Site-Directed Mutagenesis

Site-directed mutagenesis (SDM) is a molecular biology technique used to introduce specific mutations into a DNA sequence. It allows researchers to alter the genetic code at precise locations, making it a powerful tool for studying gene function, protein structure, and genetic engineering.

Key Steps in Site-Directed Mutagenesis:

1. **Primer Design:** Short DNA primers are designed to contain the desired mutation (e.g., nucleotide substitutions, insertions, or deletions).
2. **PCR Amplification:** The mutated primers are used in polymerase chain reaction (PCR) to amplify the target DNA sequence, incorporating the mutation.
3. **Template Removal:** The original (non-mutated) template DNA is degraded using an enzyme like **DpnI**, which specifically digests methylated (parental) DNA.
4. **Transformation:** The mutated DNA is introduced into bacteria (e.g., *E. coli*) via transformation for replication.
5. **Screening & Verification:** Colonies are screened for successful mutations using sequencing or restriction digestion.

Applications of Site-Directed Mutagenesis:

- **Protein Engineering:** Modify enzyme activity, stability, or binding affinity.
- **Gene Function Studies:** Identify critical regions of a gene or protein.
- **Drug Resistance Studies:** Understand antibiotic resistance mechanisms.
- **Vaccine Development:** Create attenuated viruses for vaccines.

There are various methods for SDM, including **Quik Change PCR**, **overlap extension PCR**, and **CRISPR-based mutagenesis**. Each has specific advantages depending on the application.