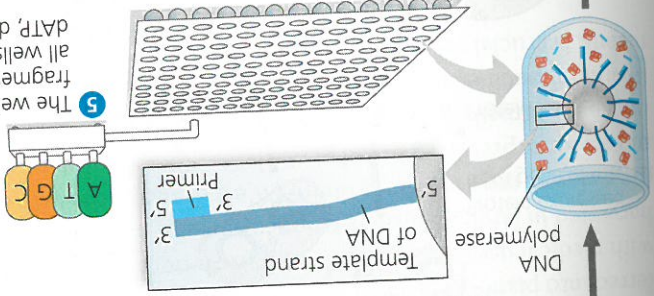
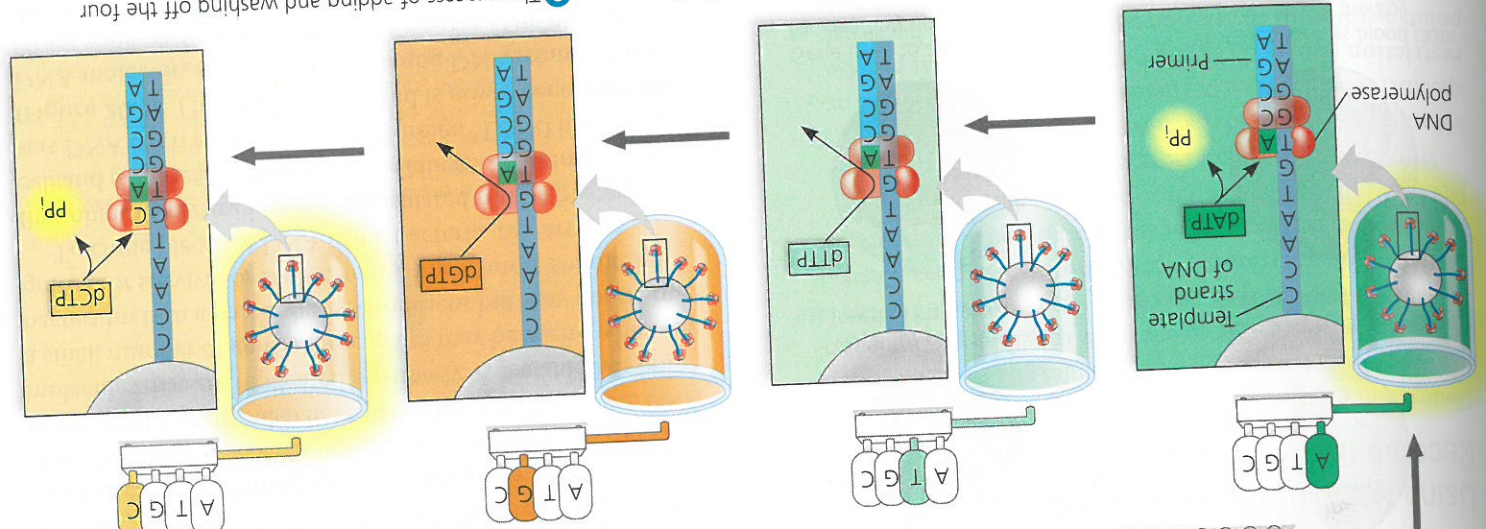


Next-Generation Sequencing

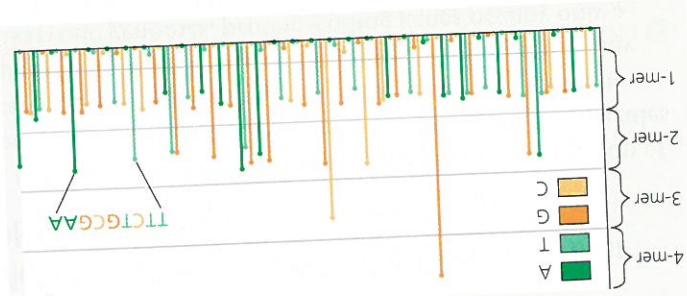
- 1 Genomic DNA is fragmented, and fragments of 400 to 1,000 base pairs are selected.
- 2 Each fragment is isolated with a bead in a droplet of aqueous solution.
- 3 The fragment is copied over and over by a technique called PCR (to be described later). All the 5' ends of one strand are specifically "captured" by the bead. Eventually, 10⁶ identical copies of the same single strand, are attached to the bead.
- 4 The bead is placed into a small well with DNA polymerases and primers that can hybridize to the 3' end of the single (template) strand.



5 The well is one of 2 million on a multiwell plate, each containing a different DNA fragment to be sequenced. A solution of one of the four nucleotides is added to all wells and then washed off. This is done sequentially for all four nucleotides: dATP, dTTP, dGTP, and then dCTP. The entire process is then repeated.



INTERPRET THE DATA If the template strand has two or more identical nucleotides in a row, their complementary nucleotides will be added one after the other in the same flow step. How are two or more of the same nucleotide (in a row) detected in the flow-gram? (See sample on the above, starting from the left. (Ignore the very short lines.)



Results Each of the 2,000,000 wells in the multiwell plate, which holds a different fragment, yields a different sequence. The results for one fragment are shown below as a "flow-gram." The sequences of the entire set of fragments are analyzed using computer software, which "stitches" them together into a whole sequence—here, an entire genome.

Application In current next-generation sequencing techniques, each fragment is 400–1,000 nucleotides long; by sequencing the fragments in parallel, 700–900 million nucleotides can be sequenced in 10 hours.

Technique See numbered steps and diagrams.