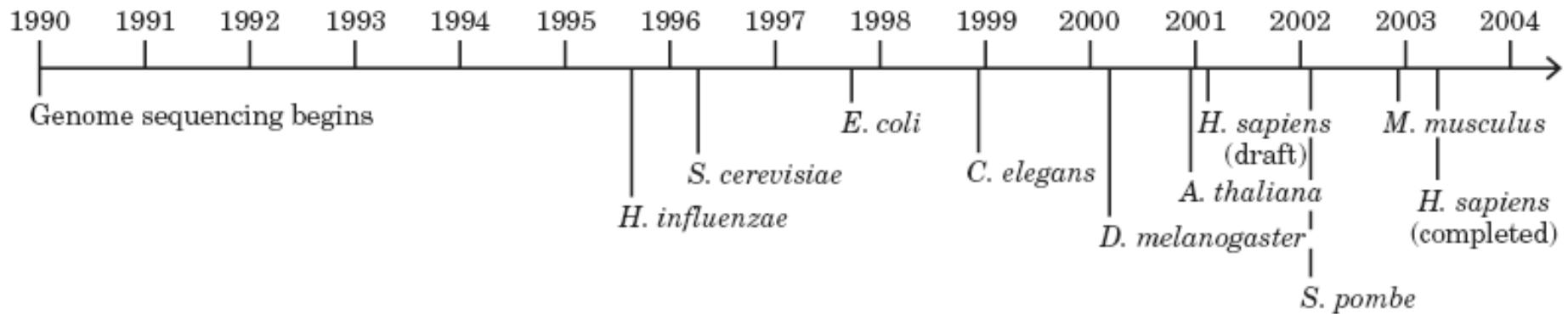
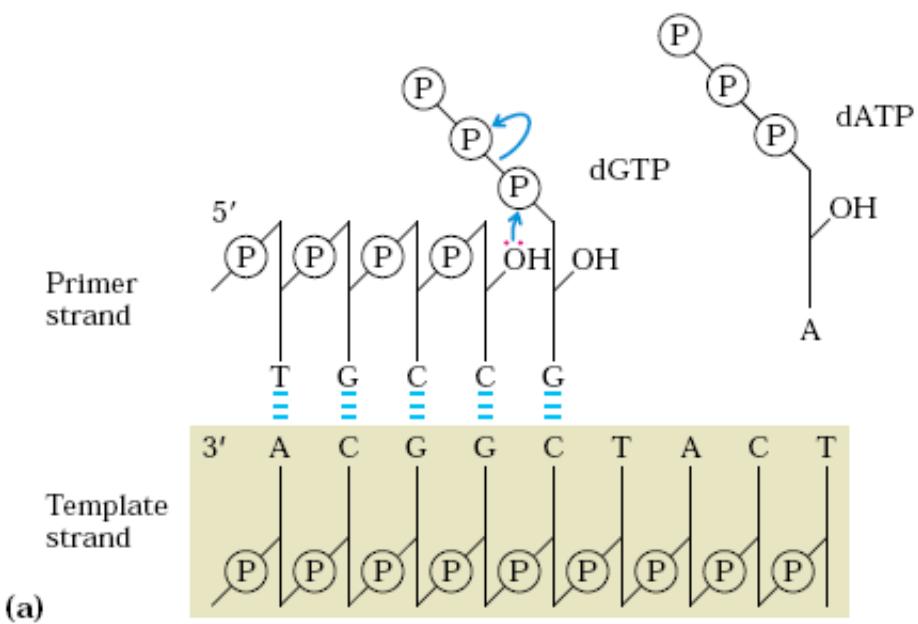


