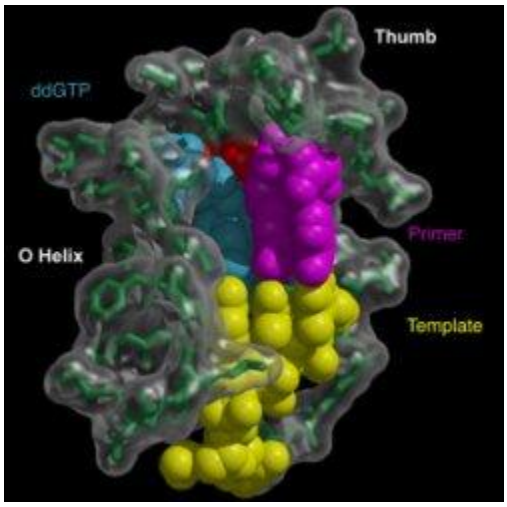
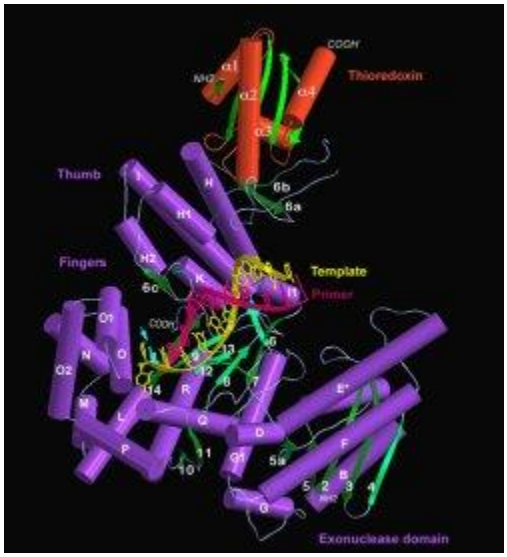
6.7 Engineered Enzyme Accelerates DNA Sequencing





Models of the structure of T7 DNA polymerase bound to a DNA substrate molecule.

The international effort to decode the human genome is ahead of schedule, thanks in part to new tools that have accelerated DNA sequencing and reduced its cost. (The DOE sequencing team can generate more data in 8 days now than it did in all of 1998, its first full year of operation.) One such tool developed in the mid-1990s is an enzyme, a type of DNA polymerase. Polymerases, which in their natural form help cells replicate genetic material, are used in sequencing to incorporate fluorescent tags into DNA to identify the locations of specific chemical units. Naturally occurring polymerases tend to reject fluorescent labels. But Stanley Tabor and Charles Richardson of Harvard Medical School, with Office of Science support, applied fundamental discoveries of the structure and mechanisms of polymerases to create a new DNA polymerase, in which a single chemical change eliminated the bias against fluorescent bases—thereby producing more usable data than was generated previously. This discovery, along with other new knowledge about organisms living in extreme environments, was used by a company to engineer a polymerase that both incorporates the labels and has the thermostability needed for sequencing.

Scientific Impact: Tabor and Richardson's discovery solved a long-standing problem in genetic research. The commercial enzyme, ThermoSequenase, now used by all DNA sequencing centers, has improved the quality of sequencing data and reduced the costs.

Social Impact: ThermoSequenase has captured a very large share of the DNA-polymerase world market, now more than $350 million and growing. It is among the tools used in decoding the human genome, recently completed in "working draft" form, which is expected to lead to new understanding of, and treatments for, human diseases.

References: Tabor, S., and Richardson, C. C. (1995) "A single residue in DNA polymerases of the Escherichia coli DNA polymerase I family is critical for distinguishing between deoxy- and dideoxyribonucleotides," *Proc. Natl. Acad. Sci.* USA 92, 6339-6343.

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Patents: Tabor, S., and Richardson, C. C. (1997) DNA polymerases with a modified nucleotide binding site for DNA sequencing. U. S. patent number 5,614,365.

Tabor, S., and Richardson, C. C. (1997) Method of sequencing based on uniform band intensities. U. S. patent number 5,674,716.

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