229

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Fast and reliable screening and identification of the most relevant beer spoilage bacteria plus detection of spoilage yeasts in beer by real-time PCR

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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 products. 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.

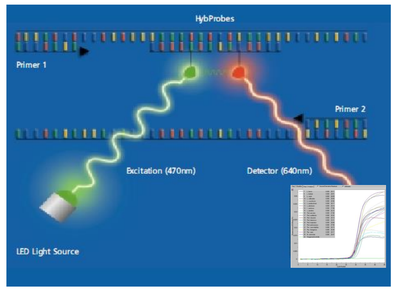


Fig. 1: **Principle of LightCycler® detection using hybridization probes and FRET.**

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.

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, L. acetotolerans***
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***

STEP 1: DETECTION

The **food**proof® Beer Screening Kit is a ready-to-use system for the routine lab of a brewery. Time to result just 2h after enrichment.

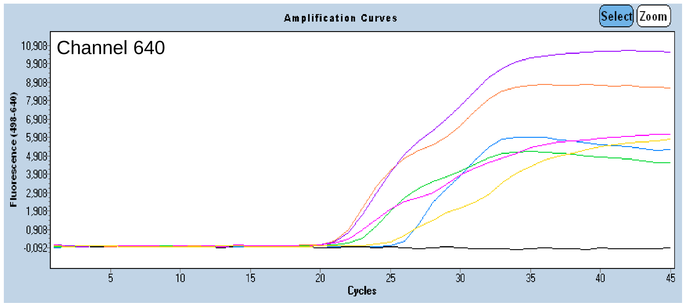


Fig. 2: **PCR amplification curves in channel 640 nm allow to screen for the presence of one or more of the 30 beer spoilers.**

STEP 2: IDENTIFICATION

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

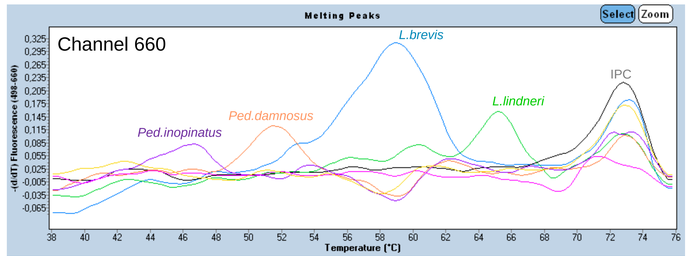


Fig. 3: **Melting curve differentation of *Pediococcus inopinatus*, *Pediococcus damnosus*, *Lactobacillus brevis*, and *Lactobacillus lindneri* in Channel 660. The internal positive control also peaks in this channel.**

food**proof® Spoilage Yeast Detection LyoKits I and II**

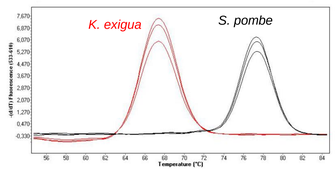
- Detect yeast spoilers even in great excess of brewing strain
- Lyophilized for easy handling
- Hydrolysis probes – run on most qPCR cyclers
- Life / Dead discrimination with Reagent D

FAM	HEX		ROX	Cy5
Dekkera/Brett	***Zygosaccharomyces***		***Saccharomyces***	
B. naardenensis	*Z. bailii*	*Z. parabailii*	*S. arboricola*	*Internal Control*
B. nanus	*Z. bisporus*	*Z. pseudobailii*	*S. bayanus†*	
D. anomala	*Z. gambellarensis*	*Z. pseudorouxii*	*S. cariocanus*	
D. bruxellensis	*Z. kombuchaensis*	*Z. rouxii*	*S. castelli*	
D. custersiana	*Z. lentus*	*Z. sapae*	*S. cerevisiae**	
	Z. machadoi	*Z. siamensis*	*S. chevalieri*	
	Z. mellis		*S. eubayanus*	
			S. kudriavzevii	
			S. mikatae	
			S. paradoxus‡	
			S. pastorianus	
			S. uvarum	

† Including *"S. douglasii"*
‡ Including *"S. globosus"*
* Including *S. cerevisiae var. diastaticus, "S. boulardii", "S. ellipsoideus" and "S. norbensis"*

Fig. 4: **Strains detected by food**proof®**Spoilage Yeast Detection LyoKit I**

FAM	HEX	ROX	Cy5
Saccharomyces cerevisiae var. diastaticus	*Wickerhamomyces anomalus* *(= Pichia anomala)*	*Kazachstania exigua* *(= S. exiguus)* *Schizosaccharomyces pombe*	*Internal Control*



K. exigua and *S. pombe* can be differentiated via melting curve analysis

Fig. 5: **Strains detected by food**proof®**Spoilage Yeast Detection LyoKit II**