

The 16S Direct workflow

Microbial identification using 16S gene sequencing on the SeqStudio Genetic Analyzer and analysis with the SmartGene web application

In this application note we present:

- A fast and economical workflow called “16S Direct” for bacterial identification at the species level by PCR and Sanger sequencing of the 16S rRNA gene
- The use of the Applied Biosystems™ BigDye™ Direct kit for high-resolution Sanger sequencing
- The use of the innovative Applied Biosystems™ SeqStudio™ Genetic Analyzer for capillary electrophoresis
- The use of the SmartGene™ web application service for easy sequence data management and organism identification
- The option to use the Applied Biosystems™ MicroSEQ™ Full Gene 16S rDNA PCR Kit and Sequencing Kit for customers who prefer quality-controlled reagents



Introduction

Rapid and accurate identification of infectious, fastidious, or noncultivable bacteria is a major challenge for microbiology laboratories and for public health surveillance. Based on the groundbreaking phylogenetic research work of Woese and others [1,2], sequencing of the 16S ribosomal RNA (rRNA) gene has emerged as the preferred method for taxonomic classification and identification of bacteria [3]. Today, Sanger sequencing of the 16S gene is recognized as the gold standard for identification at the species level.

The Applied Biosystems™ MicroSEQ™ 16S gene PCR sequencing system has long been trusted for microbial identification. In addition, several databases from public institutions and commercial companies are available for comparing 16S sequences with typed or annotated entries. Until recently, automated Sanger sequencing required the use of high-throughput instruments such as the Applied Biosystems™ 3500 or 3730 Genetic Analyzer. However, many investigators or laboratories may not need high-throughput solutions.

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