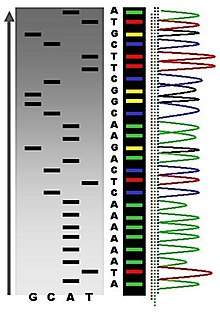
* https://en.wikipedia.org/wiki/DNA\_sequencing

DNA sequencing

**DNA sequencing** is the process of determining the precise order of [nucleotides](https://en.wikipedia.org/wiki/Nucleotides) within a [DNA](https://en.wikipedia.org/wiki/DNA) molecule. It includes any method or technology that is used to determine the order of the four bases—[adenine](https://en.wikipedia.org/wiki/Adenine), [guanine](https://en.wikipedia.org/wiki/Guanine), [cytosine](https://en.wikipedia.org/wiki/Cytosine), and [thymine](https://en.wikipedia.org/wiki/Thymine)—in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery.[[1]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-1)

Knowledge of DNA sequences has become indispensable for basic biological research, and in numerous applied fields such as [medical diagnosis](https://en.wikipedia.org/wiki/Medical_diagnosis), [biotechnology](https://en.wikipedia.org/wiki/Biotechnology), [forensic biology](https://en.wikipedia.org/wiki/Forensic_biology), [virology](https://en.wikipedia.org/wiki/Virology) and biological [systematics](https://en.wikipedia.org/wiki/Systematics). The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or [genomes](https://en.wikipedia.org/wiki/Genomes) of numerous types and species of life, including the [human genome](https://en.wikipedia.org/wiki/Human_genome) and other complete DNA sequences of many animal, plant, and [microbial](https://en.wikipedia.org/wiki/Microbe) species.

[](https://en.wikipedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg)

An example of the results of automated chain-termination DNA sequencing.

The first DNA sequences were obtained in the early 1970s by academic researchers using laborious methods based on [two-dimensional chromatography](https://en.wikipedia.org/wiki/Two-dimensional_chromatography). Following the development of [fluorescence](https://en.wikipedia.org/wiki/Fluorescence)-based sequencing methods with a [DNA sequencer](https://en.wikipedia.org/wiki/DNA_sequencer),[[2]](https://en.wikipedia.org/wiki/DNA_sequencing" \l "cite_note-olsvik1993-2) DNA sequencing has become easier and orders of magnitude faster.[[3]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid18992322-3)

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## Applications[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=1" \o "Edit section: Applications)]

DNA sequencing may be used to determine the sequence of individual [genes](https://en.wikipedia.org/wiki/Gene), larger genetic regions (i.e. clusters of genes or [operons](https://en.wikipedia.org/wiki/Operons)), full chromosomes or [entire genomes](https://en.wikipedia.org/wiki/Whole_genome_sequencing), of any organism. DNA sequencing is also the most efficient way to sequence [RNA](https://en.wikipedia.org/wiki/RNA) or [proteins](https://en.wikipedia.org/wiki/Protein) (via their [open reading frames](https://en.wikipedia.org/wiki/Open_reading_frame)). In fact, DNA sequencing has become a key technology in many areas of biology and other sciences such as medicine, [forensics](https://en.wikipedia.org/wiki/Forensics), or [anthropology](https://en.wikipedia.org/wiki/Anthropology).

### Molecular biology[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=2" \o "Edit section: Molecular biology)]

Sequencing is used in [molecular biology](https://en.wikipedia.org/wiki/Molecular_biology) to study genomes and the proteins they encode. Information obtained using sequencing allows researchers to identify changes in genes, associations with diseases and phenotypes, and identify potential drug targets.

### Evolutionary biology[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=3" \o "Edit section: Evolutionary biology)]

Since DNA is an informative macromolecule in terms of transmission from one generation to another, DNA sequencing is used in [evolutionary biology](https://en.wikipedia.org/wiki/Evolutionary_biology) to study how different organisms are related and how they evolved.

### Metagenomics[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=4" \o "Edit section: Metagenomics)]

*Main article:*[*Metagenomics*](https://en.wikipedia.org/wiki/Metagenomics)

The field of [metagenomics](https://en.wikipedia.org/wiki/Metagenomics) involves identification of organisms present in a body of water, [sewage](https://en.wikipedia.org/wiki/Sewage), dirt, debris filtered from the air, or swab samples from organisms. Knowing which organisms are present in a particular environment is critical to research in [ecology](https://en.wikipedia.org/wiki/Ecology), [epidemiology](https://en.wikipedia.org/wiki/Epidemiology), [microbiology](https://en.wikipedia.org/wiki/Microbiology), and other fields. Sequencing enables researchers to determine which types of microbes may be present in a [microbiome](https://en.wikipedia.org/wiki/Microbiome), for example.

### Medicine[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=5" \o "Edit section: Medicine)]

Medical technicians may sequence genes (or, theoretically, full genomes) from patients to determine if there is risk of genetic diseases. This is a form of [genetic testing](https://en.wikipedia.org/wiki/Genetic_testing), though some genetic tests may not involve DNA sequencing.

### Forensics[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=6" \o "Edit section: Forensics)]

DNA sequencing may be used along with [DNA profiling](https://en.wikipedia.org/wiki/DNA_profiling) methods for [forensic identification](https://en.wikipedia.org/wiki/Forensic_identification) and [paternity testing](https://en.wikipedia.org/wiki/DNA_paternity_testing). DNA testing has evolved tremendously in the last few decades to ultimately link a DNA print to what is under investigation. The DNA patterns in fingerprint, saliva, hair follicles, etc. uniquely separate each living organism from one another. Testing DNA is a technique in which can detect specific genomes in a DNA strand to produce a unique and individualized pattern. Every living organism ever created has a one of a kind DNA pattern, which can be determined through DNA testing. It's extremely rare that two people have the exact same DNA pattern, therefore DNA testing is highly successful.

## The four canonical bases[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=7" \o "Edit section: The four canonical bases)]

*Main article:*[*Nucleotide*](https://en.wikipedia.org/wiki/Nucleotide)

The canonical structure of DNA has four bases: [thymine](https://en.wikipedia.org/wiki/Thymine) (T), [adenine](https://en.wikipedia.org/wiki/Adenine) (A), [cytosine](https://en.wikipedia.org/wiki/Cytosine) (C), and [guanine](https://en.wikipedia.org/wiki/Guanine) (G). DNA sequencing is the determination of the physical order of these bases in a molecule of DNA. However, there are many other bases that may be present in a molecule. In some viruses (specifically, [bacteriophage](https://en.wikipedia.org/wiki/Bacteriophage)), cytosine may be replaced by hydroxy methyl or hydroxy methyl glucose cytosine.[[4]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-4) In mammalian DNA, variant bases with [methyl](https://en.wikipedia.org/wiki/Methyl) groups or phosphosulfate may be found.[[5]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-5)[[6]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-6) Depending on the sequencing technique, a particular modification, e.g., the 5mC ([5 methyl cytosine](https://en.wikipedia.org/wiki/5_methyl_cytosine)) common in humans, may or may not be detected.[[7]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-7)

## History[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=8" \o "Edit section: History)]

Deoxyribonucleic acid ([DNA](https://en.wikipedia.org/wiki/DNA)) was first discovered and isolated by [Friedrich Miescher](https://en.wikipedia.org/wiki/Friedrich_Miescher) in 1869, but it remained understudied for many decades because proteins, rather than DNA, were thought to hold the genetic blueprint to life. This situation changed after 1944 as a result of some experiments by [Oswald Avery](https://en.wikipedia.org/wiki/Oswald_Avery), [Colin MacLeod](https://en.wikipedia.org/wiki/Colin_Munro_MacLeod), and [Maclyn McCarty](https://en.wikipedia.org/wiki/Maclyn_McCarty) demonstrating that purified DNA could change one strain of bacteria into another. This was the first time that DNA was shown capable of transforming the properties of cells.

In 1953, [James Watson](https://en.wikipedia.org/wiki/James_Watson) and [Francis Crick](https://en.wikipedia.org/wiki/Francis_Crick) put forward their [double-helix](https://en.wikipedia.org/wiki/Double-helix) model of DNA, based on crystallized X-ray structures being studied by [Rosalind Franklin](https://en.wikipedia.org/wiki/Rosalind_Franklin). According to the model, DNA is composed of two strands of nucleotides coiled around each other, linked together by hydrogen bonds and running in opposite directions. Each strand is composed of four complementary nucleotides – adenine (A), cytosine (C), guanine (G) and thymine (T) – with an A on one strand always paired with T on the other, and C always paired with G. They proposed such a structure allowed each strand to be used to reconstruct the other, an idea central to the passing on of hereditary information between generations.[[8]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid13168976-8)

[](https://en.wikipedia.org/wiki/File:Frederick_Sanger2.jpg)

[Frederick Sanger](https://en.wikipedia.org/wiki/Frederick_Sanger), a pioneer of sequencing. Sanger is one of the few scientists who was awarded two Nobel prizes, one for the [sequencing of proteins](https://en.wikipedia.org/wiki/Protein_sequencing), and the other for the sequencing of DNA.

