

An instrument at ETS Laboratories sets up PCR reactions for Scorpions analysis, which uses rapid genetic detection to give winemakers precise insights into a range of spoilage microbes so they can act before a wine is contaminated.

ACT FAST

THE LATEST LAB TESTING DEVELOPMENTS ARE QUICK AND AFFORDABLE.

BY DR. JAMIE GOODE

Things are changing rapidly in the world of enology lab testing. Long-established, traditional analytic techniques are being replaced by technology that, just a few years ago, was the preserve of university research labs. And there's more change to come, including the application of DNA sequencing.

Back in July, I visited the Australian Wine Research Institute, and one of the scientists I interviewed was yeast researcher Dr. Anthony Borneman. Typically, in the past, the way to see what yeasts were present involved the use of plating. You'd take a sterile petri dish with a nutrient medium, and a small sample of soil or wine (depending on what you were looking at). Then you'd allow whatever is present in the soil or wine to grow on the nutrient medium in big enough quantities to identify it. "Plating gets you just 1% of what's present in the soils," says Borneman. "In wine, it's better, but you still miss a lot." But today, DNA sequencing is becoming much more affordable and rapid, and this has the potential to identify everything that's present.

Borneman showed me a small, USB-powered device, the size of a small chocolate bar, three inches long. This is the MinION (pronounced min-eye-on), produced by a United Kingdom-based company called Oxford Nanopore. It's a DNA sequencer with the potential to open up incredible new opportunities for studying what's actually happening in the winery. You could use this portable DNA sequencer to do real-time sequencing of microbes present in the winery and vineyard.

HOW IT WORKS

DNA consists of long chains of four different subunits, called nucleotides or bases. They're usually known by a single letter

code: A, T, C or G, which stand for adenosine, thymine, cytosine and guanine, respectively. The sequence of these letters forms genes, which then form proteins. The human genome, for example, consists of some 3 billion base pairs and 20,000 genes. Since next-generation sequencing methods emerged in 2005, the cost of sequencing has fallen dramatically, from around \$1,000 to less than \$0.10 per million base pairs. The cost of sequencing has been driven down by a factor of 10 every 18 months, which is a staggering rate of change.

The MinION makes use of a new technology called nanopore sequencing. A nanopore is a tiny protein with a hole in the middle of it that sits inside an artificial membrane. An electric current is applied to the pore and, as a strand of DNA passes through, the current will change according to the sequence of the DNA. This portable sequencer can cheaply and reliably sequence bacteria and yeast genomes. Other low-cost benchtop machines are made by MiSeq (Illumina, San Diego).

AT A GLANCE

- + Traditional lab testing techniques are being replaced.
- + DNA sequencing has amazing potential for real-time analysis.
- + Other analytic techniques can zero in on specific known problems.
- + Use of these new methods can support fast intervention during the winemaking process.



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The minION is a DNA sequencer with the potential to open up new opportunities for studying what's actually happening in the winery.

"DNA sequencing is easy," says Borneman. The hurdles here are the sample preparation [which isn't trivial], and then the process of making sense of the raw data to produce something actionable. It's feasible that, soon, wineries with one of these devices and some decent software on their laptops could be monitoring to see which microbes are present in the winery and vineyard. This is quite exciting, and it would have been like science fiction a decade ago. It could let winemakers intervene in fermentations because they'd know exactly which microbes are present. It would also let viticulturists know what was happening with microbes in the vineyard. For example, they could see whether fungal pathogens were showing resistance to certain agrochemical classes. Genomic differences in yeast strains could be used as molecular markers of traits, which could rapidly increase the speed of strain selection. Aside from lab testing, other applications of cheap sequencing could be to screen consumers for flavor preferences (increasingly, the genetic

contribution to flavor perception is being revealed), or for testing winemakers for specific anosmias (inability to smell certain molecules) that could lead them to miss certain faults or taints in wine.

OTHER METHODS

While DNA sequencing isn't yet a mainstream lab analysis technology, another DNA method has become widespread and affordable. This is PCR (polymerase chain reaction) analysis. It's a powerful technique that's changed how laboratories and even wineries analyze for *Brettanomyces* (known more commonly as *Brett*), which is the scourge of those who make red wine worldwide.

PCR was invented in the early 1980s. The concept behind it is quite simple, but relies on an unusually resilient enzyme called Taq polymerase, which was isolated from bugs growing in a hot spring in Yellowstone National Park. The idea behind PCR is to take two primers, which are bits of DNA that identify a specific sequence.

These primers are then put in a small test tube with the four DNA bases, the Taq polymerase enzyme and the DNA sample to be tested, which can be very small. The test tube is then put in a thermal cycler, which heats the sample to 90° C (or 194° F, enough to uncouple the DNA strand), then cools it to 50° C (122° F) for the primers to fuse on the now-single strands of DNA, then warms it to 72° C (162° F) for the DNA strands to reform and replicate through the action of the polymerase. This cycle is repeated, each time producing new strands of DNA as defined by the primers. After many repeats, the result is millions or even billions of copies of the bit of DNA of interest. The key to this is having a DNA polymerase that can withstand such high temperatures. As proteins, most enzymes lose their structure above 45° C (113° F).

