



PCR Analysis for Spoilage Yeast and Bacteria

Spoilage yeast and bacteria contamination can quickly become a costly problem for a winery. Once these organisms are introduced, they can easily be spread through hoses, equipment, humans, and insect vectors. As soon as contamination is detected, proper procedures should be taken to minimize spoilage.

To help winemakers discover contamination quickly, Vinquiry offers individual **Real-Time PCR** assays that cover a comprehensive range of wine contaminants. Real-Time PCR is a rapid method to identify organisms by the amplification and detection of unique sequences of their DNA while the PCR reaction is taking place. Vinquiry has validated Real-Time PCR assays for two spoilage yeasts, *Brettanomyces/Dekkera* species and *Zygosaccharomyces bailii*. These assays are based upon published articles by David Mills and Trevor Phister. In addition, we have developed assays for the Lactobacillaceae (the family of bacteria that includes *Lactobacillus* and *Pediococcus* species) and for *Pediococcus* species alone. These assays have been validated with dozens of pure cultures of *Lactobacillus* and *Pediococcus* isolated from local wines, as well as with cultures of *Lactobacillus* and *Pediococcus* received from Lallemand. An assay for the *Acetobacter* species is currently being validated and will be available soon.

Available Assays

- **PCR for *Brettanomyces/Dekkera*:** detects *Brettanomyces/Dekkera* yeast which can alter the sensory characteristics of wine. When growing in wine, *Brettanomyces* produces off-aromas, including 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG). The resulting sensory contributions are often described as “Band-aid,” barnyard,” or “mousy.”
- **PCR for Lactobacillaceae:** detects both *Pediococcus* and *Lactobacillus* species, but not *Oenococcus*, *Acetobacter* or wine yeasts. This assay would not distinguish between *Pediococcus parvulus* and *Lactobacillus plantarum*, for example, but would estimate the combined population of both.
- **PCR for *Pediococcus*:** detects *Pediococcus* species but not other wine bacteria or yeasts.
- **PCR for *Zygosaccharomyces bailii*:** specific for the most common spoilage yeast brought into the winery in grape juice concentrate. *Zygosaccharomyces* is resistant to the common preservatives and inhibitors used in winemaking. This yeast thrives under the high osmotic pressure of concentrate as well as the high alcohol content of finished wine, making concentrate almost a selective medium for *Zygosaccharomyces*.

Analysis

About an hour after the DNA is isolated, millions of copies of the unique genetic sequence from the target organism are generated, detected and quantified with fluorescent probes. The fluorescence produced is proportionate to the number of copies generated, which in turn is proportionate to the number of the target cells in the sample. The duration of time it takes to reach a predetermined threshold of fluorescence can be compared to a standard curve which is constructed from a dilution series of a known concentration of the target organisms subjected to the same process.

Sampling

PCR Analysis requires 50mL of sample. In order to ensure that the sample is representative of the wine, it is best to sample after stirring or racking. Please send samples in plastic containers only. Complementary plastic sample bottles are available from all four Vinquiry locations. Please call the phone number below to have sample bottles shipped to you.

For further information on PCR Analysis, please call 707-838-6312.