the primer, using a mix of dNTPs, which includes radioactive ^{35}S αdATP, to label the complementary DNA. This reaction is very fast and need only be allowed to continue for a matter of min. The labeled DNA is then transferred to tubes containing one of the four termination mixes. These are mixes of dNTPs containing a specific dideoxynucleotide, which, when incorporated into the complementary DNA strand, will stop the polymerase action by preventing any further nucleotides from binding to it. Termination reactions for each nucleotide A, C, G, and T are then loaded in four adjacent lanes of a denaturing polyacrylamide gel and separated by electrophoresis. Autoradiography is used to detect the separated fragments, so that a permanent record of the sequence can be analyzed.

Single-stranded sequencing uses the same labeling and termination reactions, but no denaturation step is required. Single-stranded DNA (ssDNA) can be made efficiently by using a helper phage. Vectors that contain an origin of replication enable a helper phage to export a single-stranded molecule that will include the insert sequence. This protocol offers a greater degree of reliability and consistency than double-stranded sequencing, and it is much quicker to perform. The least time-consuming method of sequencing, however, is automatic cycle sequencing.

Dye-terminated cycle sequencing utilizes the polymerase chain reaction (PCR). Double-stranded or single-stranded template can be used. The polymerase used is a thermal-stable *Taq*, and the labeling and termination reactions take place in a thermal cycler, with a fluorescent dye label, not a radioactive one. The resulting fragments are separated by electrophoresis and a chromatogram of the fluorescent dyes present is created and analyzed by computer. The sequence is read automatically and stored as a computer data file.

It is possible to sequence directly the inserts generated for cloning by first-strand cDNA by employing reverse-transcriptase PCR (RT-PCR). Here, first-strand cDNA is created using a primer with a known anchor sequence. This cDNA is then annealed to a second primer and used in a PCR reaction. The first cycle of the PCR will form a double-stranded molecule from the first-strand cDNA, and then PCR will proceed as normal. PCR products can be cloned into vectors specially designed for this purpose (for example, TA pCRII, Invitrogen), or they can be sequenced directly.

In order to sequence PCR products directly, it is necessary to remove all traces of dNTPs, single-stranded template, and primers from the template to be used. This can be done in a variety of ways, by enzymatic treatment, gel, or column purification. If the quantity of PCR product available is high, then it is possible to use manual sequencing techniques to obtain a result. However, automatic sequencing of such products is recommended.