

Risk-based Identification of Spoilage Bacteria and Wild Yeast for Quality Professionals

Empowering proactive decision-making for your brewery



THE CHALLENGE

Increasing competition and complexity require a new approach

The brewing industry has grown exponentially over the past decade with new entrants crowding the market. Today more than ever, due to the variety of beer styles and brewing complexity, quality management has become a critical differentiator. The need for accurate, actionable information during production and post-packaging is essential to maintain quality and protect brand image.

Traditional quality testing methods, if implemented properly, can identify issues, but they fail to do so quickly and efficiently. Delayed information leads to reactive quality decisions made after the damage has been done. To address this paradigm, several rapid microbial detection technologies have recently emerged. These rapid methods can provide same or next day information regarding contamination events, empowering quality personnel to make proactive and preventive decisions. However, these technologies typically require cumbersome workflows that are costly and complicated to use. Also, test results can be ambiguous with regard to the spoilage risk of a particular microorganism.



THE PRODUCT

Delivering functional genetic analysis for the most critical beer spoilers

Our passion for innovation, our successful development of the Veripro platform and the need for a rapid, multiplexed, high-throughput assay for risk-based identification of beer spoilers, led us to develop brewPRO™. The first of the brewPRO assays, brewPRO for *Lactobacillus* and *Pediococcus*, provides risk-based detection and characterization while maintaining the ease of use and cost effectiveness required by progressive breweries. This information enables an assessment of spoilage risk based on the abundance of genes that confer resistance to inhibitory hops compounds.

The multiplex assay simultaneously detects validated Veripro gene targets in a single assay:

- Presence of *Lactobacillus* and *Pediococcus* species on the FAM channel
- Presence of hop resistance plasmids *horA* and *horC* that enable persistence and beer spoilage on the ROX channel
- Internal amplification control (IAC) to demonstrate successful amplification on the HEX channel

THE TECHNOLOGY

Synergizing DNA Signature Capturing Technology™ and RT-PCR

Invisible Sentinel's continued focus on innovation led to the development of a new Real-Time (RT) PCR technology platform, Veripro®. This new platform incorporates reagents from our core system, Veriflow®, into assays compatible with RT-PCR instruments for the ultimate user experience. Veripro maintains the same robustness, speed to results, and ease of use of the Veriflow platform, but leverages RT-PCR's ability to multiplex and takes advantage of the platform's capacity for high throughput analyses.

Veripro features a multiplexed configuration that simultaneously detects multiple genetic targets including an internal amplification control, in a single, pre-aliquotted tube using four optical channels: FAM, HEX, ROX, and Cy5. Results can be monitored in real-time to detect the presence and relative abundance of contaminants. A simplified data analysis approach enables clear interpretation of results, including spoilage potential, to empower rapid and effective quality decisions.

brewPRO Bacteria Screen Performance Specifications	
Sensitivity (LOD)	≤ 10 cells/ml or ≤ 1 cell per 100ml with enrichment
Time to Results	< 3 hours or 18-24 hours with enrichment
Matrix Compatibility	Beer, colony PCR, environmental, fermentation, yeast slurry & post-pasteurization (Viability application)
Assay Configuration	Multiplex, qualitative and risk assessment
Target Selection	<i>Lactobacillus</i> and <i>Pediococcus</i> -specific genomic target (FAM) <i>Lactobacillus</i> and <i>Pediococcus</i> -specific hop resistance genes <i>horA</i> and <i>horC</i> (ROX) Internal Amplification Control (HEX)
Specificity	<i>Lactobacillus</i> species Including: <i>L. brevis</i> , <i>L. buchneri</i> , <i>L. rhamnosus</i> , <i>L. jensenii</i> , <i>L. backii</i> , <i>L. paracollinoides</i> , <i>L. lindneri</i> , <i>L. delbrueckii</i> , <i>L. fructivorans</i> , <i>L. plantarum</i> , <i>L. pentosus</i>
	<i>Pediococcus</i> species Including: <i>P. damnosus</i> , <i>P. inopinatus</i> , <i>P. parvulus</i> , <i>P. pentosaceus</i> , <i>P. acidilactici</i> , <i>P. clausenii</i>