The foundation for sequencing proteins was first laid by the work of [Fred Sanger](https://en.wikipedia.org/wiki/Fred_Sanger) who by 1955 had completed the sequence of all the amino acids in [insulin](https://en.wikipedia.org/wiki/Insulin), a small protein secreted by the pancreas. This provided the first conclusive evidence that proteins were chemical entities with a specific molecular pattern rather than a random mixture of material suspended in fluid. Sanger's success in sequencing insulin greatly electrified x-ray crystallographers, including Watson and Crick who by now were trying to understand how DNA directed the formation of proteins within a cell. Soon after attending a series of lectures given by Fred Sanger in October 1954, Crick began to develop a theory which argued that the arrangement of nucleotides in DNA determined the sequence of amino acids in proteins which in turn helped determine the function of a protein. He published this theory in 1958.[[9]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-9)

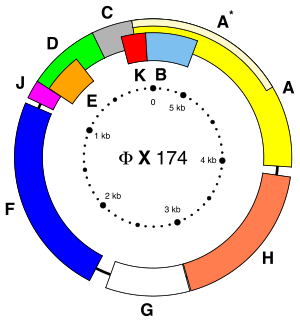
### RNA sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=9" \o "Edit section: RNA sequencing)]

[RNA sequencing](https://en.wikipedia.org/wiki/RNA_sequencing) was one of the earliest forms of nucleotide sequencing. The major landmark of RNA sequencing is the sequence of the first complete gene and the complete genome of [Bacteriophage MS2](https://en.wikipedia.org/wiki/Bacteriophage_MS2), identified and published by [Walter Fiers](https://en.wikipedia.org/wiki/Walter_Fiers) and his coworkers at the [University of Ghent](https://en.wikipedia.org/wiki/University_of_Ghent) ([Ghent](https://en.wikipedia.org/wiki/Ghent), [Belgium](https://en.wikipedia.org/wiki/Belgium)), in 1972[[10]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-10) and 1976.[[11]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-11)Traditional RNA sequencing methods require the creation of a cDNA molecule which must be sequenced.[[12]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-12)

## Early DNA sequencing methods[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=10" \o "Edit section: Early DNA sequencing methods)]

The first method for determining DNA sequences involved a location-specific primer extension strategy established by [Ray Wu](https://en.wikipedia.org/wiki/Ray_Wu) at [Cornell University](https://en.wikipedia.org/wiki/Cornell_University) in 1970.[[13]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-13) DNA polymerase catalysis and specific nucleotide labeling, both of which figure prominently in current sequencing schemes, were used to sequence the cohesive ends of lambda phage DNA.[[14]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-14)[[15]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-15)[[16]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid4553110-16) Between 1970 and 1973, Wu, R Padmanabhan and colleagues demonstrated that this method can be employed to determine any DNA sequence using synthetic location-specific primers.[[17]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid4560009-17)[[18]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid4358929-18)[[19]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-19) [Frederick Sanger](https://en.wikipedia.org/wiki/Frederick_Sanger) then adopted this primer-extension strategy to develop more rapid DNA sequencing methods at the [MRC Centre](https://en.wikipedia.org/wiki/Medical_Research_Council_(United_Kingdom)), [Cambridge](https://en.wikipedia.org/wiki/Cambridge), UK and published a method for "DNA sequencing with chain-terminating inhibitors" in 1977.[[20]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Sanger1977-20) [Walter Gilbert](https://en.wikipedia.org/wiki/Walter_Gilbert) and [Allan Maxam](https://en.wikipedia.org/wiki/Allan_Maxam) at [Harvard](https://en.wikipedia.org/wiki/Harvard_University) also developed sequencing methods, including one for "DNA sequencing by chemical degradation".[[21]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Maxam77-21)[[22]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-22) In 1973, Gilbert and Maxam reported the sequence of 24 basepairs using a method known as wandering-spot analysis.[[23]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-23)Advancements in sequencing were aided by the concurrent development of [recombinant DNA](https://en.wikipedia.org/wiki/Recombinant_DNA) technology, allowing DNA samples to be isolated from sources other than viruses.

### Sequencing of full genomes[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=11" \o "Edit section: Sequencing of full genomes)]

[](https://en.wikipedia.org/wiki/File:Genome_map_of_the_bacteriophage_%CE%A6X174_showing_overlapping_genes.svg)

The 5,386 bp genome of [bacteriophage φX174](https://en.wikipedia.org/wiki/Bacteriophage_%CF%86X174). Each coloured block represents a gene.

The first full DNA genome to be sequenced was that of [bacteriophage φX174](https://en.wikipedia.org/wiki/Bacteriophage_%CF%86X174) in 1977.[[24]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-24) [Medical Research Council](https://en.wikipedia.org/wiki/Medical_Research_Council_(UK)) scientists deciphered the complete DNA sequence of the [Epstein-Barr virus](https://en.wikipedia.org/wiki/Epstein-Barr_virus) in 1984, finding it contained 172,282 nucleotides. Completion of the sequence marked a significant turning point in DNA sequencing because it was achieved with no prior genetic profile knowledge of the virus.[[25]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-25)

A non-radioactive method for transferring the DNA molecules of sequencing reaction mixtures onto an immobilizing matrix during electrophoresis was developed by Pohl and co-workers in the early 1980s.[[26]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-26)[[27]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-27) Followed by the commercialization of the DNA sequencer "Direct-Blotting-Electrophoresis-System GATC 1500" by [GATC Biotech](https://en.wikipedia.org/wiki/GATC_Biotech), which was intensively used in the framework of the EU genome-sequencing programme, the complete DNA sequence of the yeast [*Saccharomyces cerevisiae*](https://en.wikipedia.org/wiki/Saccharomyces_cerevisiae) chromosome II.[[28]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Feldmann_1994-28) [Leroy E. Hood](https://en.wikipedia.org/wiki/Leroy_E._Hood)'s laboratory at the [California Institute of Technology](https://en.wikipedia.org/wiki/California_Institute_of_Technology) announced the first semi-automated DNA sequencing machine in 1986.[[29]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-29) This was followed by [Applied Biosystems](https://en.wikipedia.org/wiki/Applied_Biosystems)' marketing of the first fully automated sequencing machine, the ABI 370, in 1987 and by Dupont's Genesis 2000[[30]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-30) which used a novel fluorescent labeling technique enabling all four dideoxynucleotides to be identified in a single lane. By 1990, the U.S. [National Institutes of Health](https://en.wikipedia.org/wiki/National_Institutes_of_Health) (NIH) had begun large-scale sequencing trials on [*Mycoplasma capricolum*](https://en.wikipedia.org/wiki/Mycoplasma_capricolum), [*Escherichia coli*](https://en.wikipedia.org/wiki/Escherichia_coli), [*Caenorhabditis elegans*](https://en.wikipedia.org/wiki/Caenorhabditis_elegans), and [*Saccharomyces cerevisiae*](https://en.wikipedia.org/wiki/Saccharomyces_cerevisiae) at a cost of US$0.75 per base. Meanwhile, sequencing of human [cDNA](https://en.wikipedia.org/wiki/CDNA) sequences called [expressed sequence tags](https://en.wikipedia.org/wiki/Expressed_sequence_tag) began in [Craig Venter](https://en.wikipedia.org/wiki/Craig_Venter)'s lab, an attempt to capture the coding fraction of the [human genome](https://en.wikipedia.org/wiki/Human_genome).[[31]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid2047873-31) In 1995, Venter, [Hamilton Smith](https://en.wikipedia.org/wiki/Hamilton_O._Smith), and colleagues at [The Institute for Genomic Research](https://en.wikipedia.org/wiki/The_Institute_for_Genomic_Research) (TIGR) published the first complete genome of a free-living organism, the bacterium [*Haemophilus influenzae*](https://en.wikipedia.org/wiki/Haemophilus_influenzae). The circular chromosome contains 1,830,137 bases and its publication in the journal Science[[32]](https://en.wikipedia.org/wiki/DNA_sequencing" \l "cite_note-32) marked the first published use of whole-genome shotgun sequencing, eliminating the need for initial mapping efforts.

