Q&A from the January 24, 2019 Webinar: *Is PCR Right For Your Brewery QC Program* with presenter Kevin McCabe

You designed your own program; can you elaborate on whether you principally target genes or organism specific sequences?

I use both, and each where I feel they're best exploited. For lacto and aceto, I use 16S rDNA universal primers and custom designed probes that suit our needs with respect to KYLA; 1 primer pair, and I get as many bacteria as I want to develop specific probes for in one tube (up to 4/tube, of course on our instrument). I have backup horA and horC (2 different fluorophore custom probes) for hops resistance if I get a positive lacto, each targeting those specific genes. In my mind, there's no point multiplexing the horA/C into the first test, as we mostly get negatives, in which case it's a waste of the horA/C reagents. If I get a positive for lacto, then I'll check for horA/C using the same DNA extraction and see where we stand.

Can you discuss why yeast supply labs are reluctant to use PCR to check the quality of their products? Do you purchase yeast from an external source? If so, how do you check the purity of incoming cultures?

All our prop is in house from stocks stored in a liquid nitrogen dewar. If we get yeast from outside, you'd bet I'd be checking it for diastaticus before it ended up in a brew, package, etc. I can't speak as to why yeast labs are hesitant to use PCR in your experience. I could imagine, low level contamination of diastaticus in a yeast slurry would present a needle in a haystack problem. You can only put so much template DNA in a PCR, or you ruin the efficiency. So it comes back to, "will you have that diastaticus target DNA molecule (or a few, even better) in the few microliters your put in the PCR?"

Is it the best way to do that test? There are some diastaticus culture methods out there, but I haven't used them, so I can't really compare the two. That said, I think there are possibly combinations of short enrichment for diastaticus and PCR that could get to a good, more rapid test. This is one of those times where selective enrichment followed by PCR might be a good solution.

Is there a section of yeast DNA that would be a good target to differentiate strains?

There is some use of intron sequences for this. https://aem.asm.org/content/aem/62/12/4514.full.pdf

They also have used repeat sequences as well. https://onlinelibrary.wiley.com/doi/epdf/10.1002/j.2050-0416.1996.tb00921.x and **https://doi.org/10.1002/jsfa.2740620113**

Then, there are those that have applied microsatellite markers. https://www.researchgate.net/publication/14928451_Rapid_identification_and_differentiation_of_yeasts_by_DNA_and_PCR_fingerprinting

If you have specific concerns about specific strain features (e.g. production of phenolics) and cross contamination, you could target genes in those metabolic pathways.

I'd love to run the Verstrepen data set from their cell paper https://www.cell.com/fulltext/S0092-8674(16)31071-6) through a program like CodaChrome https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-15-65 to look for fast clock genes quickly. Last I checked my colleague who developed CodaChrome, was possibly, maybe, kinda considering adapting CodaChrome, which had only been used for bacterial genomes, to being able to run yeast genomes. I haven't pushed him on it in a while, but it could be a neat tool for IDing good candidate genes for your strains.

Have you seen an increased sensitivity for detecting spoilers using PCR that you could not achieve with plating?

Short and unsatisfying answer is, "it depends."

The trick is, "are you getting a target template into your PCR." I'd say right now, our PCR is on par with culture, with respect to CFU/mL for most of what we can culture. This all comes back to how much of that original sample, once through DNA extraction, ends up in your PCR tube. Then, "how sensitive is your assay?"

If you can get that template in your tube, then, I think the better question is how readily can you culture that microbe? Megasphera and pectinatus are really tough without strict O2 exclusion. Some lacto's can be tricky to grow, especially if you haven't been nice to them. Are any of the organisms viable but not culturable (VBNC) and might grow out in your beer?
https://en.wikipedia.org/wiki/Viable_but_nonculturable

The next question is speed. All sensitivities being equal, if you can get there, PCR is going to win hands down with speed.