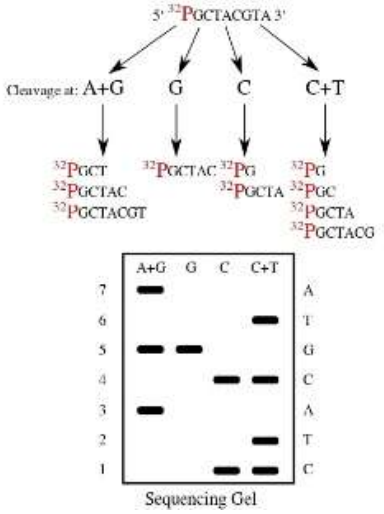
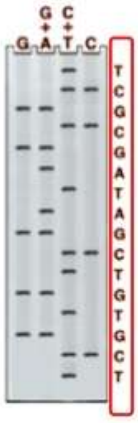
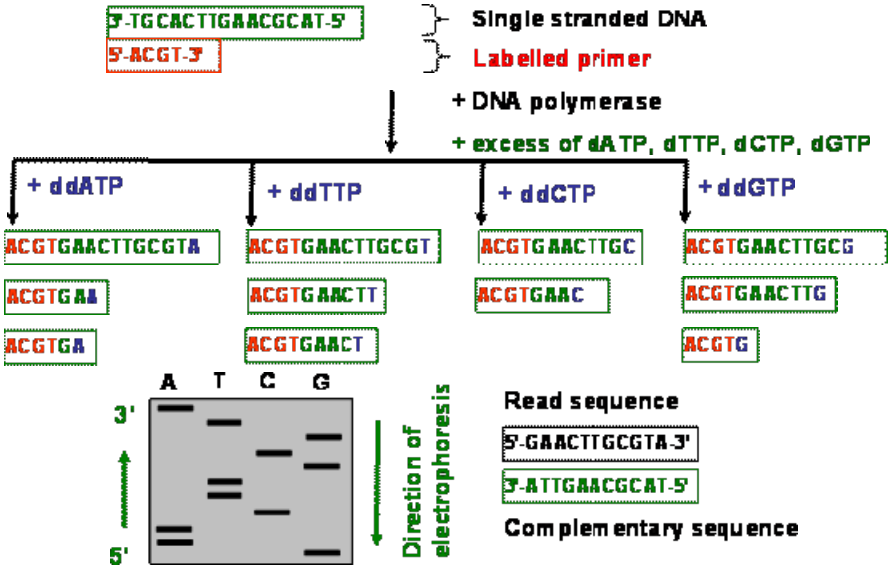
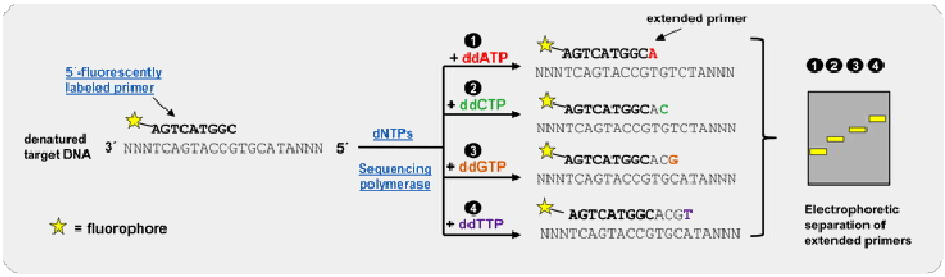
# Maxam-Gilbert method