By 2001, shotgun sequencing methods had been used to produce a draft sequence of the human genome.[[33]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Lander_2001-33)[[34]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Venter_2001-34)

### High-throughput sequencing (HTP) methods[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=12" \o "Edit section: High-throughput sequencing (HTP) methods)]

Several new methods for DNA sequencing were developed in the mid to late 1990s and were implemented in commercial [DNA sequencers](https://en.wikipedia.org/wiki/DNA_sequencers) by the year 2000. Together these were called the "next-generation" or "second-generation" sequencing methods.

On October 26, 1990, [Roger Tsien](https://en.wikipedia.org/wiki/Roger_Tsien), Pepi Ross, Margaret Fahnestock and Allan J Johnston filed a patent describing stepwise ("base-by-base") sequencing with removable 3' blockers on DNA arrays (blots and single DNA molecules).[[35]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-TsienPatent-35) In 1996, [Pål Nyrén](https://en.wikipedia.org/wiki/P%C3%A5l_Nyr%C3%A9n) and his student [Mostafa Ronaghi](https://en.wikipedia.org/wiki/Mostafa_Ronaghi) at the Royal Institute of Technology in [Stockholm](https://en.wikipedia.org/wiki/Stockholm) published their method of [pyrosequencing](https://en.wikipedia.org/wiki/Pyrosequencing).[[36]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Ronaghi-36)

On April 1, 1997, Pascal Mayer and Laurent Farinelli submitted patents to the World Intellectual Property Organization describing DNA colony sequencing.[[37]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-DNA_colony_patents-37) The DNA sample preparation and random surface-PCR arraying methods described in this patent, coupled to Roger Tsien et al.'s "base-by-base" sequencing method, is now implemented in [Illumina](https://en.wikipedia.org/wiki/Illumina_(company))'s Hi-Seq genome sequencers.

Lynx Therapeutics published and marketed [Massively parallel signature sequencing](https://en.wikipedia.org/wiki/Massively_parallel_signature_sequencing) (MPSS), in 2000. This method incorporated a parallelized, adapter/ligation-mediated, bead-based sequencing technology and served as the first commercially available "next-generation" sequencing method, though no [DNA sequencers](https://en.wikipedia.org/wiki/DNA_sequencers) were sold to independent laboratories.[[38]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Brenner_2000-38)

The large quantities of data produced by DNA sequencing have also required development of new methods and programs for sequence analysis. Phil Green and Brent Ewing of the University of Washington described their [phred quality score](https://en.wikipedia.org/wiki/Phred_quality_score) for sequencer data analysis in 1998.[[39]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-39)

## Basic methods[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=13" \o "Edit section: Basic methods)]

### Maxam-Gilbert sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=14" \o "Edit section: Maxam-Gilbert sequencing)]

*Main article:*[*Maxam-Gilbert sequencing*](https://en.wikipedia.org/wiki/Maxam-Gilbert_sequencing)

[Allan Maxam](https://en.wikipedia.org/wiki/Allan_Maxam) and [Walter Gilbert](https://en.wikipedia.org/wiki/Walter_Gilbert) published a DNA sequencing method in 1977 based on chemical modification of DNA and subsequent cleavage at specific bases.[[21]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Maxam77-21) Also known as chemical sequencing, this method allowed purified samples of double-stranded DNA to be used without further cloning. This method's use of radioactive labeling and its technical complexity discouraged extensive use after refinements in the Sanger methods had been made.

Maxam-Gilbert sequencing requires radioactive labeling at one 5' end of the DNA and purification of the DNA fragment to be sequenced. Chemical treatment then generates breaks at a small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T). The concentration of the modifying chemicals is controlled to introduce on average one modification per DNA molecule. Thus a series of labeled fragments is generated, from the radiolabeled end to the first "cut" site in each molecule. The fragments in the four reactions are electrophoresed side by side in denaturing acrylamide gels for size separation. To visualize the fragments, the gel is exposed to X-ray film for autoradiography, yielding a series of dark bands each corresponding to a radiolabeled DNA fragment, from which the sequence may be inferred.[[21]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Maxam77-21)

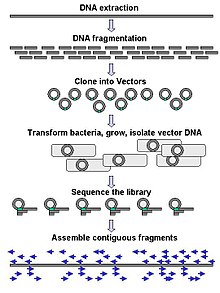
### Chain-termination methods[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=15" \o "Edit section: Chain-termination methods)]

*Main article:*[*Sanger sequencing*](https://en.wikipedia.org/wiki/Sanger_sequencing)

The [chain-termination method](https://en.wikipedia.org/wiki/Sanger_sequencing) developed by [Frederick Sanger](https://en.wikipedia.org/wiki/Frederick_Sanger) and coworkers in 1977 soon became the method of choice, owing to its relative ease and reliability.[[20]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Sanger1977-20)[[40]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Sanger75-40) When invented, the chain-terminator method used fewer toxic chemicals and lower amounts of radioactivity than the Maxam and Gilbert method. Because of its comparative ease, the Sanger method was soon automated and was the method used in the first generation of [DNA sequencers](https://en.wikipedia.org/wiki/DNA_sequencer).

Sanger sequencing is the method which prevailed from the 1980s until the mid-2000s. Over that period, great advances were made in the technique, such as fluorescent labelling, capillary electrophoresis, and general automation. These developments allowed much more efficient sequencing, leading to lower costs. The Sanger method, in mass production form, is the technology which produced the [first human genome](https://en.wikipedia.org/wiki/Human_Genome_Project) in 2001, ushering in the age of [genomics](https://en.wikipedia.org/wiki/Genomics). However, later in the decade, radically different approaches reached the market, bringing the cost per genome down from $100 million in 2001 to $10,000 in 2011.[[41]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-41)

## Advanced methods and *de novo* sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=16" \o "Edit section: Advanced methods and de novo sequencing)]

[](https://en.wikipedia.org/wiki/File:DNA_Sequencing_gDNA_libraries.jpg)

Genomic DNA is fragmented into random pieces and cloned as a bacterial library. DNA from individual bacterial clones is sequenced and the sequence is assembled by using overlapping DNA regions.(click to expand)

Large-scale sequencing often aims at sequencing very long DNA pieces, such as whole [chromosomes](https://en.wikipedia.org/wiki/Chromosome), although large-scale sequencing can also be used to generate very large numbers of short sequences, such as found in [phage display](https://en.wikipedia.org/wiki/Phage_display). For longer targets such as chromosomes, common approaches consist of cutting (with [restriction enzymes](https://en.wikipedia.org/wiki/Restriction_enzyme)) or shearing (with mechanical forces) large DNA fragments into shorter DNA fragments. The fragmented DNA may then be [cloned](https://en.wikipedia.org/wiki/Clone_(genetics)) into a [DNA vector](https://en.wikipedia.org/wiki/Vector_DNA) and amplified in a bacterial host such as [*Escherichia coli*](https://en.wikipedia.org/wiki/Escherichia_coli). Short DNA fragments purified from individual bacterial colonies are individually sequenced and [assembled electronically](https://en.wikipedia.org/wiki/Sequence_assembly) into one long, contiguous sequence. Studies have shown that adding a size selection step to collect DNA fragments of uniform size can improve sequencing efficiency and accuracy of the genome assembly. In these studies, automated sizing has proven to be more reproducible and precise than manual gel sizing.[[42]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid23147856-42)[[43]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid22713159-43)[[44]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid22675423-44)

The term "*de novo* sequencing" specifically refers to methods used to determine the sequence of DNA with no previously known sequence. *De novo* translates from Latin as "from the beginning". Gaps in the assembled sequence may be filled by [primer walking](https://en.wikipedia.org/wiki/Primer_walking). The different strategies have different tradeoffs in speed and accuracy; [shotgun methods](https://en.wikipedia.org/wiki/Shotgun_sequencing) are often used for sequencing large genomes, but its assembly is complex and difficult, particularly with [sequence repeats](https://en.wikipedia.org/wiki/Microsatellite_(genetics)) often causing gaps in genome assembly.

