



# Microbiology & Microbiome Modulators.

## Services

### 1) Bacterial identification by 16S rRNA Sequencing

Amplification and sequencing of the gene encoding 16S ribosomal RNA using universal primers has become widely used in clinical laboratories for identification of bacteria at the genus and species levels, either after their isolation in culture or directly from clinical samples.

EQUIPMENT: Genetic analyzer ABI 3130XL) using the advanced chemical BigDye Terminator 3.1 (Applied Biosystems).

#### Applications:

- To provide genus and species identification of isolates that do not fit any recognized biochemical profiles.
- To confirm the suspected identity of an unknown isolate.

### 2) Bacterial identification and relative quantification by Real-Time PCR

This technique allows the detection of bacterial strains directly from clinical samples or from small amounts of cultured bacterial cells, thus improving the sensitivity and decreasing the time required for bacterial identification. PCR has been particularly useful in this regard, which relies on primer sequences designed to facilitate bacterial identification at any level of specificity: strain, species or genus.

EQUIPMENT: 7500 Real Time PCR (Applied Biosystems)

#### Applications:

In recent years, real-time PCR methods have been developed and described for the rapid detection and identification of several bacterial strains. Real-time PCR is a promising tool for distinguishing specific sequences from a complex mixture of DNA and therefore is useful for determining the presence and quantity of pathogen-specific or other unique sequences within a sample. Real-time PCR facilitates a rapid detection of low amounts of bacterial DNA accelerating therapeutic decisions and enabling an earlier adequate antibiotic treatment.

### **3) Bacterial identification by Fragment analysis**

Terminal restriction fragment (TRF) length polymorphisms (T-RFLP) due to 16S ribosomal DNA (rDNA) sequence diversity to rapidly identify bacterial pathogens. TRF profiles for each organism are determined by sizing fragments from restriction digests of PCR products derived from two sets of 16S rDNA-specific fluorescent dye-labeled primers.

This service includes:

- Amplification and labelling of the PCR product
- Electrophoresis of the samples
- Analysis of the results with Gene Mapper software.

#### **Applications:**

##### - Amplified Fragment Length Polymorphism (AFLP) Mapping

Amplified fragment length polymorphism (AFLP®) analysis is a genetic mapping technique that uses selective amplification of a subset of restriction enzyme-digested DNA fragments to generate a unique fingerprint for a particular genome. The power of AFLP analysis derives from its ability to quickly generate large numbers of marker fragments for any organism, without prior knowledge of the genomic sequence.

##### - Microsatellite Analysis Application

Microsatellite genotyping is the genotyping of tandem repeats such as Short Tandem Repeats (STRs) or Variable Nucleotide Tandem Repeats (VNTRs). STRs and VNTRs are polymorphic DNA loci present throughout the genome. Microsatellite genotyping is a widely accepted tool for a variety of applications such as, linkage mapping studies, association studies, and identification of organisms.

### **4) Total Bacteria Count**

Bacterial plate counts are recommended to be performed on every batch of product following manufacture. Bacterial counts are conducted by taking a sample of the product and placing it in a suitable neutralizer broth, and then plating dilutions to appropriate bacterial growth agar plates. Those plates are then incubated and analyzed to determine cfus (colony formation units).

## **5) Antagonistic activity of bacteria**

Some bacterial strains have the capability of inhibiting pathogenic microorganism with antimicrobial metabolites, including lactic acid, acetic acid, and other organic acids, hydrogen peroxide, bacteriocins and bacteriocin-like substances. We offer different methods to determine the antagonistic activity of bacteria strains against pathogens.

## **6) Assessment of bacterial susceptibility to antimicrobials**

Standardized method to identify resistance to antimicrobials of human and veterinary importance in bacterial strains intended for use as feed additives following EFSA (European Food Safety Authority) guidance.

As a basic requirement, the minimum inhibitory concentration of the antimicrobials should be determined for each of the following substances: ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol and, in specific cases, tylosine, apramycin, nalidixic acid, sulfonamide and trimethoprim. Any bacterial strain carrying an acquired resistance to antimicrobial that is shown to be due to the acquisition of genetic determinant presents the greatest potential for horizontal spread and should not be used as a feed additive.

