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# Implementation of real-time PCR to ensure bacteria free yeast propagations in a mid-sized craft brewery

**Ben Bailey, Tröegs Brewing Co., Hershey, PA, USA**

## Introduction

Yeast propagation is one of the most sensitive steps in fermentation management, due to the fact that the yeast and any possible infections are subsequently used in multiple fermentations. The short time available during propagation for microbiological analysis is insufficient for traditional methods. By adding real-time PCR (Polymerase Chain Reaction) to our microbiological tools, it is possible to screen every yeast propagation for beer spoilage bacteria prior to its release for fermentation.

## Method

In order to test for beer spoilage bacteria during propagation, aseptic samples from each propagator are incubated with NBB-C[1] as an enrichment medium for PCR analysis, which can be carried out after only three days. In addition to the PCR analysis, propagator samples are plated on two Universal Beer Agar with Actidione (UBA+) plates and incubated aerobically and anaerobically to test for beer spoilage bacteria using traditional methods. We utilize several differential media to screen for wild yeasts: Lysine Agar, Copper Sulfate Agar (Taylor and Marsh), as well as Lin's Wild Yeast Medium. These plates are incubated aerobically and can be evaluated after 3 days. Other differential media that can be used for wild yeast detection can be seen in **figure 1**.

| | Lysine Agar | Copper Sulfate Agar (Lin) | Crystal Violet Agar | Lin's Wild Yeast Medium | Schwarz Differential Medium | Copper Sulfate Agar (Taylor and Marsh) |
|---|---|---|---|---|---|---|
| Brewing Culture Yeast | - | - | - | - | - | - |
| Saccharomyces Wild Yeast | - | - | + | + | +/- | + |
| Non-Saccharomyces Wild Yeast | + | + | - | - | + | + |

**Figure 1**. Differential Media for Wild Yeast Detection **[1]**

The aerobic UBA+ plates and the wild yeast plates can be evaluated within a few minutes by trained personnel. Traditional microbiological techniques cannot confirm the presence of beer spoilage bacteria in less than five to seven days, although our brewery requires yeast from propagation to be released for fermentation within three days. While PCR analysis does require more training, it only takes about an hour to prepare the DNA extraction with results available after an additional 45 minutes. The DNA extraction method is shown in **figure 2.**

1. Nutrient media for beer-spoiling bacteria (concentrate)

**DNA extraction diagram for PCR screening of beer spoilage bacteria**

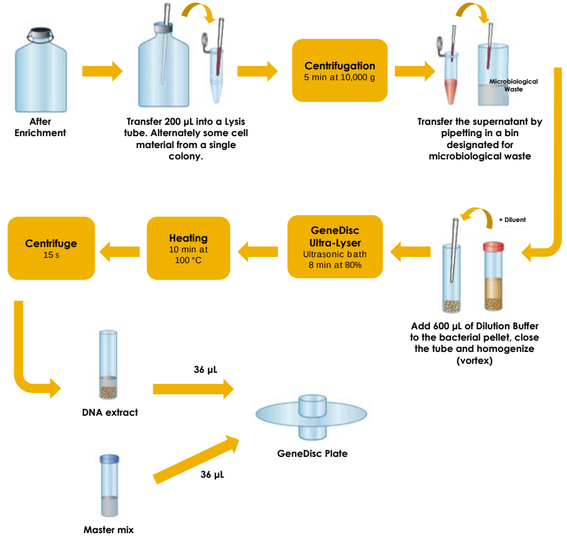


**Figure 2**. DNA extraction diagram for beer spoilage bacteria (courtesy of Pall Corp.)

The Pall GeneDisc[2] PCR system is unique in its use of the so-called GeneDisc plates. These discs are pre-loaded with "key components like primers and probes along with appropriate controls […] which ensures reproducible performance and eliminates operator errors that can occur during multiple reagent additions."**[2]** The GeneDisc plates are designed to run six samples simultaneously, however our brewery never has more than 3 propagation samples at one time. In order to maximize the value provided by the PCR analysis, we save any anaerobic plates which have potential beer spoilage bacteria colonies and have them screened on the machine.

2. GeneDisc is a registered trademark of the Pall Corporation

An infection of beer spoilage bacteria during propagation is especially problematic for those breweries that do not stabilize their beers by pasteurization or sterile filtration. Within the seven days required for traditional beer spoilage bacteria incubation, up to nine brews can be fermented from a single propagation in our brewery. PCR analysis gives us the ability to prevent a single one of these brews from being infected by beer spoilage bacteria. The potential loss caused by an infection of all nine brews would more than offset the costs of the PCR equipment.

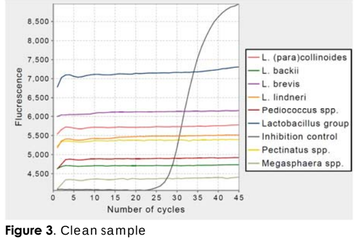


**Figure 3**. Clean sample

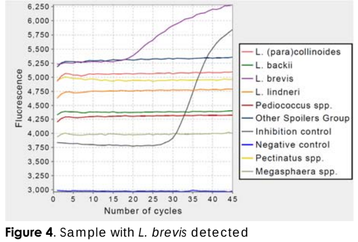


**Figure 4**. Sample with *L. brevis* detected

**Figures 3 and 4** show examples of a sample showing no detectable beer spoilage bacteria and a sample with *Lactobacillus brevis* respectively.

The graph and a correlating screen confirming the absence or presence of beer spoilage bacteria make the evaluation of the data very easy for lab personnel.

PCR analysis can identify the group or species of an organism, which helps determine the risk of spoilage when dispositioning beer in any stage of production, including packaged beer. Each brewery should become familiar with which types of bacteria are likely to cause damage in their various brands in order to react appropriately to the data provided by PCR analysis.

## Conclusion

Using PCR for the detection of beer spoilage bacteria, along with traditional microbiological methods for the detection of wild yeast, we are able to detect the majority of microbiological issues in our propagations before any yeast leaves the propagator. This nearly eliminates the yeast propagation step as a source of contamination in fermentation. Waiting 5–7 days for traditional microbiological methods to produce results for bacterial infection during propagation would not be possible because the yeast is ready to be pitched into a fermenter within three days. Despite the relatively high cost of PCR in comparison with traditional methods, the reduction in risk of financial loss due to beer spoilage bacteria can be justified in even mid-sized breweries and should certainly be considered in larger breweries. As costs for this equipment continue to decrease, many smaller to mid-sized breweries may be able to justify the cost of real-time PCR analysis based on the loss it can prevent by reducing the time required to test for beer spoilage bacteria.

## References

1. Back, Werner (1994). Differenzierung zwischen Brauerei-Kulturhefen und Fremdhefen. In: Farbatlas und Handbuch der Getränkebiologie, Band 1, pp. 44-48. Fachverlag Hans Carl, Nürnberg.
2. "GeneDisc® Rapid Microbiology System." Pall Corporation, n.d. Web. 05 May 2014. <http://www.pall.com/main/biopharmaceuticals/genedisc-rapid-microbiology-system-53215.page#WAT>.

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## Contact

The author can be contacted at: bbailey@troegs.com