Most sequencing approaches use an *in vitro* cloning step to amplify individual DNA molecules, because their molecular detection methods are not sensitive enough for single molecule sequencing. Emulsion PCR[[45]](https://en.wikipedia.org/wiki/DNA_sequencing" \l "cite_note-Williams2006ePCR-45) isolates individual DNA molecules along with primer-coated beads in aqueous droplets within an oil phase. A [polymerase chain reaction](https://en.wikipedia.org/wiki/Polymerase_chain_reaction) (PCR) then coats each bead with clonal copies of the DNA molecule followed by immobilization for later sequencing. Emulsion PCR is used in the methods developed by Marguilis et al. (commercialized by [454 Life Sciences](https://en.wikipedia.org/wiki/454_Life_Sciences)), Shendure and Porreca et al. (also known as "[Polony sequencing](https://en.wikipedia.org/wiki/Polony_(biology))") and [SOLiD sequencing](https://en.wikipedia.org/wiki/ABI_Solid_Sequencing), (developed by [Agencourt](https://en.wikipedia.org/wiki/Agencourt), later [Applied Biosystems](https://en.wikipedia.org/wiki/Applied_Biosystems), now [Life Technologies](https://en.wikipedia.org/wiki/Life_Technologies_(Thermo_Fisher_Scientific))).[[46]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Margulies_2005-46)[[47]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-polony_sequencing-47)[[48]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-solid_sequencing-48) Emulsion PCR is also used in the GemCode and Chromium platforms developed by [10x Genomics](https://en.wikipedia.org/wiki/10x_Genomics).[[49]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-10x-epcr-49)

### Shotgun sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=17" \o "Edit section: Shotgun sequencing)]

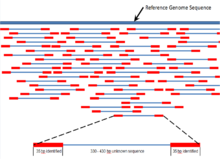
*Main article:*[*Shotgun sequencing*](https://en.wikipedia.org/wiki/Shotgun_sequencing)

Shotgun sequencing is a sequencing method designed for analysis of DNA sequences longer than 1000 base pairs, up to and including entire chromosomes. This method requires the target DNA to be broken into random fragments. After sequencing individual fragments, the sequences can be reassembled on the basis of their overlapping regions.[[50]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-50)

### Bridge PCR[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=18" \o "Edit section: Bridge PCR)]

Another method for [*in vitro*](https://en.wikipedia.org/wiki/In_vitro) clonal amplification is bridge PCR, in which fragments are amplified upon primers attached to a solid surface[[37]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-DNA_colony_patents-37)[[51]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-DNA_colony_presentation-51)[[52]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Mosaic_patent-52)and form "[DNA colonies](https://en.wikipedia.org/wiki/DNA_sequencing#subsection_Illumina_.28Solexa.29_sequencing)" or "DNA clusters". This method is used in the [Illumina](https://en.wikipedia.org/wiki/Illumina_(company)) Genome Analyzer [sequencers](https://en.wikipedia.org/wiki/DNA_sequencing#subsection_Illumina_.28Solexa.29_sequencing). Single-molecule methods, such as that developed by [Stephen Quake](https://en.wikipedia.org/wiki/Stephen_Quake)'s laboratory (later commercialized by [Helicos](https://en.wikipedia.org/wiki/Helicos_Biosciences)) are an exception: they use bright fluorophores and laser excitation to detect base addition events from individual DNA molecules fixed to a surface, eliminating the need for molecular amplification.[[53]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-53)

## High-throughput methods[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=19" \o "Edit section: High-throughput methods)]

[](https://en.wikipedia.org/wiki/File:Mapping_Reads.png)

Multiple, fragmented sequence reads must be assembled together on the basis of their overlapping areas.

High-throughput (formerly "next-generation") sequencing applies to genome sequencing, genome resequencing, [transcriptome](https://en.wikipedia.org/wiki/Transcriptome) profiling ([RNA-Seq](https://en.wikipedia.org/wiki/RNA-Seq)), DNA-protein interactions ([ChIP-sequencing](https://en.wikipedia.org/wiki/ChIP-sequencing)), and [epigenome](https://en.wikipedia.org/wiki/Epigenome) characterization.[[54]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid19900591-54) Resequencing is necessary, because the genome of a single individual of a species will not indicate all of the genome variations among other individuals of the same species.

The high demand for low-cost sequencing has driven the development of high-throughput sequencing technologies that [parallelize](https://en.wikipedia.org/wiki/Multiplex_(assay)) the sequencing process, producing thousands or millions of sequences concurrently.[[55]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-hall2007-55)[[56]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-church2006-56) High-throughput sequencing technologies are intended to lower the cost of DNA sequencing beyond what is possible with standard dye-terminator methods.[[57]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid18165802-57) In ultra-high-throughput sequencing as many as 500,000 sequencing-by-synthesis operations may be run in parallel.[[58]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-kalb1992-58)[[59]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-tenBosch2008-59)[[60]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Tucker2009-60)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Comparison of high-throughput sequencing methods**[[61]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-quail2012-61)[[62]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-lin2012-62) | | | | | | | |
| **Method** | **Read length** | **Accuracy (single read not consensus)** | **Reads per run** | **Time per run** | **Cost per 1 million bases (in US$)** | **Advantages** | **Disadvantages** |
| **Single-molecule real-time sequencing (Pacific Biosciences)** | 10,000 bp to 15,000 bp avg (14,000 bp [N50](https://en.wikipedia.org/wiki/N50_statistic)); maximum read length >40,000 bases[[63]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-63)[[64]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-autogenerated1-64)[[65]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-65) | 87% single-read accuracy[[66]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid23644548-66) | 50,000 per SMRT cell, or 500–1000 megabases[[67]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-flxlexblog.wordpress.com-67)[[68]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-rasko2011-68) | 30 minutes to 4 hours[[69]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-tran2012-69) | $0.13–$0.60 | Longest read length. Fast. Detects 4mC, 5mC, 6mA.[[70]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-70) | Moderate throughput. Equipment can be very expensive. |
| **Ion semiconductor (Ion Torrent sequencing)** | up to 400 bp | 98% | up to 80 million | 2 hours | $1 | Less expensive equipment. Fast. | Homopolymer errors. |
| **Pyrosequencing (454)** | 700 bp | 99.9% | 1 million | 24 hours | $10 | Long read size. Fast. | Runs are expensive. Homopolymer errors. |
| **Sequencing by synthesis (Illumina)** | MiniSeq, NextSeq: 75-300 bp; MiSeq: 50-600 bp; HiSeq 2500: 50-500 bp; HiSeq 3/4000: 50-300 bp; HiSeq X: 300 bp | 99.9% (Phred30) | MiniSeq/MiSeq: 1-25 Million; NextSeq: 130-00 Million, HiSeq 2500: 300 million - 2 billion, HiSeq 3/4000 2.5 billion, HiSeq X: 3 billion | 1 to 11 days, depending upon sequencer and specified read length[[71]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-vliet2010-71) | $0.05 to $0.15 | Potential for high sequence yield, depending upon sequencer model and desired application. | Equipment can be very expensive. Requires high concentrations of DNA. |
| **Sequencing by ligation (SOLiD sequencing)** | 50+35 or 50+50 bp | 99.9% | 1.2 to 1.4 billion | 1 to 2 weeks | $0.13 | Low cost per base. | Slower than other methods. Has issues sequencing palindromic sequences.[[72]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Yu-Feng_Huang.2C_Sheng-Chung_Chen.2C_Yih-Shien_Chiang.2C_Tzu-Han_Chen_.26_Kuo-Ping_Chiu_2012_S10-72) |
| **Nanopore Sequencing**[[73]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-73)**)** | Dependent on library prep, not the device, so user chooses read length. (up to 500 kb reported) | ~92–97% single read (up to 99.96% consensus) | dependent on read length selected by user | data streamed in real time. Choose 1 min to 48 hrs | $500–999 per Flow Cell, base cost dependent on expt | Very long reads, Portable (Palm sized) | Lower throughput than other machines, Single read accuracy in 90s. |
| **Chain termination (Sanger sequencing)** | 400 to 900 bp | 99.9% | N/A | 20 minutes to 3 hours | $2400 | Long individual reads. Useful for many applications. | More expensive and impractical for larger sequencing projects. This method also requires the time consuming step of plasmid cloning or PCR. |

### Massively parallel signature sequencing (MPSS)[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=20" \o "Edit section: Massively parallel signature sequencing (MPSS))]