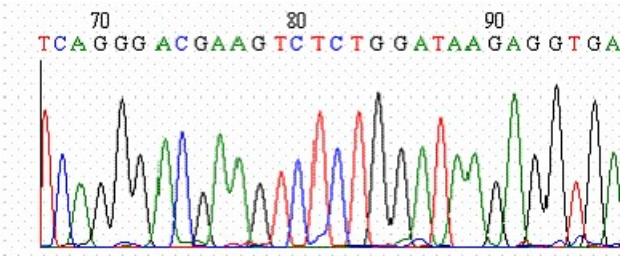


# Sanger sequencing




# Sanger sequencing

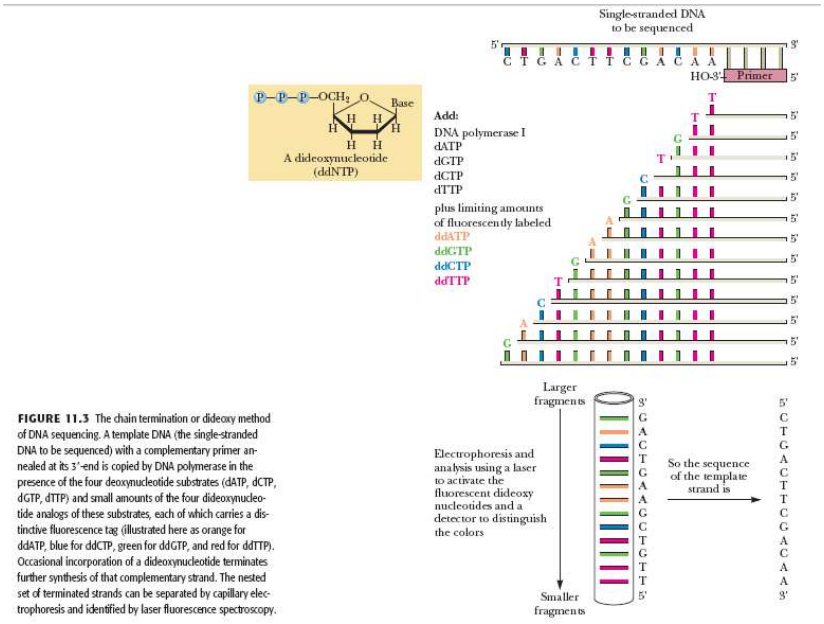



**FIGURE 11.3** The chain termination or dideoxy method of DNA sequencing. A template DNA (the single-stranded DNA to be sequenced) with a complementary primer annealed at its 3'-end is copied by DNA polymerase in the presence of the four deoxynucleotide substrates (dATP, dCTP, dGTP, dTTP) and small amounts of the four dideoxynucleotide analogs of these substrates, each of which carries a distinctive fluorescence tag (illustrated here as orange for ddATP, blue for ddCTP, green for ddGTP, and red for ddTTP). Occasional incorporation of a dideoxynucleotide terminates further synthesis of that complementary strand. The nested set of terminated strands can be separated by capillary electrophoresis and identified by laser fluorescence spectroscopy.

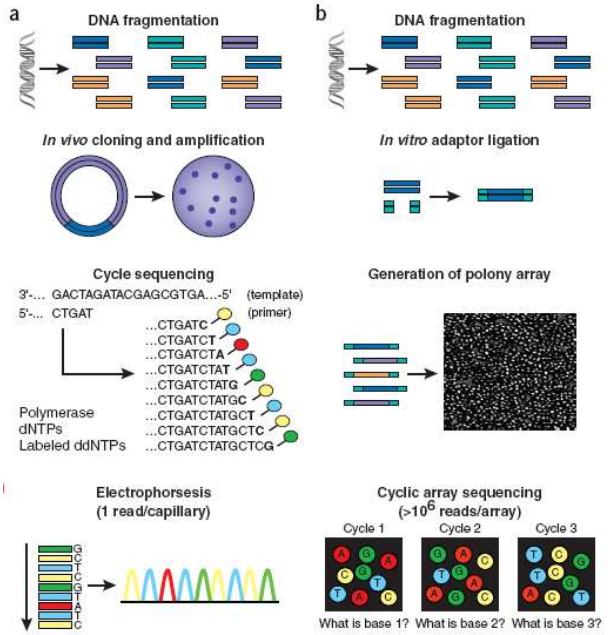



**Figure 1** Work flow of conventional versus second-generation sequencing. (a) With high-throughput shotgun Sanger sequencing, genomic DNA is fragmented, then cloned to a plasmid vector and used to transform *E. coli*. For each sequencing reaction, a single bacterial colony is picked and plasmid DNA isolated. Each cycle sequencing reaction takes place within a microliter-scale volume, generating a ladder of ddNTP-terminated, dye-labeled products, which are subjected to high-resolution electrophoretic separation within one of 96 or 384 capillaries in one run of a sequencing instrument. As fluorescently labeled fragments of discrete sizes pass a detector, the four-channel emission spectrum is used to generate a sequencing trace. (**b**) In shotgun sequencing with cyclic-array methods, common adaptors are ligated to fragmented genomic DNA, which is then subjected to one of several protocols that results in an array of millions of spatially immobilized PCR colonies or 'polonies'[15]. Each polony consists of many copies of a single shotgun library fragment. As all polonies are tethered to a planar array, a single microliter-scale reagent volume (e.g., for primer hybridization and then for enzymatic extension reactions) can be applied to manipulate all array features in parallel. Similarly, imaging-based detection of fluorescent labels incorporated with each extension can be used to acquire sequencing data on all features in parallel. Successive iterations of enzymatic interrogation and imaging are used to build up a contiguous sequencing read for each array feature.