THE ANALYSIS

Scientific approach provides unique information to assess spoilage risk

The discovery and characterization of hop resistance genes demonstrated that members of a single *Lactobacillus* or *Pediococcus* species vary widely in their ability to cause beer spoilage. Two hop resistant related genes known as *horA* and *horC* have been shown to enable lactic acid bacteria, such as *Lactobacillus* spp and *Pediococcus* spp, to grow in beer. The function of *horA* and *horC* is to encode transporters on the surface of cells that expel toxic hops compounds to enable survival in the presence of hops. These two genes were found to have nearly 100% correlation with species of beer spoiling lactic acid bacteria in several studies.

However, mere detection of hop resistance genes is insufficient to fully understand the risk and potential for spoilage these organisms present. brewPRO Bacteria screen reports BOTH the presence of *horA* and/or *horC* AND the relative abundance of these gene targets. This information serves as an indicator of bacterial persistence in beer and spoilage potential. The relative abundance is calculated by comparing the cycle threshold value

(Ct/Cp value) of hop resistance gene targets to the Ct/Cp value of ribosomal gene targets in order to assess spoilage risk. The higher the relative abundance, the more likely the organism can thrive and spoil even high IBU & ABV beers.

Unparalleled Analysis	Unrivalled Ease of Use
<ul style="list-style-type: none">• Superior, risk-based identification of the presence of <i>Lactobacillus</i> and <i>Pediococcus</i> in samples ranging from environmental to raw materials to in-process beer through finished products• Proprietary analysis that quantifies the relative abundance of hop resistance genes• Results in under 3 hours for wide variety of sample types• Automated data collection and analysis	<ul style="list-style-type: none">• Simplified workflow with easy sample preparation - no DNA purification• Multiplexed format• High throughput with fast time to results• Easy to train personnel• Compatible with multiple instruments



THE PRODUCT

Delivering multi-plexed detection of detrimental wild yeast species

Recent studies have shown that not only *Brettanomyces* wild yeast contamination can cause headaches for brewers. *Saccharomyces cerevisiae* var. *diastaticus*, sometimes associated with Saison strains possessing the glucoamylase gene (*STA1*), can cause unwanted secondary fermentation and severe over-attenuation if cross contaminated with typical brewing strains. The brewPRO Wild Yeast screening assay is able to simultaneously detect both types of wild yeast in a single reaction.

The multiplex assay simultaneously detects validated Veriflow gene targets in a single assay:

- FAM: Presence of *Dekkera/Brettanomyces* species
- Cy5: *Brettanomyces bruxellensis* species
- ROX: *Saccharomyces cerevisiae* var. *diastaticus* (*STA1*)
- HEX: Internal amplification control (IAC) to demonstrate successful amplification on the HEX channel

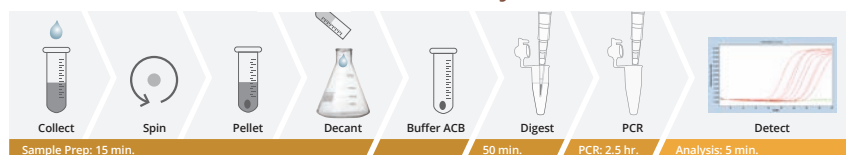
brewPRO Wild Yeast Screen Performance Specifications	
Sensitivity (LOD)	10-100 cells/ml
Time to Results	< 4 hours or 48 hours with enrichment
Matrix Compatibility	Beer, colony PCR, fermentation, environmental, yeast slurry, enrichment
Assay Configuration	Multiplex, qualitative
Target Selection	<i>Saccharomyces cerevisiae</i> var. <i>diastaticus</i> species <i>STA1</i> genetic target (FAM) <i>Dekkera/Brettanomyces</i> species (ROX) <i>Brettanomyces bruxellensis</i> (Cy5) Internal Amplification Control (HEX)
Specificity	<i>S. cerevisiae</i> var. <i>diastaticus</i>
	<i>Dekkera/Brettanomyces</i> Species Including: <i>B. bruxellensis</i> , <i>B. anomalus</i> , <i>B. naardenensis</i> , <i>B. custersiana</i>

