BioReliance

Biologics Safety Testing Services

Genetic identification of microbial organisms using sequence analysis of the ribosomal RNA gene (rDNA)

Introduction

BioReliance offer a genotypic identification method for eubacteria, yeasts and filamentous fungi which involves sequencing the highly conserved and universally functional gene that encodes a portion of the ribosome (rDNA). This genotypic method offers improved accuracy, precision and faster turnaround to result compared to classic methods that rely on morphology, biochemistry, phenotype and culture requirements.

Bacterial indentification targets the 16S rDNA which is comprised of nine hypervariable regions interspersed among nine conserved regions. Sequence analysis targets approximately 500 base pairs of the 16S rDNA gene which incorporates three of the hypervariable regions providing a high level of discrimination that is sufficient to make a successful identification for most bacterial organisms. Fungal and yeast identification target the D2 region of the nuclear large-subunit rDNA that offers ample variation to identify most yeast and fungi to the species level.

Comparison of the test sequence against the fully validated MicroSEQ® microbial libraries containing in excess of 2000 microbial sequences ensure a reliable identification from a range of key genera present within the library, some of which include *Bacillus, Brevibacillus, Chryseobacterium, Coryneforms, Paenibacillus, Pseudomonas* and *Staphylococcus.*

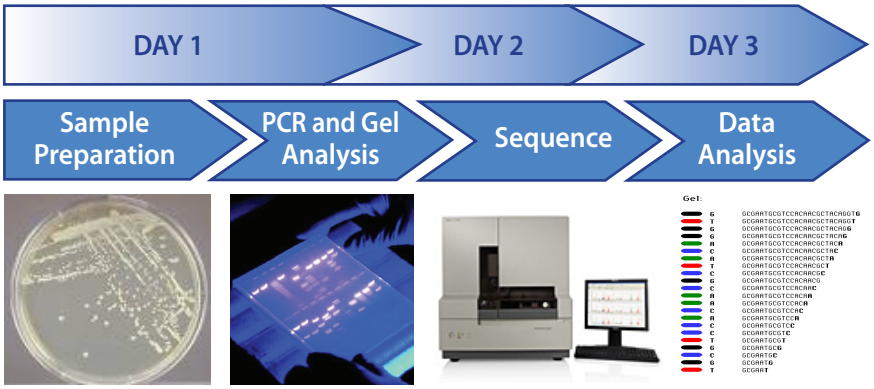
Regulatory bodies including the Food and Drug Administration (FDA) recommend the use of DNA based identification methods for bacteria such as sequence analysis of the 16S rDNA gene.[1] BioReliance offer this service as a GMP compliant and validated identification system. (**Figure 1**)

Assay method

For a successful identification pure cultures are required. The assay initiates with DNA isolation from the microbial cells by a crude lysis step, DNA is collected and prepared for PCR. Amplification is performed using a standard primer set which targets the appropriate rDNA.

PCR amplified product is analysed by agarose gel electrophoresis. The successfully amplified products are sequenced using standard sequencing primers which target the amplified PCR product in the forward and reverse orientation.

Figure 1: Workflow Process



Rapid method for the identification of microbial organisms.

Fully validated microbial library ensuring reliable results.

GMP compliant and validated genotypic microbial identification method.

Regulatory bodies recommend molecular ID methods.

BioReliance® by SAFC

Genetic identification of microbial organisms, continued

The resulting DNA sequence is analyzed using MicroSEQ® ID Analysis Software and compared to the appropriate rDNA sequence library. Data quality is assessed in the Analysis QC report and the closest matches to the test sample are detailed in the Library Search Report. Raw data is also available in the form of an electropherogram (**Figure 2**).

Assay Appropriateness and Use:

Identification of	Sample Specification
Microbial Cell Bank	**Pure microbial culture required.** For organisms that produce hyphal structures and spores, it is preferred that samples are submitted with minimal spore formation where possible e.g. hyphal mass cell pellet or freshly prepared sub-culture on solid media such as a nutrient agar plate. For organisms that produce homogenous colonies, it is preferred that samples are submitted on solid based media where possible e.g. nutrient agar plate
Microbial Isolate from Environmental Monitoring	
Microbial Isolate from Investigation	

BioReliance have a dedicated Programme Management Team who can further assist you if you require further information or wish to discuss your requirements in more detail.

Figure 2: Electropherogram data for *E. coli* bacterial positive control.

Ordering Information

Assay Number	Assay Description	Regulatory Compliance	Sample Requirements
106701GMP.BUK	Genetic identification of bacterial microorganisms using the Fast MicroSEQ® 500 Protocol (3 day turn-around time)	GMP	Provide a pure bacterial culture in one of the following formats: 1 x agar plate/slant or 1 x cell pellet containing 5×10^7 or 1×1 ml broth containing 5×10^7 cells.
106708GMP.BUK	Genotypic identification of yeast and fungi using the Fast MicroSEQ® D2 LSU rDNA system (3 day turn-around time)	GMP	Provide a pure yeast or fungal culture in one of the following formats: 1 x agar plate/slant or 1 x cell pellet containing 5×10^7 or 1x1ml broth containing 5×10^7 cells or 1×1 cm^3 hyphal mass pellet.

References:

[1] FDA Guidance for Industry—Sterile Drug Products Produced by Aseptic Processing. US FDA, September 2004, Pharmaceutical cGMPs.

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