DNA SEQUENCING

LUCIA DHIANTIKA WITASARI

Genomic sequencing timeline



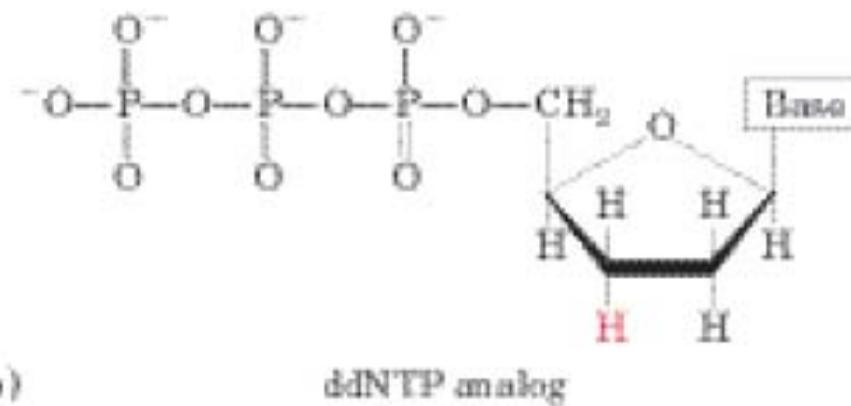


DNA polymerases require both a primer (a short oligonucleotide strand), to which nucleotides are added, and a template strand to guide selection of each new nucleotide.

In cells, the 3'-hydroxyl group of the primer reacts with an incoming deoxynucleoside triphosphate (dNTP) to form a new phosphodiester bond.

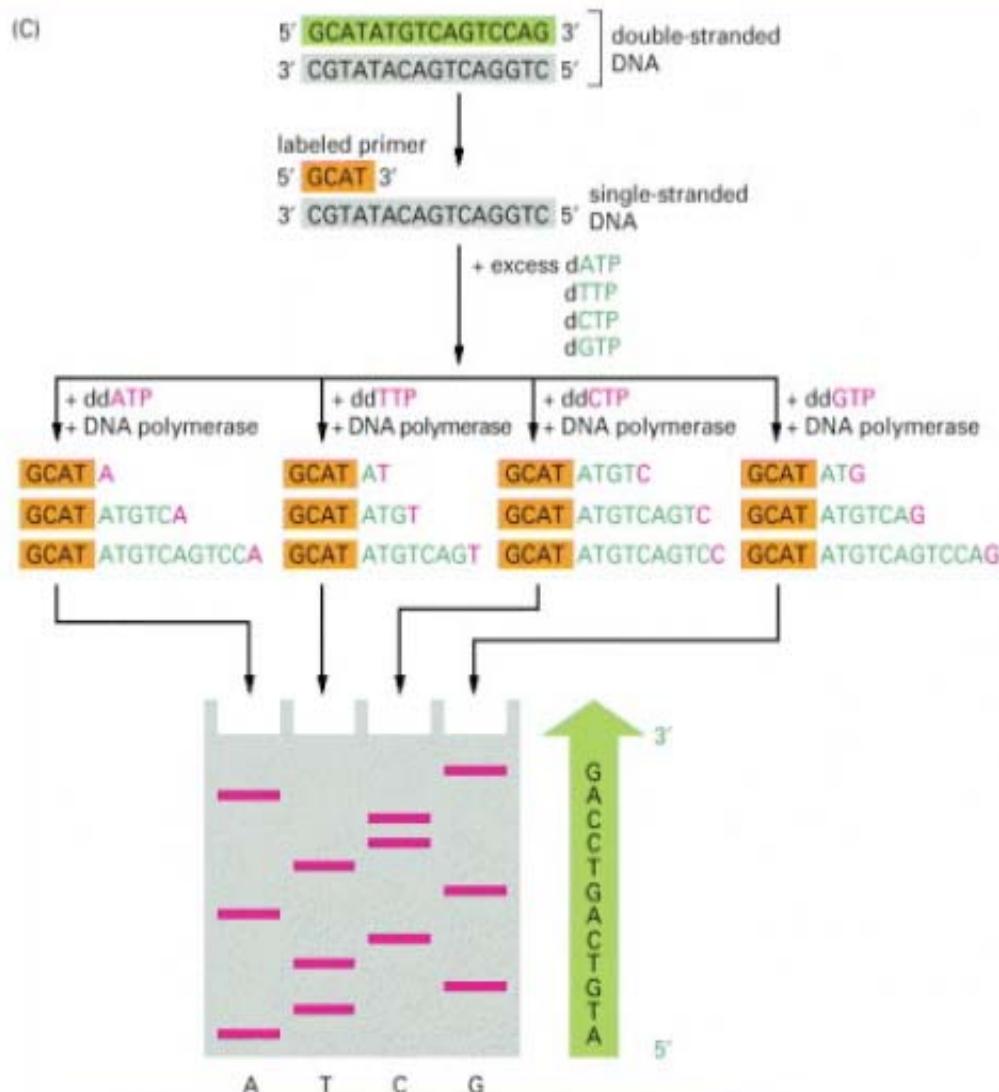
Dideoxynucleoside triphosphate (ddNTP) analogs