brewPRO Bacteria Same Day Workflow



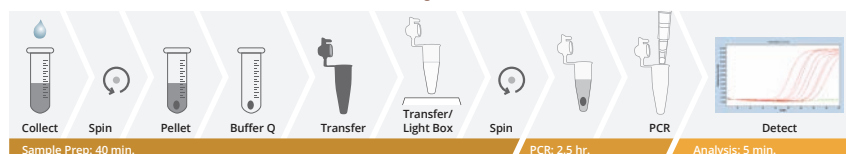
Adaptive workflow will accommodate colony pick, fermentation and yeast slurry as well as enrichment if desired

brewPRO Wild Yeast Same Day Workflow



Adaptive workflow will accommodate colony pick, fermentation and yeast slurry as well as enrichment if desired

brewPRO Bacteria Viability Workflow



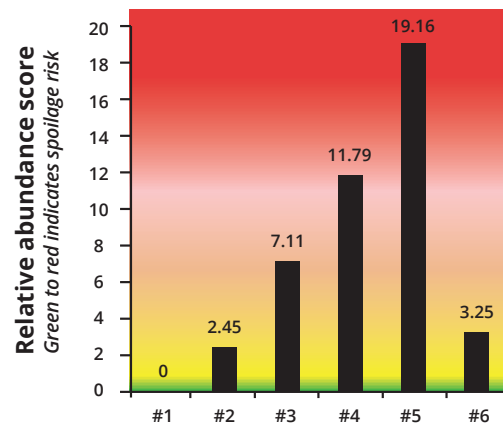
Adaptive workflow will accommodate colony pick, fermentation and yeast slurry as well as enrichment if desired

ITEM #	DESCRIPTION	SIZE
IS1063LC	brewPRO Bacteria Screen Kit for GENE-UP Thermal Cycler	1 Kit, 24 tests
IS1080LC	brewPRO Wild Yeast Screen Kit for GENE-UP Thermal Cycler	1 Kit, 24 tests
ISEV056	brewPRO Color Compensation Kit	
IS1065	Viability kit for Bacteria Screen	1 Kit, 24 reagents
ISTC007	GENE-UP Thermal Cycler	1 Kit, 24 reagents

For more information or to place an order, please contact Invisible Sentinel at 215.966.6118 or www.invisiblesentinel.com

Invisible Sentinel, a global molecular solutions company, is dedicated to providing first-in-class microbial detection tools. The company's core technology, Veriflow, is a patented, game-changing platform that integrates molecular diagnostics, antibody design, and immunoassays. Veriflow has been applied across multiple industries including food safety and beverage quality.

brewPRO test results for various brewery isolates of *L. brevis* and *L. plantarum*



Brewery Isolates #1 Isolate: *L. brevis* Brewery, #2 Isolate: *L. brevis* Brewery, #3 Isolate: *L. brevis* Brewery, #4 Isolate: *L. brevis* Brewery, #5 Isolate: *L. brevis* Brewery, #6 Isolate: *L. plantarum* Brewery

The higher the relative abundance score the higher the risk of spoilage. Assessment of spoilage risk should also take into consideration beer style.

The graph above highlights the value of this information by comparing the relative abundance of several brewery isolates of *Lactobacillus* species. *L. brevis* is the predominant beer spoiler among *Lactobacillus* species, and its detection can strike immediate fear into brewers. However, not all *L. brevis* isolates are created equally. Several *L. brevis* isolates were tested with brewPRO and test results demonstrate that each isolate poses a significantly different spoilage risk in beer. An isolate of *L. plantarum*, a subspecies not typically associated with beer spoilage, was also tested and results showed hop resistance capability and a relative abundance score that would make it a cause for concern in certain beer styles. With this comprehensive information on spoilage potential, brewers can make more accurate decisions, and remediation activities can be tailored – improving overall beer quality and operational efficiencies. This unique information empowers quality teams to make real-time decisions about their processes by providing data on the specific nature of the organism in their facility from pitch through packaging.



GeneUp Thermal Cycler & IS Viability PhotoLysis Devices