to execute the assay. There are many components that go into each reaction, increasing the overall chance of human error and the entire assay required a cold chain. Significant research developments in the area of simplifying the assay, such as the development of a kit with premixed components and attempts to eliminate the cold chain, would drive SHERLOCK and BMS detection into the real-world. As for the BMS themselves, we observed a great deal of variability depending on the sequence of a given barcode. Some in particular would result in a significant background signal when using SHERLOCK even in the absence of the barcode in the sample. In our hands this was resulting

in confounding and sometimes false positive results. Further refinement is needed to validate and optimise barcode sequences that can be used for object provenance.

What are the potential risks of using this technology in supply chains or forensics, and how could these be limited?

The biggest risk or drawback of this technology is the need to know where the BMS should be applied and then when and where to sample for them. This may limit the overall applicability to crops and other highly consistent supply chain goods where object provenance is important. For forensics applications it could be of benefit to apply BMS at locations of particularly high significance as an extra layer of protection should tracking be needed. Similarly as mentioned above, additional research is needed to optimise the barcodes that can be used for supply chain applications or forensics. Without barcodes that function with high sensitivity and specificity there is a risk for false positives/negatives that could present significant risks for real-world applications of this technology.

What other applications could these technologies be used for, particularly SHERLOCK?

SHERLOCK and similar CRISPR/Cas detection technologies are revolutionising the entire field of biological detection. Numerous studies have demonstrated that CRISPR/Cas can be used for detecting pathogens ranging from the flu, to COVID-19, to biological threat agents.

Details



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Funding

The researcher would like to acknowledge DARPA BTO for funding this independent verification and validation work.

Collaborators

The lab of Michael Springer

Bio

Dr Nathan McDonald is a Principal Investigator for the United States Army DEVCOM Chemical Biological Center. In this role he leads multiple programs that harness the power of synthetic biology to deliver solutions that help protect the nation and community at large.

Further reading

• McDonald, ND, et al, (2022) *Evaluating*

the persistence and stability of a DNA-barcoded microbial system in a mock home environment. *Synthetic Biology*, 7(1), 1–10.

• Qian, J, et al, (2020) *An enhanced isothermal amplification assay for viral detection*. *Nature Communications*, 11, 5920.

• Qian, J, et al, (2020) *Barcoded microbial system for high-resolution object provenance*. *Science*, 368, 1135–1140.

• Ulrich, N, et al, (2018) *Experimental studies addressing the longevity of Bacillus subtilis spores – The first data from a 500-year experiment*. *PLOS ONE*, 13, e0208425.

• Jiang, C, et al, (2018) *Dynamic human environmental exposome revealed by longitudinal personal monitoring*. *Cell*, 175, 277–291.e31.

• Lax, S, et al, (2015) *Forensic analysis of the microbiome of phones and shoes*. *Microbiome*, 3, 21.

• Lax, S, et al, (2014) *Longitudinal analysis of microbial interaction between humans and the indoor environment*. *Science*, 345, 1048–1052.

• Scallan, E, et al, (2011) *Foodborne illness acquired in the United States—unspecified agents*. *Emerging Infectious Diseases*, 17, 16–22.

• Hedberg, CW, et al, (2008) *Timeliness of enteric disease surveillance in 6 US states*. *Emerging Infectious Diseases*, 14, 311–313.

Competing interest statement

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorising documents.

