How much does the DNA extraction route impact the results of microbiome research?

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Comparing DNA extractions for microbiome studies. Credit: Tess Deyett

Let's face it, we are a results-driven society. Too focused on the outcome, people don't often think about the "how." For instance, did you think about "how" you got to work this morning or did you just get there? In microbiome studies the results are the graphs and the data but the "how," like commuting, is often just part of a routine.

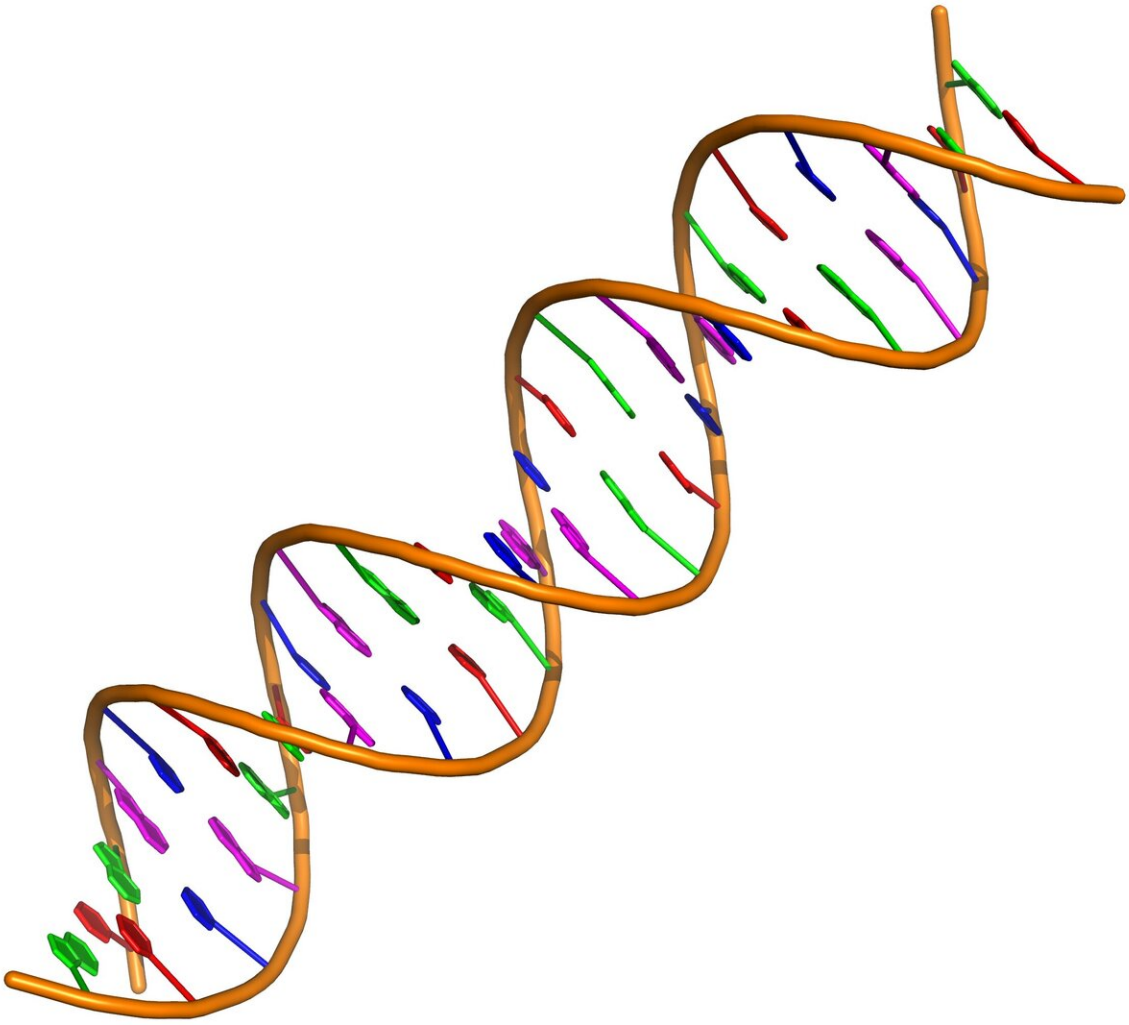
However, a fundamental aspect of analyzing microbiomes (regardless of the host) relies on DNA extraction and amplification methods. Dozens of DNA extraction methods currently on the market are each capable of extracting DNA and producing reputable results, but each has a slightly different "how." Labs often have their "go-to" kit selected by the lab leader and passed down from generation to generation of graduate students. And once you choose a method it's best to stick to that method simply because everyone knows a different kit will produce slightly different results. But which method is the best? How do you choose? How much does the "how" really impact the results? And when should you switch?