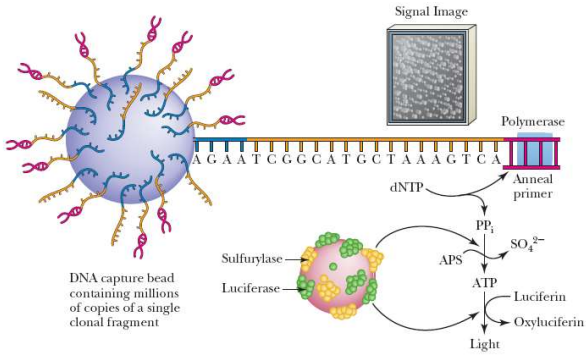



**FIGURE 11.4** Ultra-high-throughput DNA sequencing by the 454 method. Light emission signals nucleotide incorporation in a DNA polymerase-catalyzed primed synthesis reaction.

Reaction 1:

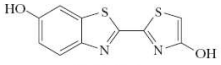
$$PP_i^{2-} + APS \rightarrow ATP + SO_4^{2-}$$

(This is the reverse of the ATP sulfurylase reaction shown as reaction 1 in Figure 25.34.)

Reaction 2:

$$ATP + luciferin + O_2 \rightarrow AMP + PP_i + CO_2 + oxyluciferin + light$$


**Luciferin**




**Oxyluciferin**

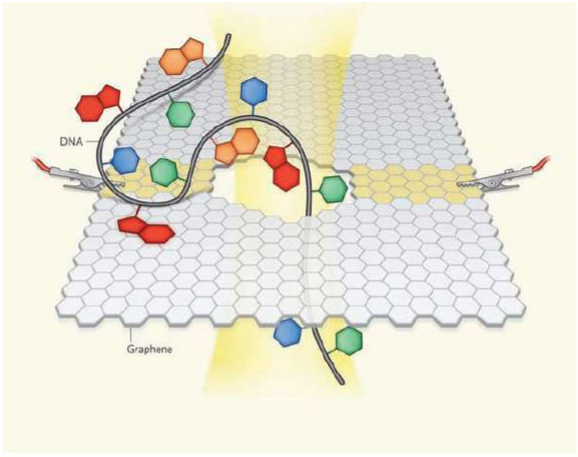



**FIGURE 11.5** A graphene monolayer is one possible platform for nanopore-based DNA sequencing. An electrical potential is placed across a graphene monolayer punctured with nanopores. As single-stranded DNA is pulled through a nanopore, the fluctuations in ion flow (shown as yellow shading) reveal which base (A, C, G, or T) is momentarily occupying the pore (adapted from Figure 1 in Bayley, H., 2010. Nanotechnology: Holes with an edge. *Nature* **467**:164–165).

**a** | The 454 sequencing method is a highly parallel, two-step approach. First, the DNA is sheared and oligonucleotide adaptors are attached. Each fragment is attached to a bead and the beads are PCR amplified within droplets of an oil–water emulsion. This generates multiple copies of the same DNA sequence on each bead. Second, the beads are captured in picolitre-sized wells in a fabricated substrate and pyrosequencing (pyrophosphate-based sequencing) is performed in parallel on each DNA fragment as shown (the DNA fragment has been artificially elongated in the figure). Nucleotide incorporation is detected by the release of inorganic pyrophosphate (PPi), which leads to the enzymatic generation of photons: PPi is released and converted to ATP and luciferase uses the ATP to generate light. The cycle is iteratively repeated for each of the four bases. The average read length has already increased from 110 bp to approximately 250 bp, and future developments will probably increase it further to over 400 bp.. **b** | SOLiD technology has an amplification procedure that is conceptually similar to that of 454, but the sequencing strategy is radically different. Beads are deposited onto glass slides and the sequence is determined by sequential hybridization and ligation of partially random oligonucleotides with a central determined base (or pair of bases) that is identified by a specific fluorophore. After the colour is recorded, the ligated oligonucleotide is cleaved and removed and the process is then repeated. The reads that are generated are currently ~25 bp, but will probably increase to more than 50 bp in the future. **c** | The first step of SOLEXA sequencing is based on the amplification of DNA on a solid surface using fold-back PCR and anchored primers. Multiple cycles of the solid-phase amplification followed by denaturation create clusters of ~1,000 copies of single-stranded DNA molecules. Sequencing is performed sequentially using primers, DNA polymerase and four fluorophore-labelled, reversibly terminating nucleotides. After the incorporation of a nucleotide, the image is captured and the identity of the first base is recorded. The terminators and fluorophores are then removed and the incorporation, detection and identification steps are repeated. The average read length is currently ~40 bp, but this will also probably increase in the future.