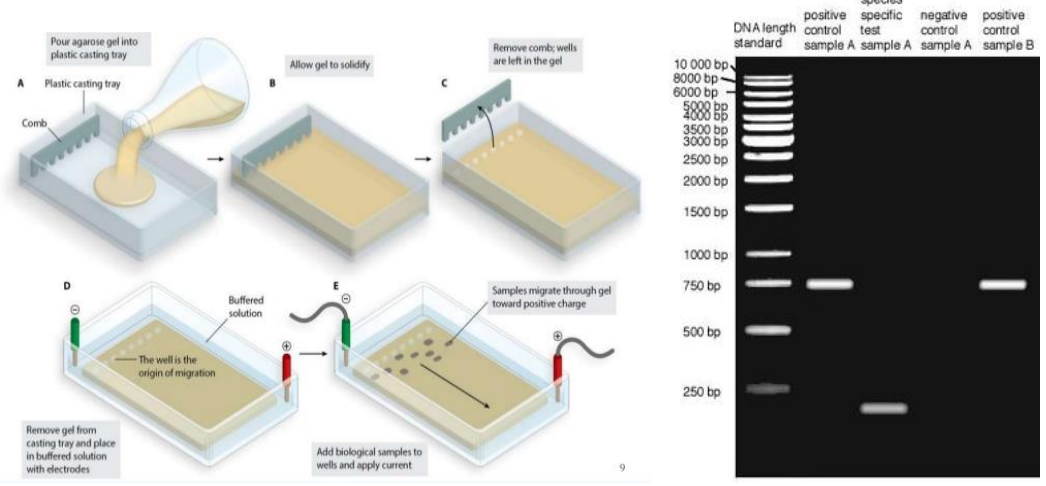
Agarose Gel Electrophoresis

When significant quantity of DNA was produced during the PCR recation, they can be analyzed through gene electrophoresis. This technique uses a carbohydrate [illegible] is derived from seaweeds. Agarose is mixed wi[illegible]Tris-EDTA-acetate (TAE) or Tris-EDTA-borate (TBE[illegible]til it melts and mixes with the buffer. The resul[illegible]es is then casted on the agarose gel electroph[illegible]d is allowed to polymerize. Polymerization sol[illegible]gel . The agarose gel contains wells where sampl[illegible]s portion of the gel where the wells are located[illegible]egative electrode of the electrophoresis apparat[illegible]ode on the other side is positively charged. Th[illegible]ed into the same buffer that was used for its preparation. DNA samples are placed into the wells. The net charge of the DNA is negative due to its phosphate backbone, such that when the current is allowed to pass trough the agarose gel, the negatively-charged DNA molecules will be attracted to move towards the positively-charged electrode. Larger DNA fragments will migrate through the gel slowly, while shorter and lighter DNA amplicons will move faster across the gel. In order to know how many bases are contained by the amplicon, molecular weight markers are used in every electrophoresis experiment. The migration of the DNA sample within the gel is monitored through a gel leading

dye that is mixed with the DN[illegible] reactions are colorless solutions. After se[illegible] according to their molecular sizes, the aga[illegible]zed using gel documentation equipment. In mo[illegible]lating agents are added in the agarose gel. T[illegible]hemselves into the double helix DNA and exh[illegible] struck by UV radiation. The more intens[illegible] the higher concentration of DNA has been generated by the PCR reaction. Furthermore, agarose gel electrophoresis can also be used to purify DNA fragments before they analyzed through DNA sequencing.



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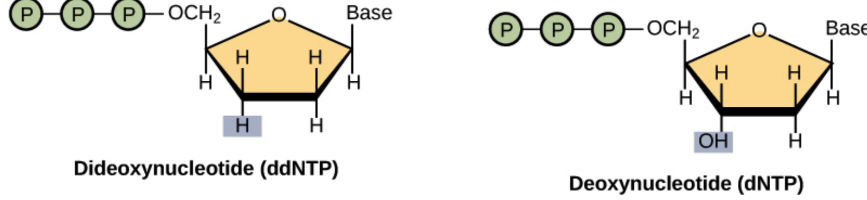
9.6 Sanger-Coulson Method for DNA Sequencing

After gel electrophoresis, the nucleotide sequence of the amplicons are determined. One of [illegible] method in determination of a DNA sequence is th [illegible] son method. The amplicon is aliquoted into fou [illegible] amplified through PCR using the same primers [illegible] amplicon. Each reaction tube contains a spe [illegible] leotide or ddNTPs (i.e., ddATP, ddGTP, ddCTP and [illegible] n in Figure X, the ddNTP lacks oxygen atom at [illegible] ugar. This prevents attachment of the next nucl [illegible] ncorporated in the growing DNA sequence by the DNA polymerase.



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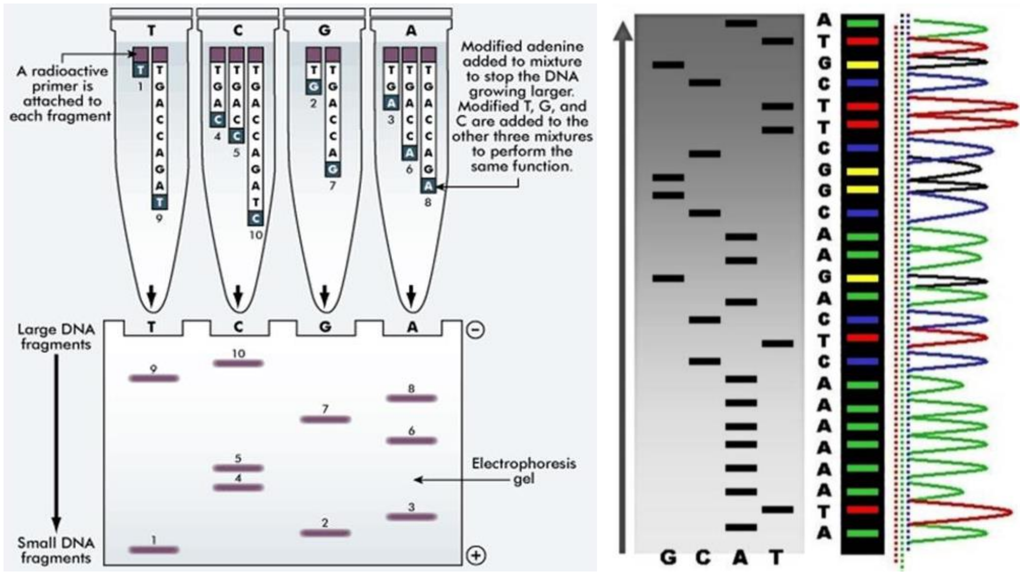
As a result, replication stops on every position where the ddNTP are incorporated, leading to formation of DNA amplicons with varied molecular weights in four separate tubes. The amplicons are run in an agarose or a capillary gel for separation. Results are read from the bottom part of the gel where the DNA fragments are shortest. Nowadays, results of DNA sequencing, called chromatogram are read using computer programs,



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especially for very long DNA sequences. Through these basic concepts of gene manipulation : engineers are capable of producing organisms wi ypes as well as with other applications that addr the agricultural, medical, educational and indust opment of pest and drought resistant and high yie e insects for pest control, glowing bacteria that in absence of microscopes, larger-than-usual frui to address global hunger, medicines specifically ns, and many other products and commodities are generated through alteration of gene structure and function of organisms.