This technology has found many uses, the most famous of which is in forensic science, where only the tiniest bit of DNA is needed to produce results. For wine, it's been exploited by Invisible Sentinel, whose best-known product is called VinoBRETT, powered by a technology it's named Veriflow. The basis for this PCR test is primers based on a small strand of DNA from *Brettanomyces bruxellensis*.

Traditionally, testing for *Brett* has involved plating, a procedure that takes seven to 10 days. This timescale isn't really fast enough for winemakers to be reactive to any problems. The new, PCR-based tests provide results within

four hours. Gordon Burns of ETS Laboratories is very upbeat about these new technologies. "The biggest thing in ETS is molecular biology and microbiology, using specific probes and real-time PCR," he says. "If you asked as recently as five or six years ago to look for microbes in wine, about 10% was molecular biology and 90% was traditional plating. This has now inverted to 90% using molecular probes. This is so important because of the limitations of plating."

enter a sort of limbo state in which they're still alive but aren't dividing or metabolizing. But if conditions change, they're able to start growing again. They're also smaller than regular cells and can pass more easily through filters. This makes them particularly problematic, and only PCR can spot them.

Burns says that for these molecular tests, ETS has a two-day turnover, but it often turns out to be a same-day service. "So it's in time for winemakers to do something



ETS analyst Sedonia Yoshida prepares primers and reagents for Scorpions analysis.

As well as taking a long time, plating is also limited to looking for actively growing bugs. One of the problems with *Brett* is what's called the viable but non-culturable state (VBNC). This is when *Brett* cells

about it," he says. "It becomes predictive, not forensic. All microbiology in winemaking from the time of Pasteur has been forensic: Why did this wine spoil? Now techniques are fast."

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If customers have VinoBRETT in house, it's even quicker, although the results are less certain than using a laboratory that regularly runs quality control and uses a more thorough approach, and there's always a danger of a false negative result (where you think your wine is clean, but in fact it does have *Brett*). Sample preparation is simple: The wine just needs to be centrifuged. Then there are two-and-one-half hours of thermocycling, after which the sample is poured onto a test cassette window for a readout, much as you might do with a pregnancy test. However, unlike with a pregnancy, you can have no *Brett*, a little bit of *Brett*, or a lot of *Brett* — it's semi-quantitative.

The cost of bringing VinoBRETT in house isn't crazy: The reagents for 24 tests cost \$840, while the thermocycler is around \$3,500. A centrifuge is also needed, but many labs will already have one. All in all, using a lab such as ETS will cost around \$60 per sample, whereas running a less accurate in-house test without quality control will cost around \$45.

Invisible Sentinel also offers a PCR test for different kinds of malolactic bacteria that works in a similar way, called VinoPAL. This examines which species of *Lactobacillus* and *Pediococcus* are present in the wine.

DIFFERENT TECHNOLOGIES

Aside from DNA technology, the other significant technology opening up new analytic possibilities is cheaper and more accurate GC-MS (gas chromatography mass spectrometry). This powerful analytic technique allows detection of tiny quantities of compounds of interest present in wine. "Over the last 12 to 15 years, we've gone through six generations and iterations of GC-MS," says ETS' Burns. "As the instrumentation gets better, accuracy improves and reportable concentrations get lower every two or three years." An interesting related technology is MS-MS, where MS (mass spectrometry) is followed by

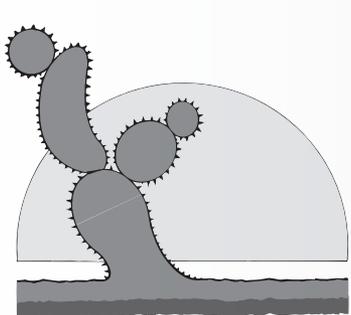
another MS, which hones in on specific compounds of interest, rather than trying to identify unknown compounds. "This is hugely important," says Burns, "because it allows the development of practical analyses previously only available in research laboratories, such as low-level methoxypyrazines and looking at haloanisoles and halophenols in cooperage oak."

One very interesting application is to use a technique called cryofocusing, where headspace over a wine sample is focused by chilling the inlet to -90° C (-130 F). Then the inlet is warmed and GC-MS is used with a chemoluminescence detector to look at volatile sulphur compounds in wine, which are responsible for reduction problems. This is of intense current interest, and affordable analysis of volatile sulphur compounds is of great interest to winemakers.

Many winemakers will continue to work without carrying out any more analysis than they need to do legally. But these new technologies, bringing previously unaffordable analyses into the reach of even small- and medium-sized wineries, offer new insights for winemakers and promise to be valuable tools that could especially assist those looking to intervene as little as possible in the winemaking process.

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Dr. Jamie Goode, author of the books *"The Science of Wine: From Vine to Glass"* and *"Authentic Wine: Toward Natural and Sustainable Winemaking,"* publishes the online wine magazine *WineAnorak.com*. He also writes for *The World of Fine Wine* and *Sommelier Journal*, among other publications.

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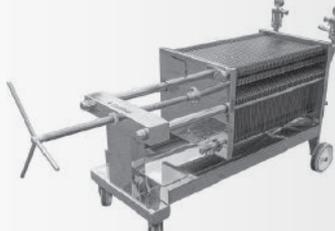


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