“But,” adds Wargo, who started PRIME TR, “there’s a lot of complexity because any time these tissues were collected, it wasn’t necessarily for the sole intent of looking at microbes, and so they may have been collected in a way that really complicates that. There could be a lot of contaminants.”

This is a particular challenge because the microbial communities found in tumors are relatively small—bacteria are far less abundant than in the gut, for instance. Such low-biomass samples, as they’re called, are highly sensitive to contamination, which, according to Straussman, is entirely unavoidable. “A few years ago, we went into a lot of hassle trying to just clean everything completely . . . [and] we found out we just cannot do it,” he says. “There’s always some bacterial DNA contamination.”

So when he, Wargo, and their colleagues decided to survey 1,526 samples of seven different tumor types—including breast, lung, pancreatic, and brain—to rigorously address the question of whether bacteria were commonplace in cancer, they needed to control for this inevitability. They did so by incorporating negative controls in each step of the process, including extracting the DNA, cycling the samples through PCR, and sequencing the resulting nucleic acids. They even took bits of the paraffin block that the tumor tissue was preserved in, to control for contaminants introduced when the samples were first taken. “So at the end of the day,” Straussman says, “we know what the background noise is and what [are] the true sequences that are coming from the tumor.”

After eliminating more than 90 percent of the reads based on these controls, the group was able to identify a distinct bacterial signature for each tumor type, with breast cancer exhibiting a particularly abundant and diverse microbiome. Then, to ascertain if the bacteria were actually living within the tumors, the team cultured tumor slices and treated them with a fluorescently stained enantiomer of the amino acid alanine, which bacteria incorporate into their cell walls. When they saw the glow of the bacteria under the microscope, it was “nice proof of the fact that live bacteria are present in these tumors,” says Straussman.

The results, published in *Science* in May 2020, took “another step further into the right direction, which is they did not only rely on bioinformatics tools to determine the presence of these bacterial signals; they coupled that with imaging,” says Ajami, who consults or advises for a few biotech companies involved in microbiome research and development. “So they were able to identify intratumoral bacteria—and in some cases, intracellular bacteria—that were present in these tumors.” For good measure, Straussman’s team cultured bacteria from breast tumor samples taken from five women undergoing surgery and was able to cultivate hundreds of colonies, including members of three main phyla, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (recently renamed *Pseudomonadota*, *Bacillota*, and *Actinomycetota*).

Straussman is not alone in his mission to document and interrogate microorganisms in tumors. More and more studies present new data and novel approaches to help propel the conversation beyond potential contamination concerns. Margaret Sällberg Chen, a clinical immunologist and cancer microbiome researcher at the Karolinska Institutet in Sweden, worked with physicians there to sample bacteria from precancerous pancreatic cysts—in which they’d previously identified bacterial DNA—in the operating room. “When they lifted out the pancreas, they could take the sample and inoculate it directly to the culture medium,” she explains. The work, published in November 2021, resulted in successful cultures of *Enterococcus*, *Enterobacter*, and *Klebsiella* bacteria, among other groups. Because some of these species die upon exposure to oxygen, time was of the essence when sampling the tumors and preserving their microbial communities, says Sällberg Chen. “By having this very fast culturing method, we succeeded to cultivate some of the pancreatic microbiome.”

While much more research is needed to pin down the function of microbes in cancer, researchers who spoke with *The Sci-