The first of the high-throughput sequencing technologies, [massively parallel signature sequencing](https://en.wikipedia.org/wiki/Massively_parallel_signature_sequencing) (or MPSS), was developed in the 1990s at Lynx Therapeutics, a company founded in 1992 by [Sydney Brenner](https://en.wikipedia.org/wiki/Sydney_Brenner) and [Sam Eletr](https://en.wikipedia.org/wiki/Applied_Biosystems#History). MPSS was a bead-based method that used a complex approach of adapter ligation followed by adapter decoding, reading the sequence in increments of four nucleotides. This method made it susceptible to sequence-specific bias or loss of specific sequences. Because the technology was so complex, MPSS was only performed 'in-house' by Lynx Therapeutics and no DNA sequencing machines were sold to independent laboratories. Lynx Therapeutics merged with Solexa (later acquired by [Illumina](https://en.wikipedia.org/wiki/Illumina_(company))) in 2004, leading to the development of sequencing-by-synthesis, a simpler approach acquired from [Manteia Predictive Medicine](https://en.wikipedia.org/wiki/Manteia_Predictive_Medicine), which rendered MPSS obsolete. However, the essential properties of the MPSS output were typical of later high-throughput data types, including hundreds of thousands of short DNA sequences. In the case of MPSS, these were typically used for sequencing [cDNA](https://en.wikipedia.org/wiki/CDNA) for measurements of [gene expression](https://en.wikipedia.org/wiki/Gene_expression) levels.[[38]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Brenner_2000-38)

### Polony sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=21" \o "Edit section: Polony sequencing)]

*Main article:*[*Polony sequencing*](https://en.wikipedia.org/wiki/Polony_sequencing)

The [Polony sequencing](https://en.wikipedia.org/wiki/Polony_sequencing) method, developed in the laboratory of [George M. Church](https://en.wikipedia.org/wiki/George_M._Church) at Harvard, was among the first high-throughput sequencing systems and was used to sequence a full [*E. coli*](https://en.wikipedia.org/wiki/E._coli) genome in 2005.[[74]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Shendure2005-74) It combined an in vitro paired-tag library with emulsion PCR, an automated microscope, and ligation-based sequencing chemistry to sequence an *E. coli* genome at an accuracy of >99.9999% and a cost approximately 1/9 that of Sanger sequencing.[[74]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Shendure2005-74) The technology was licensed to Agencourt Biosciences, subsequently spun out into Agencourt Personal Genomics, and eventually incorporated into the [Applied Biosystems](https://en.wikipedia.org/wiki/Applied_Biosystems) SOLiD platform. Applied Biosystems was later acquired by [Life Technologies](https://en.wikipedia.org/wiki/Life_Technologies_(Thermo_Fisher_Scientific)), now part of [Thermo Fisher Scientific](https://en.wikipedia.org/wiki/Thermo_Fisher_Scientific).

### 454 pyrosequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=22" \o "Edit section: 454 pyrosequencing)]

*Main article:*[*454 Life Sciences § Technology*](https://en.wikipedia.org/wiki/454_Life_Sciences#Technology)

A parallelized version of [pyrosequencing](https://en.wikipedia.org/wiki/Pyrosequencing) was developed by [454 Life Sciences](https://en.wikipedia.org/wiki/454_Life_Sciences), which has since been acquired by [Roche Diagnostics](https://en.wikipedia.org/wiki/Roche_Diagnostics). The method amplifies DNA inside water droplets in an oil solution (emulsion PCR), with each droplet containing a single DNA template attached to a single primer-coated bead that then forms a clonal colony. The sequencing machine contains many [picoliter](https://en.wikipedia.org/wiki/Picoliter)-volume wells each containing a single bead and sequencing enzymes. Pyrosequencing uses [luciferase](https://en.wikipedia.org/wiki/Luciferase) to generate light for detection of the individual nucleotides added to the nascent DNA, and the combined data are used to generate sequence [reads](https://en.wikipedia.org/wiki/Read_(biology)).[[46]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Margulies_2005-46) This technology provides intermediate read length and price per base compared to Sanger sequencing on one end and Solexa and SOLiD on the other.[[57]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid18165802-57)

### Illumina (Solexa) sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=23" \o "Edit section: Illumina (Solexa) sequencing)]

*Main article:*[*Illumina dye sequencing*](https://en.wikipedia.org/wiki/Illumina_dye_sequencing)

[Solexa](https://en.wikipedia.org/wiki/Solexa), now part of [Illumina](https://en.wikipedia.org/wiki/Illumina_(company)), was founded by [Shankar Balasubramanian](https://en.wikipedia.org/wiki/Shankar_Balasubramanian) and [David Klenerman](https://en.wikipedia.org/wiki/David_Klenerman) in 1998, and developed a sequencing method based on reversible dye-terminators technology, and engineered polymerases.[[75]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Bentley_2008-75) The reversible terminated chemistry concept was invented by Bruno Canard and Simon Sarfati at the Pasteur Institute in Paris.[[76]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-76)[[77]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-77) It was developed internally at Solexa by those named on the relevant patents. In 2004, Solexa acquired the company [Manteia Predictive Medicine](https://en.wikipedia.org/wiki/Manteia_Predictive_Medicine) in order to gain a massivelly parallel sequencing technology invented in 1997 by Pascal Mayer and Laurent Farinelli.[[37]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-DNA_colony_patents-37) It is based on "DNA Clusters" or "DNA colonies", which involves the clonal amplification of DNA on a surface. The cluster technology was co-acquired with Lynx Therapeutics of California. Solexa Ltd. later merged with Lynx to form Solexa Inc.

[](https://en.wikipedia.org/wiki/File:Illumina_HiSeq_2500.jpg)

An Illumina HiSeq 2500 sequencer

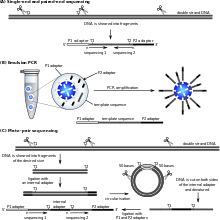
In this method, DNA molecules and primers are first attached on a slide or flow cell and amplified with [polymerase](https://en.wikipedia.org/wiki/Polymerase) so that local clonal DNA colonies, later coined "DNA clusters", are formed. To determine the sequence, four types of reversible terminator bases (RT-bases) are added and non-incorporated nucleotides are washed away. A camera takes images of the [fluorescently labeled](https://en.wikipedia.org/wiki/Fluorescent_labeling) nucleotides. Then the dye, along with the terminal 3' blocker, is chemically removed from the DNA, allowing for the next cycle to begin. Unlike pyrosequencing, the DNA chains are extended one nucleotide at a time and image acquisition can be performed at a delayed moment, allowing for very large arrays of DNA colonies to be captured by sequential images taken from a single camera.

[](https://en.wikipedia.org/wiki/File:Illumina_MiSeq_sequencer.jpg)

An Illumina MiSeq sequencer

Decoupling the enzymatic reaction and the image capture allows for optimal throughput and theoretically unlimited sequencing capacity. With an optimal configuration, the ultimately reachable instrument throughput is thus dictated solely by the analog-to-digital conversion rate of the camera, multiplied by the number of cameras and divided by the number of pixels per DNA colony required for visualizing them optimally (approximately 10 pixels/colony). In 2012, with cameras operating at more than 10 MHz A/D conversion rates and available optics, fluidics and enzymatics, throughput can be multiples of 1 million nucleotides/second, corresponding roughly to 1 human genome equivalent at 1x [coverage](https://en.wikipedia.org/wiki/Coverage_(genetics)) per hour per instrument, and 1 human genome re-sequenced (at approx. 30x) per day per instrument (equipped with a single camera).[[78]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid18576944-78)

### SOLiD sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=24" \o "Edit section: SOLiD sequencing)]

[](https://en.wikipedia.org/wiki/File:Library_preparation_for_the_SOLiD_platform.svg)

Library preparation for the SOLiD platform

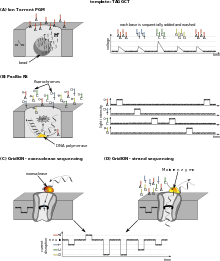
*Main article:*[*2 base encoding*](https://en.wikipedia.org/wiki/2_base_encoding)

[Applied Biosystems](https://en.wikipedia.org/wiki/Applied_Biosystems)' (now a [Life Technologies](https://en.wikipedia.org/wiki/Life_Technologies_(Thermo_Fisher_Scientific)) brand) SOLiD technology employs [sequencing by ligation](https://en.wikipedia.org/wiki/Sequencing_by_ligation). Here, a pool of all possible oligonucleotides of a fixed length are labeled according to the sequenced position. Oligonucleotides are annealed and ligated; the preferential ligation by [DNA ligase](https://en.wikipedia.org/wiki/DNA_ligase) for matching sequences results in a signal informative of the nucleotide at that position. Before sequencing, the DNA is amplified by emulsion PCR. The resulting beads, each containing single copies of the same DNA molecule, are deposited on a glass slide.[[79]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid18477713-79) The result is sequences of quantities and lengths comparable to Illumina sequencing.[[57]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid18165802-57) This [sequencing by ligation](https://en.wikipedia.org/wiki/Sequencing_by_ligation) method has been reported to have some issue sequencing palindromic sequences.[[72]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Yu-Feng_Huang.2C_Sheng-Chung_Chen.2C_Yih-Shien_Chiang.2C_Tzu-Han_Chen_.26_Kuo-Ping_Chiu_2012_S10-72)

