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Bacterial genomic DNA is extracted from whole cells by using a commercial system (Applied Biosystems). The DNA is used as the template for PCR to amplify a segment of about 500 or 1,500 bp of the 16S rRNA gene sequence. Broad-based or universal primers complementary to conserved regions are used so that the region can be amplified from any bacteria. The PCR products are purified to remove excess primers and nucleotides. The next step is a process called cycle sequencing. It is similar to PCR, in that it uses DNA (purified products of the first PCR cycle) as the template. One key difference is that only one primer is used in each cycle sequencing reaction so that the amplification of product is linear, not exponential. Another key difference is that dideoxynucleotides are used which interrupts the extension of the DNA strand when incorporated so no new template is formed (the same template is reused for as many cycles as programmed, usually 25 cycles) and the product is a mixture of DNA of various lengths.

As each of the four added labelled terminator bases has different fluorescent dye, each of which absorbs at a different wavelength, the terminal base of each fragment can be determined by a fluorometer.

The products are purified to remove unincorporated dye terminators, and the length of each is determined using capillary electrophoresis (ABI PRISM 3130 genetic analyzer with 4 capillaries) and the sequence of the bases can be determined. The two strands of the DNA are sequenced separately, generating both forward and reverse (complementary) sequences.