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# Rapid detection and identification of 30 beer-spoilage bacteria in one test

Cordt Grönewald and Julia Hoffmann, BIOTECON Diagnostics, Potsdam, Germany

**Summary**:
The detection and identification of beer spoilage bacteria by conventional methods in a routine lab of a brewery is a time consuming and laborious task. Real-time PCR using the **food**proof® Beer Screening Kit provides easy, fast, and reliable results in 24-48h. BIOTECON Diagnostics has developed a test based on PCR and the LightCycler® technology from Roche. It allows the detection of 30 beer-spoilage bacteria, including 12 single identifications, in just one test. The method does not require any molecular biological skills from the user and is adjusted to the routine lab allowing a throughput of up to 30 or 96 samples (depending on the instrument) per PCR run. Real-time PCR is performed on a LightCycler® and uses hybridization probes and FRET (fluorescence resonance energy transfer) to detect the DNA amplificates. After the PCR run, the absence or presence of beer spoilers can be detected immediately. Subsequent melting curve analysis allows the user to differentiate bacteria from a positive result without any further hands-on time. Differences such as length, G-C-content, and base sequence, make the signal obtained by melting curve investigation distinct for nearly every probe-DNA combination. BIOTECON Diagnostics thus provides a rapid and easy method for the screening of the most troublesome beer spoilers along with their subsequent identification.

Real-time PCR performed on a LightCycler® using hybridization probes and FRET (fluorescence resonance energy transfer) to detect the PCR products is illustrated in Figure 1.
In each cycle the probes anneal adjacent to each other. The first fluorescent dye is excited by light at 470 nm, the energy is transferred to the second fluorescent dye, which lies in close proximity when bound to the DNA, and is emitted at 640 nm.

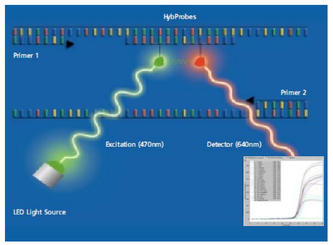


*Fig. 1: Principle of LightCycler® detection using hybridization probes and FRET.*

## STEP 1: DETECTION

The **food**proof® Beer Screening Kit is a ready-to-use system for the routine lab of a brewery. The sample preparation steps are as simple as follows:
· Filtration of the sample (bottled beer, yeast cultures, etc.)
· Sample enrichment in broth for 24 h to 48 h
· Centrifugation of 1 ml for 10 min at 15,000 x g
· Discard supernatant
· Resuspension of the pellet in lysis buffer, followed by heat treatment for 10 min (≥95°C)
· Pipetting sample into the PCR mix and starting the real-time PCR run.
The screening result is shown in Figure 2. It shows the presence or absence of one or more of the 30 beer spoilage bacteria listed in Table 1.

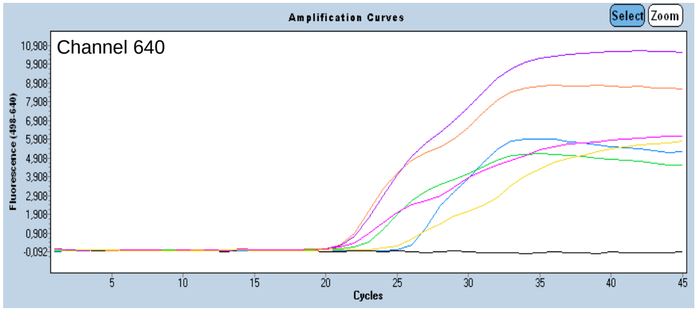


*Fig. 2: PCR amplification curves in channel 640nm allow to screen for the presence of one or more of the 30 beer spoilers.*

## STEP 2: IDENTIFICATION

After amplification of bacterial DNA for detection of the beer spoilers, the LightCycler® carries out a melting curve analysis for their identification. Melting curve analysis investigates the melting behavior of the probes on the DNA. The behavior varies depending on the binding strength of a specific probe. This analysis is performed automatically without any additional hands-on-time.

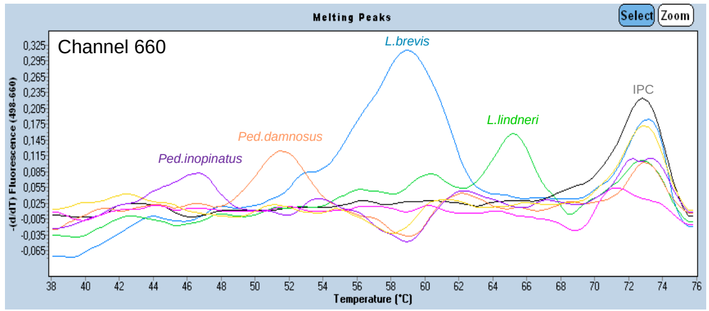


*Fig. 3: Melting curve differentation of Pediococcus inopinatus, Pediococcus damnosus, Lactobacillus brevis, and Lactobacillus lindneri in Channel 660. The internal positive control also peaks in this channel.*

Due to differences such as length, G-C-content, and base sequence, the signal obtained by melting curve analysis is distinct for every probe-DNA combination. In this kit, the melting curves are used to differentiate beer spoilers. Figure 3 shows an example of melting curves for *Ped. inopinatus*, *Ped. damnosus*, *L. brevis*, and *L. lindneri*) in channel 660. The curves are clearly differentiable. Thus, it is possible to identify contaminants without any additional work. Table 1 shows the spectrum of bacteria detected by the kit in one PCR reaction.

*Table 1. Strains detected by the food*proof® *Beer Screening Kit*

| | |
|---|---|
| ***Lactobacillus*** | ***L. brevis, L. lindneri, L. casei, L. paracasei, L. coryniformis, L. buchneri, L. parabuchneri (frigidus), L. pentosus, L. collinoides, L. paracollinoides, L. plantarum, L. paraplantarum, L. perolens, L. harbinensis (L. perolens* DSM 12745*), L. sp. (*DSM 6265 *L. brevisimilis), L. rossiae, L. backii*** |
| ***Pectinatus*** | ***Pec. cerevisiiphilus, Pec. frisingensis, Pec. haikarae, Pec. sp.* DSM 20764** |
| ***Megasphaera*** | ***M. cerevisiae, M. paucivorans, M. sueciensis*** |
| ***Pediococcus*** | ***Ped. damnosus, Ped. inopinatus, Ped. parvulus, Ped. pentosaceus, Ped. acidilactici, Ped. claussenii*** |