### Ion Torrent semiconductor sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=25" \o "Edit section: Ion Torrent semiconductor sequencing)]

*Main article:*[*Ion semiconductor sequencing*](https://en.wikipedia.org/wiki/Ion_semiconductor_sequencing)

Ion Torrent Systems Inc. (now owned by [Life Technologies](https://en.wikipedia.org/wiki/Life_Technologies_(Thermo_Fisher_Scientific))) developed a system based on using standard sequencing chemistry, but with a novel, semiconductor based detection system. This method of sequencing is based on the detection of [hydrogen ions](https://en.wikipedia.org/wiki/Hydrogen_ion) that are released during the [polymerisation](https://en.wikipedia.org/wiki/DNA_polymerase) of [DNA](https://en.wikipedia.org/wiki/DNA), as opposed to the optical methods used in other sequencing systems. A microwell containing a template DNA strand to be sequenced is flooded with a single type of [nucleotide](https://en.wikipedia.org/wiki/Nucleotide). If the introduced nucleotide is [complementary](https://en.wikipedia.org/wiki/Complementarity_(molecular_biology)) to the leading template nucleotide it is incorporated into the growing complementary strand. This causes the release of a hydrogen ion that triggers a hypersensitive ion sensor, which indicates that a reaction has occurred. If [homopolymer](https://en.wikipedia.org/wiki/Homopolymer) repeats are present in the template sequence, multiple nucleotides will be incorporated in a single cycle. This leads to a corresponding number of released hydrogens and a proportionally higher electronic signal.[[80]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-rusk-80)

[](https://en.wikipedia.org/wiki/File:From_second_to_fourth-generation_sequencing,_illustration_on_TAGGCT_template.svg)

Sequencing of the TAGGCT template with IonTorrent, PacBioRS and GridION

### DNA nanoball sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=26" \o "Edit section: DNA nanoball sequencing)]

*Main article:*[*DNA nanoball sequencing*](https://en.wikipedia.org/wiki/DNA_nanoball_sequencing)

[DNA nanoball sequencing](https://en.wikipedia.org/wiki/DNA_nanoball_sequencing) is a type of high throughput sequencing technology used to determine the entire [genomic sequence](https://en.wikipedia.org/wiki/Genomic_sequence) of an organism. The company [Complete Genomics](https://en.wikipedia.org/wiki/Complete_Genomics) uses this technology to sequence samples submitted by independent researchers. The method uses [rolling circle replication](https://en.wikipedia.org/wiki/Rolling_circle_replication) to amplify small fragments of genomic DNA into DNA nanoballs. Unchained sequencing by ligation is then used to determine the nucleotide sequence.[[81]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Drmanac_2010-81) This method of DNA sequencing allows large numbers of DNA nanoballs to be sequenced per run and at low [reagent](https://en.wikipedia.org/wiki/Reagent) costs compared to other high-throughput sequencing platforms.[[82]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-82) However, only short sequences of DNA are determined from each DNA nanoball which makes mapping the short reads to a [reference genome](https://en.wikipedia.org/wiki/Reference_genome) difficult.[[81]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Drmanac_2010-81) This technology has been used for multiple genome sequencing projects and is scheduled to be used for more.[[83]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-83)

### Heliscope single molecule sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=27" \o "Edit section: Heliscope single molecule sequencing)]

*Main article:*[*Helicos single molecule fluorescent sequencing*](https://en.wikipedia.org/wiki/Helicos_single_molecule_fluorescent_sequencing)

Heliscope sequencing is a method of single-molecule sequencing developed by [Helicos Biosciences](https://en.wikipedia.org/wiki/Helicos_Biosciences). It uses DNA fragments with added poly-A tail adapters which are attached to the flow cell surface. The next steps involve extension-based sequencing with cyclic washes of the flow cell with fluorescently labeled nucleotides (one nucleotide type at a time, as with the Sanger method). The reads are performed by the Heliscope sequencer.[[84]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-84)[[85]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-85) The reads are short, averaging 35 bp.[[86]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-86) In 2009 a human genome was sequenced using the Heliscope, however in 2012 the company went bankrupt.[[87]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-87)

### Single molecule real time (SMRT) sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=28" \o "Edit section: Single molecule real time (SMRT) sequencing)]

*Main article:*[*Single molecule real time sequencing*](https://en.wikipedia.org/wiki/Single_molecule_real_time_sequencing)

SMRT sequencing is based on the sequencing by synthesis approach. The DNA is synthesized in zero-mode wave-guides (ZMWs) – small well-like containers with the capturing tools located at the bottom of the well. The sequencing is performed with use of unmodified polymerase (attached to the ZMW bottom) and fluorescently labelled nucleotides flowing freely in the solution. The wells are constructed in a way that only the fluorescence occurring by the bottom of the well is detected. The fluorescent label is detached from the nucleotide upon its incorporation into the DNA strand, leaving an unmodified DNA strand. According to [Pacific Biosciences](https://en.wikipedia.org/wiki/Pacific_Biosciences) (PacBio), the SMRT technology developer, this methodology allows detection of nucleotide modifications (such as cytosine methylation). This happens through the observation of polymerase kinetics. This approach allows reads of 20,000 nucleotides or more, with average read lengths of 5 kilobases.[[67]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-flxlexblog.wordpress.com-67)[[88]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-88) In 2015, Pacific Biosciences announced the launch of a new sequencing instrument called the Sequel System, with 1 million ZMWs compared to 150,000 ZMWs in the PacBio RS II instrument.[[89]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-89)[[90]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-90) SMRT sequencing is referred to as "[third-generation](https://en.wikipedia.org/wiki/Third-generation_sequencing)" or "long-read" sequencing.

### Nanopore DNA sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=29" \o "Edit section: Nanopore DNA sequencing)]

*Main article:*[*Nanopore sequencing*](https://en.wikipedia.org/wiki/Nanopore_sequencing)

The DNA passing through the nanopore changes its ion current. This change is dependent on the shape, size and length of the DNA sequence. Each type of the nucleotide blocks the ion flow through the pore for a different period of time. The method does not require modified nucleotides and is performed in real time. Nanopore sequencing is referred to as "[third-generation](https://en.wikipedia.org/wiki/Third-generation_sequencing)" or "long-read" sequencing, along with SMRT sequencing.

Early industrial research into this method was based on a technique called 'Exonuclease sequencing', where the readout of electrical signals occurring at nucleotides passing by alpha-[hemolysin](https://en.wikipedia.org/wiki/Hemolysin) pores covalently bound with [cyclodextrin](https://en.wikipedia.org/wiki/Cyclodextrin).[[91]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-91) However the subsequently commercial method, 'strand sequencing' sequencing DNA bases in an intact strand.

Two main areas of nanopore sequencing in development are solid state nanopore sequencing, and protein based nanopore sequencing. Protein nanopore sequencing utilizes membrane protein complexes such as ∝-[Hemolysin](https://en.wikipedia.org/wiki/Hemolysin), MspA (Mycobacterium Smegmatis Porin A) or CssG, which show great promise given their ability to distinguish between individual and groups of nucleotides.[[92]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Torre_2012-92) In contrast, solid-state nanopore sequencing utilizes synthetic materials such as silicon nitride and aluminum oxide and it is preferred for its superior mechanical ability and thermal and chemical stability.[[93]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Pathak_2012-93)The fabrication method is essential for this type of sequencing given that the nanopore array can contain hundreds of pores with diameters smaller than eight nanometers.[[92]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Torre_2012-92)

The concept originated from the idea that single stranded DNA or RNA molecules can be electrophoretically driven in a strict linear sequence through a biological pore that can be less than eight nanometers, and can be detected given that the molecules release an ionic current while moving through the pore. The pore contains a detection region capable of recognizing different bases, with each base generating various time specific signals corresponding to the sequence of bases as they cross the pore which are then evaluated.[[93]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Pathak_2012-93) Precise control over the DNA transport through the pore is crucial for success. Various enzymes such as exonucleases and polymerases have been used to moderate this process by positioning them near the pore’s entrance.[[94]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Korlach_2008-94)

