

ETS Scorpion™ Detection of Fermentation Contamination Organisms

New Capabilities

Despite the best practice of modern fermentation methods, microbial contamination often occurs during ethanol production. The contamination microbes are capable of survival and growth during the biofuel production process, potentially reducing ethanol yield and fermentation efficiency.

Microbiological contamination is often undetected until related problems in the fermentation process become noticeable such as excessive foaming or yield loss. ETS now offers Scorpions™ polymerase chain reaction assays, based on specific genetic markers, to detect biofuel fermentation contamination microorganisms. This new, genetic analysis method detects microbial populations directly from the fermentation. Results are routinely reported within 4-5 hours giving production staff the ability to address problems before contamination gets out of control.

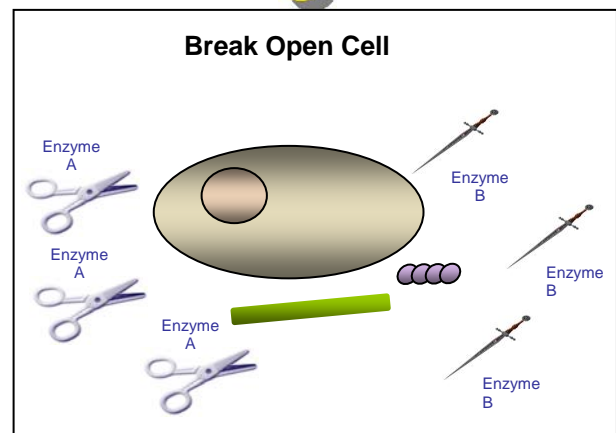
Targeting Specific Contaminant Microbes

ETS has developed the Scorpion™ assay to provide production microbiologists a tool for rapid identification and quantitation of contaminant microbes in their fermentation process. The assays utilize a combination of gene amplification and hybridization to accurately quantify the total number of viable cells in a sample.

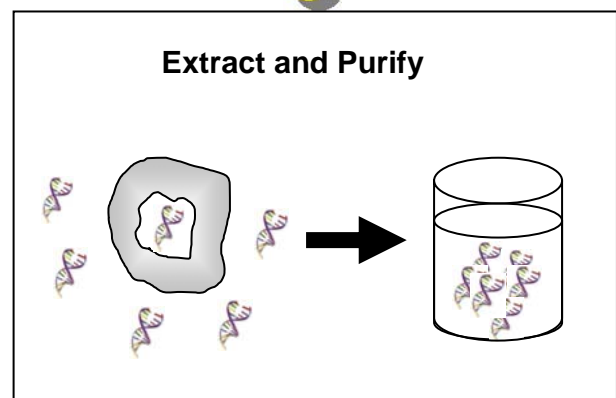
ETS Fermentation Contamination Assays screen for a range of bacteria in the biofuel production process, including acetic acid producing bacteria such as *Acetobacter* and *Gluconobacter*, and selected species of *Lactobacillus* and *Pediococcus*. They also detect the contaminant yeasts *Brettanomyces* and *Zygosaccharomyces*. An additional assay is available to quantitate levels of *Saccharomyces*.

Genetic probes, targeted to specific contaminant organisms, give the industrial microbiologist the ability to monitor only those organisms that have the potential to adversely impact the fermentation process.

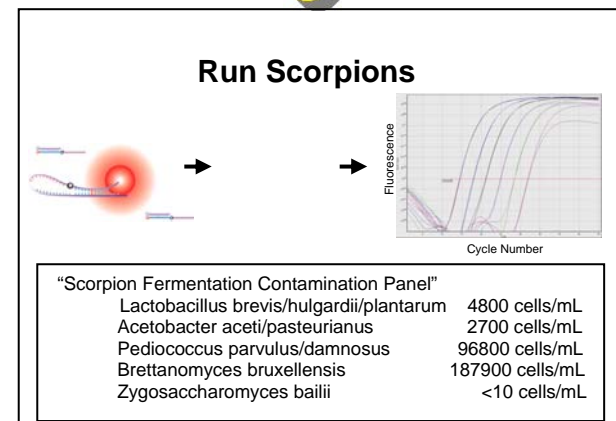
1



2



3



ETS Scorpion™ Detection of Specific Fermentation Contamination Microbes

The ETS Scorpions™ Diagnostic system enables the industrial microbiologist or process engineer to specifically monitor those microorganisms that pose a threat to the quality of their biofuel fermentations, including: *Brettanomyces*, *Zygosaccharomyces*, *Lactobacillus*, *Pediococcus* and acetic acid bacteria.

Brettanomyces is widely distributed in fermentation environments. They can produce high concentrations of acetic acid. Conditions optimal for growth of *Saccharomyces* are also conducive to *Brettanomyces* growth. These contaminant yeast compete directly with *Saccharomyces* for fermentable sugar, resulting in reduced ethanol yield.

Zygosaccharomyces is a contaminant yeast that is tolerant of high osmotic environments and is resistant to sorbate. It has been identified in biofuel fermentations. It is an aggressive, fast growing yeast that can out compete *Saccharomyces*.

Pediococcus is a lactic acid bacteria found as a contaminant in biofuel fermentations. *Pediococcus* is capable of producing large amounts of lactic acid and biogenic amines. They can interfere with fermentation as well as grow to large numbers, competing with *Saccharomyces* for fermentable sugar.

Lactobacillus is a lactic acid bacteria commonly found in biofuel fermentations. They may produce high concentrations of acetic acid in a short period of time - often in a matter of a few hours. This can rapidly affect fermentations by having an inhibitory action on *Saccharomyces*.

Acetic acid bacteria are commonly associated with corn mash and the biofuel production environment. The two groups of acetic acid

bacteria detected by the Scorpions Diagnostic are *Gluconobacter* and *Acetobacter*. These bacteria can generate high levels of acetic acid in the presence of oxygen. The presence of these acetic acid bacteria has been confirmed in many stages of the biofuel production process. They compete for fermentable sugar and can also produce inhibitory levels of acetic acid.

Advantages of Gene Specific Analysis

There are several advantages of genetic analysis as compared to traditional plating methods.

- **Time to results** - Standard time to results for Scorpions™ is 4-5 hours in house or 2 working days from a service lab.
- **Detects Viable but Nonculturable (VNC) organisms that are not detected by plating**- Many fermentation contamination microbes can survive in a VNC state under the combined stress of pH, and alcohol and will not grow under routine plating conditions. These same microbes are detectable using Scorpions technology.
- **Specific identification**- Scorpions™ technology is highly specific to the target organism, requiring three exact DNA sequences, that are aligned and spaced properly to detect the target organism. This enables identification to species for the selected fermentation contamination microbes.

Proactive use of Scorpions™ technology as a preemptive screening tool enables early detection of fermentation contamination. Early detection enables early intervention, preventing loss of yield and efficiency.