The Sanger sequencing procedure uses dideoxynucleoside triphosphate (ddNTP) analogs to interrupt DNA synthesis. (The Sanger method is also known as the dideoxy method.) When a ddNTP is inserted in place of a dNTP, strand elongation is halted after the analog is added, because it lacks the 3'-hydroxyl group needed for the next step.



DNA Sequencing

- 1) A DNA primer is annealed to the desired DNA.
- 2) DNA polymerase extends to primer, and labeled nucleotides incorporate in the newly made DNA.
- 3) 2', 3'-dideoxynucleotides are incorporated and stop DNA synthesis in four test tubes, with each tube containing one dideoxynucleotide such as ddATP, ddGTP, ddCTP, and ddTTP. For example, ddATP will stop DNA synthesis wherever an adenine nucleotide needs to be inserted.
- 4) Each resulting strand is a different length, and is separated by electrophoresis.



DNA sequence reading directly from the bottom of the gel upward, is

ATGTCAGTCAG
1 12

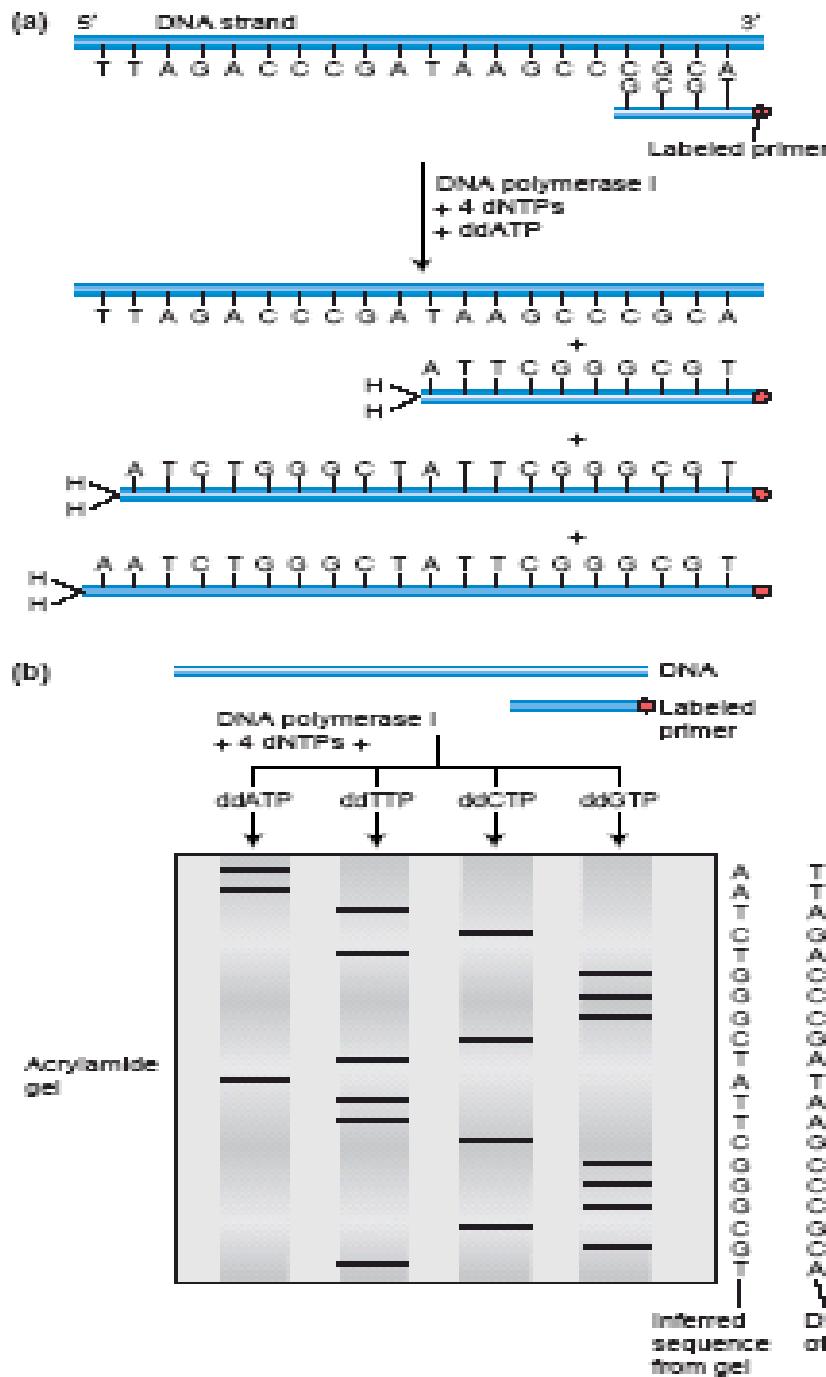
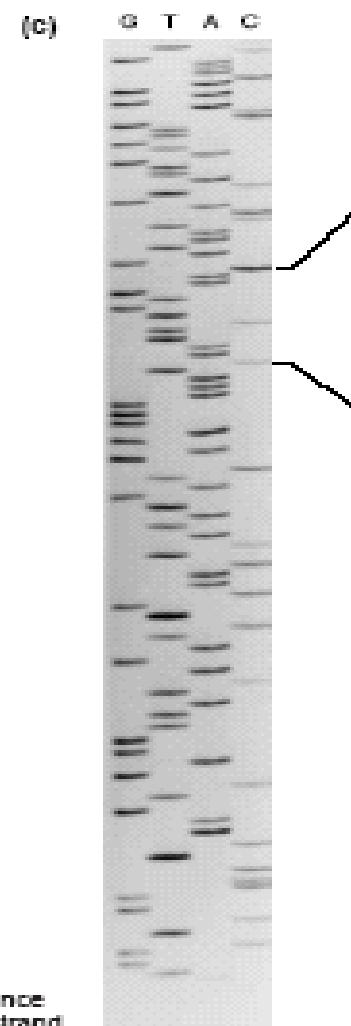


Figure 11-18. The dideoxy sequencing method. (a) A labeled primer (designed from the flanking vector sequence) is used to initiate DNA synthesis. The addition of four different dideoxynucleotides (ddATP is shown here) randomly arrests synthesis. (b) The resulting fragments are separated electrophoretically and subjected to autoradiography. The inferred sequence is shown at the right. (c) Sanger sequencing gel. [Parts a and b from J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2nd ed. Copyright 1992 by Scientific American Books; part c is from Linda Escote-Carlson.]



- DNA sequencing is readily automated by a variation of Sanger's sequencing method in which the dideoxynucleotides used for each reaction are labeled with a differently colored fluorescent tag.
- This technology allows DNA sequences containing thousands of nucleotides to be determined in a few hours

