



# Exploring Advanced Techniques in DNA Manipulation

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## DESCRIPTION

DNA manipulation has become an essential part of many scientific fields, including biotechnology, genetics, and medicine. The ability to manipulate DNA has led to groundbreaking discoveries and innovations in these fields. There are various tools and techniques used for DNA manipulation, each with its strengths and limitations. This study highlights some of the most commonly used tools and techniques for DNA manipulation.

### Tools and techniques for DNA manipulation

Polymerase Chain Reaction (PCR): PCR is a widely used technique for amplifying a specific DNA sequence. It is a simple and efficient technique that involves the use of a thermostable DNA polymerase enzyme, which is able to withstand high temperatures.

#### PCR involves three steps

The denaturation step separates the DNA strands, while the annealing step allows for the binding of specific primers to the DNA template. The extension step involves the synthesis of complementary DNA strands, leading to the amplification of the targeted DNA sequence. PCR has many applications, including gene expression analysis, genetic testing, and DNA sequencing.

**Restriction enzyme digestion:** Restriction enzymes are enzymes that recognize specific DNA sequences and cleave the DNA at those sites. These enzymes are commonly used for DNA manipulation, particularly in cloning and genetic engineering. Restriction enzyme digestion involves the incubation of DNA with a restriction enzyme, resulting in the production of fragments of DNA. These fragments can then be ligated into plasmids or other vectors for further manipulation.

**Gel electrophoresis:** Gel electrophoresis is a technique used for separating DNA fragments based on their size. The DNA fragments are loaded onto an agarose gel and subjected to an electric field. The smaller fragments move faster through the gel, while the larger fragments move more slowly. The separated DNA fragments can then be visualized using various staining methods, such as ethidium bromide staining. Gel electrophoresis is a crucial technique for DNA analysis, including DNA sequencing, PCR product analysis, and DNA fragment sizing.

DNA sequencing: DNA sequencing is a technique used to determine the order of nucleotides in a DNA molecule. There are several methods for DNA sequencing, including Sanger sequencing, next-generation sequencing (NGS), and single-molecule real-time (SMRT) sequencing. Sanger sequencing is a widely used method that involves the use of fluorescently labeled did eoxynucleotides, which terminate DNA synthesis when incorporated into the growing DNA strand. The terminated fragments are separated by gel electrophoresis, and the sequence is determined by analyzing the color of the fluorescent signal. NGS is a high-throughput method that enables the simultaneous sequencing of millions of DNA fragments. SMRT sequencing involves the use of a single molecule of DNA, which is sequenced in real-time.

Clustered regularly interspaced short palindromic repeats and CRISPR-Cas9 is a powerful genome editing tool that enables the precise modification of DNA sequences in cells and organisms. It involves the use of a guide RNA (gRNA) that targets a specific DNA sequence, and the Cas9 enzyme, which cuts the DNA at the targeted site. The DNA repair machinery then repairs the cut, leading to the insertion, deletion, or replacement of DNA sequences. CRISPR-Cas9 has many potential applications, including the treatment of genetic diseases and the development of genetically modified organisms.

DNA manipulation is a crucial technique in many scientific fields, and there are several tools and techniques available for this purpose. These tools and techniques enable scientists to amplify, analyze, sequence, and edit DNA sequences, leading to groundbreaking discoveries and innovations. Understanding the strengths and limitations of each tool and technique is crucial for selecting the most appropriate approach for a given application.

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