Experimental design and sample collection are usually discussed at great length at the start of any project, yet DNA extraction methods are often overlooked. This looming bias of ignoring the importance of the DNA extraction method became the focal question for researcher Cecelia Giangacomo and her advisor Jason G. Wallace in their latest *Phytobiomes Journal* publication, "Comparing DNA Extraction and 16S rRNA Gene Amplification Methods For Plant-Associated Bacterial Communities." Wallace states, "This research lets us know the best/most effective methods going forward. Our lab does a lot of plant microbiome work, so we want to make sure we're using those resources well. I was actually surprised no one had done this before, so we hope other labs find it useful."

DNA extraction kits are designed to be broad spectrum to work for both microbes and their host. In most plant-microbiome projects there will be a minute quantity of microbial DNA compared to the host. Thus, the biggest challenge in microbiome sequencing is optimizing the extraction of the microbial DNA in the deluge of host DNA within a sample.

Once DNA is extracted, researchers will often amplify a single piece of DNA from all the organisms in the sample. This piece of DNA is

conserved across various species of interest but is different enough between species to characterize the diversity within the sample. One of the most common targets to survey bacteria—the 16S ribosomal RNA gene—is also present in plant chloroplasts. So while this method of amplification works well in separating host from microbe in human and environmental samples it still produces host contamination (through amplification of chloroplast) in plant samples.



A double stranded DNA fragment. Credit: Vcpmartin/Wikimedia/ CC BY-SA 4.0

Wallace and his team compared four common commercially available DNA extraction methods: DNeasy Plant (Qiagen), Quick DNA (Zymo), Extract-N-Amp (Sigma-Aldrich), and Power Soil Kit (Qiagen). They also looked at four different amplification methods that target specific regions of the 16S ribosomal RNA gene to determine which method did the best job of excluding the host DNA while preserving the microbial DNA.

One way researchers can select against host DNA is through modifying the amplification process. Wallace and his colleagues looked at adding molecular clamps which would clamp down on chloroplast and mitochondrial DNA, essentially blocking amplification of unwanted DNA. They also tried amplifying a different region of the 16S gene that would discriminate against chloroplast and mitochondria leading to more amplified DNA of the microbiome. Their final method of optimizing the amplification process used sequences that "poison" unwanted DNA so it can't be amplified further.

This process is analogous to taking multiple routes on your commute. Each may have some overlapping scenery but also unique attributes; one may be more scenic, one faster, another more stressful. In Wallace's research they could use the various routes of extraction and amplification to compare how the 'how' of the methods impacts the overall results. While most researchers know there is bias to their methods, this was a direct side-by-side comparison showing how each method produced different results and changed the overall composition of the sample. "This study should help people make the best choices in terms of how to spend their time and money for microbiome research,"

says Wallace.

Wallace emphasized that there is no "perfect method", and even within their study some methods worked better for some sample types but not for others. This data provides researchers the knowledge to make better-informed decisions regarding their methods. Even the best method here may not be the best method for specific projects, samples, types, or budgets. Companies are constantly trying to optimize their products, so as we move forward this challenge will hopefully become easier for researchers.

Most importantly, this research drives home that there are differences among methods that can impact results. There is no "perfect method." It is up to the researchers to understand the nuances of their samples and choose the best method for the scientific question they hope to address. So while all roads may lead to a result, experimenting with the 'how' may be worth the effort to find the best route for microbiome research. "This isn't a paradigm shift, but it's one of the small, incremental changes that help us do research just a little bit better, and over time those add up to pretty large improvements," explains Wallace.

More information: Cecelia Giangacomo et al, Comparing DNA Extraction and 16S rRNA Gene Amplification Methods for Plant-Associated Bacterial Communities, *Phytobiomes Journal* (2020). DOI: 10.1094/PBIOMES-07-20-0055-R

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