## Methods in development[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=30" \o "Edit section: Methods in development)]

DNA sequencing methods currently under development include reading the sequence as a DNA strand transits through [nanopores](https://en.wikipedia.org/wiki/Nanopore_sequencing) (a method that is now commercial but subsequent generations such as solid-state nanopores are still in development),[[95]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-95)[[96]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Physorg-96) and microscopy-based techniques, such as [atomic force microscopy](https://en.wikipedia.org/wiki/Atomic_force_microscope) or [transmission electron microscopy](https://en.wikipedia.org/wiki/Transmission_electron_microscopy_DNA_sequencing) that are used to identify the positions of individual nucleotides within long DNA fragments (>5,000 bp) by nucleotide labeling with heavier elements (e.g., halogens) for visual detection and recording.[[97]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-97)[[98]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-98) [Third generation technologies](https://en.wikipedia.org/wiki/Third-generation_sequencing) aim to increase throughput and decrease the time to result and cost by eliminating the need for excessive reagents and harnessing the processivity of DNA polymerase.[[99]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-99)

### Tunnelling currents DNA sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=31" \o "Edit section: Tunnelling currents DNA sequencing)]

Another approach uses measurements of the electrical tunnelling currents across single-strand DNA as it moves through a channel. Depending on its electronic structure, each base affects the tunnelling current differently,[[100]](https://en.wikipedia.org/wiki/DNA_sequencing" \l "cite_note-100) allowing differentiation between different bases.[[101]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-101)

The use of tunnelling currents has the potential to sequence orders of magnitude faster than ionic current methods and the sequencing of several DNA oligomers and micro-RNA has already been achieved.[[102]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid22787559-102)

### Sequencing by hybridization[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=32" \o "Edit section: Sequencing by hybridization)]

[*Sequencing by hybridization*](https://en.wikipedia.org/wiki/Sequencing_by_hybridization) is a non-enzymatic method that uses a [DNA microarray](https://en.wikipedia.org/wiki/DNA_microarray). A single pool of DNA whose sequence is to be determined is fluorescently labeled and hybridized to an array containing known sequences. Strong hybridization signals from a given spot on the array identifies its sequence in the DNA being sequenced.[[103]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-103)

This method of sequencing utilizes binding characteristics of a library of short single stranded DNA molecules (oligonucleotides), also called DNA probes, to reconstruct a target DNA sequence. Non-specific hybrids are removed by washing and the target DNA is eluted.[[104]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Morey-104) Hybrids are re-arranged such that the DNA sequence can be reconstructed. The benefit of this sequencing type is its ability to capture a large number of targets with a homogenous coverage.[[105]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Qin-105) A large number of chemicals and starting DNA is usually required. However, with the advent of solution-based hybridization, much less equipment and chemicals are necessary.[[104]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Morey-104)

### Sequencing with mass spectrometry[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=33" \o "Edit section: Sequencing with mass spectrometry)]

[Mass spectrometry](https://en.wikipedia.org/wiki/Mass_spectrometry) may be used to determine DNA sequences. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, or [MALDI-TOF MS](https://en.wikipedia.org/wiki/Matrix-assisted_laser_desorption/ionization), has specifically been investigated as an alternative method to gel electrophoresis for visualizing DNA fragments. With this method, DNA fragments generated by chain-termination sequencing reactions are compared by mass rather than by size. The mass of each nucleotide is different from the others and this difference is detectable by mass spectrometry. Single-nucleotide mutations in a fragment can be more easily detected with MS than by gel electrophoresis alone. MALDI-TOF MS can more easily detect differences between RNA fragments, so researchers may indirectly sequence DNA with MS-based methods by converting it to RNA first.[[106]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-106)

The higher resolution of DNA fragments permitted by MS-based methods is of special interest to researchers in forensic science, as they may wish to find [single-nucleotide polymorphisms](https://en.wikipedia.org/wiki/Single-nucleotide_polymorphisms) in human DNA samples to identify individuals. These samples may be highly degraded so forensic researchers often prefer [mitochondrial DNA](https://en.wikipedia.org/wiki/Mitochondrial_DNA) for its higher stability and applications for lineage studies. MS-based sequencing methods have been used to compare the sequences of human mitochondrial DNA from samples in a [Federal Bureau of Investigation](https://en.wikipedia.org/wiki/Federal_Bureau_of_Investigation) database[[107]](https://en.wikipedia.org/wiki/DNA_sequencing" \l "cite_note-107) and from bones found in mass graves of World War I soldiers.[[108]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-108)

Early chain-termination and TOF MS methods demonstrated read lengths of up to 100 base pairs.[[109]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-109) Researchers have been unable to exceed this average read size; like chain-termination sequencing alone, MS-based DNA sequencing may not be suitable for large *de novo* sequencing projects. Even so, a recent study did use the short sequence reads and mass spectroscopy to compare single-nucleotide polymorphisms in pathogenic [*Streptococcus*](https://en.wikipedia.org/wiki/Streptococcus) strains.[[110]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-110)

### Microfluidic Sanger sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=34" \o "Edit section: Microfluidic Sanger sequencing)]

*Main article:*[*Sanger sequencing*](https://en.wikipedia.org/wiki/Sanger_sequencing)

In microfluidic Sanger sequencing the entire thermocycling amplification of DNA fragments as well as their separation by electrophoresis is done on a single glass wafer (approximately 10 cm in diameter) thus reducing the reagent usage as well as cost.[[111]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-111) In some instances researchers have shown that they can increase the throughput of conventional sequencing through the use of microchips.[[112]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-112) Research will still need to be done in order to make this use of technology effective.

### Microscopy-based techniques[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=35" \o "Edit section: Microscopy-based techniques)]

*Main article:*[*Transmission electron microscopy DNA sequencing*](https://en.wikipedia.org/wiki/Transmission_electron_microscopy_DNA_sequencing)

This approach directly visualizes the sequence of DNA molecules using electron microscopy. The first identification of DNA base pairs within intact DNA molecules by enzymatically incorporating modified bases, which contain atoms of increased atomic number, direct visualization and identification of individually labeled bases within a synthetic 3,272 base-pair DNA molecule and a 7,249 base-pair viral genome has been demonstrated.[[113]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-113)

### RNAP sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=36" \o "Edit section: RNAP sequencing)]

This method is based on use of [RNA polymerase](https://en.wikipedia.org/wiki/RNA_polymerase) (RNAP), which is attached to a [polystyrene](https://en.wikipedia.org/wiki/Polystyrene) bead. One end of DNA to be sequenced is attached to another bead, with both beads being placed in optical traps. RNAP motion during transcription brings the beads in closer and their relative distance changes, which can then be recorded at a single nucleotide resolution. The sequence is deduced based on the four readouts with lowered concentrations of each of the four nucleotide types, similarly to the Sanger method.[[114]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-114) A comparison is made between regions and sequence information is deduced by comparing the known sequence regions to the unknown sequence regions.[[115]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Pareek_CS-115)

### *In vitro* virus high-throughput sequencing[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=37" \o "Edit section: In vitro virus high-throughput sequencing)]

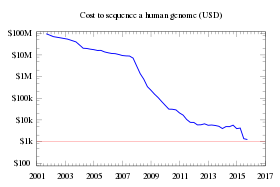
A method has been developed to analyze full sets of [protein interactions](https://en.wikipedia.org/wiki/Interactome) using a combination of 454 pyrosequencing and an *in vitro* virus [mRNA display](https://en.wikipedia.org/wiki/MRNA_display) method. Specifically, this method covalently links proteins of interest to the mRNAs encoding them, then detects the mRNA pieces using reverse transcription [PCRs](https://en.wikipedia.org/wiki/Polymerase_chain_reaction). The mRNA may then be amplified and sequenced. The combined method was titled IVV-HiTSeq and can be performed under cell-free conditions, though its results may not be representative of *in vivo* conditions.[[116]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-116)

## Sample preparation[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=38" \o "Edit section: Sample preparation)]

The success of a DNA sequencing protocol is dependent on the sample preparation. A successful DNA extraction will yield a sample with long, non-degraded strands of DNA which require further preparation according to the sequencing technology to be used. For Sanger sequencing, either cloning procedures or PCR are required prior to sequencing. In the case of next generation sequencing methods, library preparation is required before processing.[[117]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-117)

With the advent of next generation sequencing, Illumina and Roche 454 methods have become a common approach to [transcriptomic](https://en.wikipedia.org/wiki/Transcriptomics) studies (RNAseq). RNA can be extracted from tissues of interest and converted to complementary DNA (cDNA) using reverse transcriptase—a DNA polymerase that synthesizes a complimentary DNA based on existing strands of RNA in a PCR-like manner.[[118]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-118) Complementary DNA can be processed the same way as genomic DNA, allowing the expression levels of RNAs to be determined for the tissue selected.[[119]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-119)

## Development initiatives[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=39" \o "Edit section: Development initiatives)]

[](https://en.wikipedia.org/wiki/File:Historic_cost_of_sequencing_a_human_genome.svg)

Total cost of sequencing a human genome over time as calculated by the [NHGRI](https://en.wikipedia.org/wiki/National_Human_Genome_Research_Institute).

