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### POLYMERASE CHAIN REACTION (PCR) ANALYSIS FOR SPOILAGE YEAST AND BACTERIA

*New special price discount!*

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 and equipment, by cellar workers and insect vectors. As soon as contamination is detected, proper procedures should be taken to minimize potential spoilage. To help winemakers discover contamination quickly, Vinquiry has expanded its number of Individual Real-Time PCR assays, covering a comprehensive range of wine contaminants. These tests are perfect for providing rapid and reliable results that can be available in days, as opposed to a full week with traditional culture plating methods. This rapid detection allows winemakers to take the appropriate steps to limit spoilage and maintain the quality of their wine.

#### Real-Time PCR

Real-Time PCR is a rapid method to identify organisms by the amplification and detection of their unique sequences of 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 pure cultures of *Lactobacillus* and *Pediococcus* isolated from local wines, as well as with cultures of *Lactobacillus* and *Pediococcus* received from Lallemend. An assay for the *Acetobacter* species is currently being validated and will be available soon.

#### Real-Time Quantitative PCR

Real-time quantitative PCR (qPCR) provides an estimate of the population of a species or group of organisms by reference to a standard curve. The standard curve has earlier been generated from the same PCR analysis of a dilution series of a known concentration of the target organisms.

#### No False Positives

Individual Real-Time PCR assays avoid the common problem of false positives associated with Multiplex PCR, which can occur because of the use of multiple primers and probes in the same PCR reaction. Multiple primers and probes often lead to the generation of non-specific PCR products which cannot be distinguished from the desired product in Multiplex PCR. With the Individual PCR assays at Vinquiry, a melt curve at the end of the amplification cycle can determine if the product is the desired product (pure targeted DNA and not the false positive result of non-specific amplification).

#### Assays Available at Vinquiry

- **PCR for *Brettanomyces*:** detects populations of *Brettanomyces* known for altering the sensory characteristics of wine.
- **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 also 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*.

#### Recommendations

Before bottling, blending or moving red wine, Vinquiry recommends testing for *Brettanomyces* and *Pediococcus* or *Brettanomyces* and Lactobacillaceae. If juice concentrate has been added to the wine, we recommend testing for *Zygosaccharomyces bailii*.

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## **Discount Pricing**

Vinquiry is offering a special rate on PCR testing. The first assay, for a specific yeast or bacteria, is \$60 and all other assays on the same sample are only \$20 each. For further information on PCR Analysis, please call 707-838-6312.

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