In October 2006, the [X Prize Foundation](https://en.wikipedia.org/wiki/X_Prize_Foundation) established an initiative to promote the development of [full genome sequencing](https://en.wikipedia.org/wiki/Full_genome_sequencing) technologies, called the [Archon X Prize](https://en.wikipedia.org/wiki/Archon_X_Prize), intending to award $10 million to "the first Team that can build a device and use it to sequence 100 human genomes within 10 days or less, with an accuracy of no more than one error in every 100,000 bases sequenced, with sequences accurately covering at least 98% of the genome, and at a recurring cost of no more than $10,000 (US) per genome."[[120]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-120)

Each year the [National Human Genome Research Institute](https://en.wikipedia.org/wiki/National_Human_Genome_Research_Institute), or NHGRI, promotes grants for new research and developments in [genomics](https://en.wikipedia.org/wiki/Genomics). 2010 grants and 2011 candidates include continuing work in microfluidic, polony and base-heavy sequencing methodologies.[[121]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-121)

## Computational challenges[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=40" \o "Edit section: Computational challenges)]

The sequencing technologies described here produce raw data that needs to be assembled into longer sequences such as complete genomes ([sequence assembly](https://en.wikipedia.org/wiki/Sequence_assembly)). There are many computational challenges to achieve this, such as the evaluation of the raw sequence data which is done by programs and algorithms such as [Phred](https://en.wikipedia.org/wiki/Phred_base_calling) and [Phrap](https://en.wikipedia.org/wiki/Phrap). Other challenges have to deal with [repetitive](https://en.wikipedia.org/wiki/Repetitive_DNA) sequences that often prevent complete genome assemblies because they occur in many places of the genome. As a consequence, many sequences may not be assigned to particular [chromosomes](https://en.wikipedia.org/wiki/Chromosome). The production of raw sequence data is only the beginning of its detailed [bioinformatical](https://en.wikipedia.org/wiki/Bioinformatics) analysis.[[122]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-pmid24727769-122) Yet new methods for sequencing and correcting sequencing errors were developed.[[123]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-123)

### Read trimming[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=41" \o "Edit section: Read trimming)]

Sometimes, the raw reads produced by the sequencer are correct and precise only in a fraction of their length. Using the entire read may introduce artifacts in the downstream analyses like genome assembly, snp calling, or gene expression estimation. Two classes of trimming programs have been introduced, based on the window-based or the running-sum classes of algorithms.[[124]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-124) This is a partial list of the trimming algorithms currently available, specifying the algorithm class they belong to:

|  |  |  |
| --- | --- | --- |
| **Read Trimming Algorithms** | | |
| **Name of algorithm** | **Type of algorithm** | **Link** |
| Cutadapt[[125]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-cutadapt-125) | Running sum | [Cutadapt](https://code.google.com/p/cutadapt/) |
| ConDeTri[[126]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-condetri-126) | Window based | [ConDeTri](https://code.google.com/p/condetri/) |
| ERNE-FILTER[[127]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-erne-bs5-127) | Running sum | [ERNE-FILTER](http://erne.sourceforge.net/) |
| FASTX quality trimmer | Window based | [FASTX quality trimmer](http://hannonlab.cshl.edu/fastx_toolkit/download.html) |
| PRINSEQ[[128]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-prinseq-128) | Window based | [PRINSEQ](http://sourceforge.net/projects/prinseq/files/) |
| Trimmomatic[[129]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-trimmomatic-129) | Window based | [Trimmomatic](http://www.usadellab.org/cms/index.php?page=trimmomatic) |
| SolexaQA[[130]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-solexaqa-130) | Window based | [SolexaQA](http://sourceforge.net/projects/solexaqa/files/) |
| SolexaQA-BWA | Running sum | [SolexaQA-BWA](http://sourceforge.net/projects/solexaqa/files/) |
| Sickle | Window based | [Sickle](https://github.com/najoshi/sickle) |

## Ethical issues[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=42" \o "Edit section: Ethical issues)]

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| --- | --- |
| [[icon]](https://en.wikipedia.org/wiki/File:Wiki_letter_w_cropped.svg) | **This section needs expansion**. You can help by[adding to it](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=). *(May 2015)* |

*Main article:*[*Bioethics*](https://en.wikipedia.org/wiki/Bioethics)

Human genetics have been included within the field of [bioethics](https://en.wikipedia.org/wiki/Bioethics) since the early 1970s[[131]](https://en.wikipedia.org/wiki/DNA_sequencing" \l "cite_note-ethics-murray-131) and the growth in the use of DNA sequencing (particularly high-throughput sequencing) has introduced a number of ethical issues. One key issue is the ownership of an individual's DNA and the data produced when that DNA is sequenced.[[132]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-usd1000-genome-ethics-132) Regarding the DNA molecule itself, the leading legal case on this topic, [*Moore v. Regents of the University of California*](https://en.wikipedia.org/wiki/Moore_v._Regents_of_the_University_of_California) (1990) ruled that individuals have no property rights to discarded cells or any profits made using these cells (for instance, as a patented [cell line](https://en.wikipedia.org/wiki/Cell_line)). However, individuals have a right to informed consent regarding removal and use of cells. Regarding the data produced through DNA sequencing, *Moore* gives the individual no rights to the information derived from their DNA.[[132]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-usd1000-genome-ethics-132)

As DNA sequencing becomes more widespread, the storage, security and sharing of genomic data has also become more important.[[132]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-usd1000-genome-ethics-132)[[133]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-guardian-ethics-133) For instance, one concern is that insurers may use an individual's genomic data to modify their quote, depending on the perceived future health of the individual based on their DNA.[[133]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-guardian-ethics-133)[[134]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-ny-times-insurance-ethics-134) In May 2008, the [Genetic Information Nondiscrimination Act](https://en.wikipedia.org/wiki/Genetic_Information_Nondiscrimination_Act) (GINA) was signed in the United States, prohibiting discrimination on the basis of genetic information with respect to health insurance and employment.[[135]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-OMB_support-135)[[136]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-Signing-136) In 2012, the US [Presidential Commission for the Study of Bioethical Issues](https://en.wikipedia.org/wiki/Presidential_Commission_for_the_Study_of_Bioethical_Issues) reported that existing privacy legislation for DNA sequencing data such as GINA and the [Health Insurance Portability and Accountability Act](https://en.wikipedia.org/wiki/Health_Insurance_Portability_and_Accountability_Act) were insufficient, noting that whole-genome sequencing data was particularly sensitive, as it could be used to identify not only the individual from which the data was created, but also their relatives.[[137]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-nature-ethics-137)[[138]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-privacy-progress-report-138)

Ethical issues have also been raised by the increasing use of genetic variation screening, both in newborns, and in adults by companies such as [23andMe](https://en.wikipedia.org/wiki/23andMe).[[139]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-ethics-newborns-139)[[140]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-ethics-hughes-140) It has been asserted that screening for genetic variations can be harmful, increasing [anxiety](https://en.wikipedia.org/wiki/Anxiety) in individuals who have been found to have an increased risk of disease.[[141]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-ethics-bloss-141) For example, in one case noted in [*Time*](https://en.wikipedia.org/wiki/Time_(magazine)), doctors screening an ill baby for genetic variants chose not to inform the parents of an unrelated variant linked to [dementia](https://en.wikipedia.org/wiki/Dementia) due to the harm it would cause to the parents.[[142]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-ethics-time-142) However, a 2011 study in [*The New England Journal of Medicine*](https://en.wikipedia.org/wiki/The_New_England_Journal_of_Medicine) has shown that individuals undergoing disease risk profiling did not show increased levels of anxiety.[[141]](https://en.wikipedia.org/wiki/DNA_sequencing#cite_note-ethics-bloss-141)

## See also[[edit](https://en.wikipedia.org/w/index.php?title=DNA_sequencing&action=edit&section=43" \o "Edit section: See also)]

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