



FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

SITE DIRECTED MUTAGENESIS

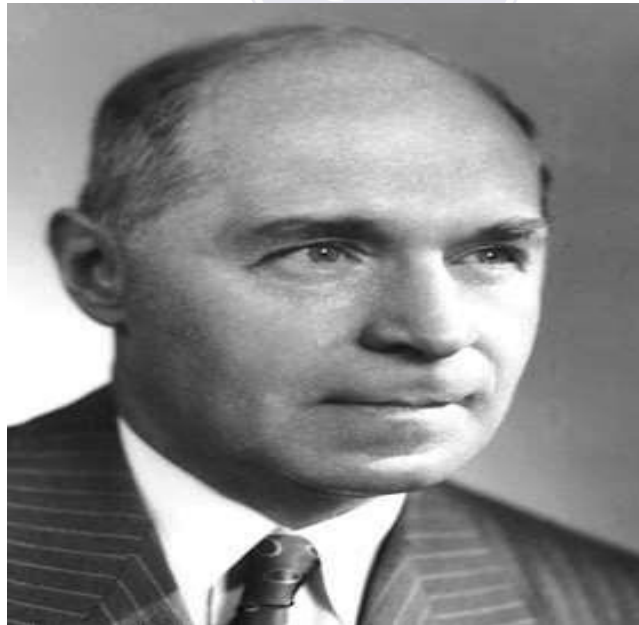
❖ The mutations induced by various physical and chemical mutagens are random in nature and it is very difficult to get a mutation at the desired site in DNA. Now, with the help of recombinant DNA methods, a mutation can be directed to a particular site. Even a single nucleotide change is possible. Several methods like oligonucleotide-mediated site-directed mutagenesis, cassette mutagenesis, PCR-based site-directed mutagenesis are available for site-directed mutagenesis.

❖ In the year 1981 Garry Ruvkun and Frederick Ausubel developed a general method for site-directed mutagenesis in prokaryotes. K. R. Thomas and Mario Capecchi in 1987 gave a site-directed method for mouse embryo derived stem cells. In the same year Oliver Smithies and coworkers described targeted correction of a mutant HPRT gene in the mouse embryonic stem cells.

❖ Mario Capecchi and Oliver Smithies were awarded Nobel Prize in 2007 for these trendsetting discoveries. In the year 2008 site-directed mutagenesis in the cystic fibrosis gene of pigs was done. The mutated pig embryos proved to be very useful for the study of cystic fibrosis disease of humans since the pig mutants, unlike the mouse mutants, were found to show symptoms like human beings suffering from this disease.

WHAT IS MUTAGENESIS

- ❖ Mutagenesis (the creation or formation of a mutation) can be used as a powerful genetic tool.
- ❖ By inducing mutations in specific ways and then observing the phenotype of the organism the function of genes and even individual nucleotides can be determined.
- ❖ Hermann Joseph Muller (or H. J. Muller, December 21, 1890 – April 5, 1967) was an American geneticist, educator, and Nobel laureate best known for his work on the physiological and genetic effects of radiation (X-ray mutagenesis).

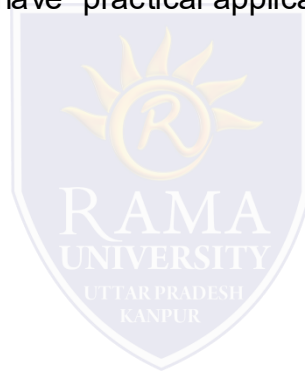


A LABORATORY TECHNIQUE

- ❖ Mutagenesis in the laboratory is an important technique whereby DNA mutations are deliberately engineered to produce mutant genes, proteins, or strains of organisms.
- ❖ Mutant strains may also be produced that have practical application or allow the molecular basis of particular cell function to be investigated.

Types of Mutagenesis

- ❖ Random Mutagenesis
- ❖ Site Directed Mutagenesis



Site-Directed Mutagenesis

Site-directed mutagenesis, also called site-specific mutagenesis or oligonucleotide-directed mutagenesis, is a molecular biology technique in which a mutation is created at a defined site in a DNA molecule.

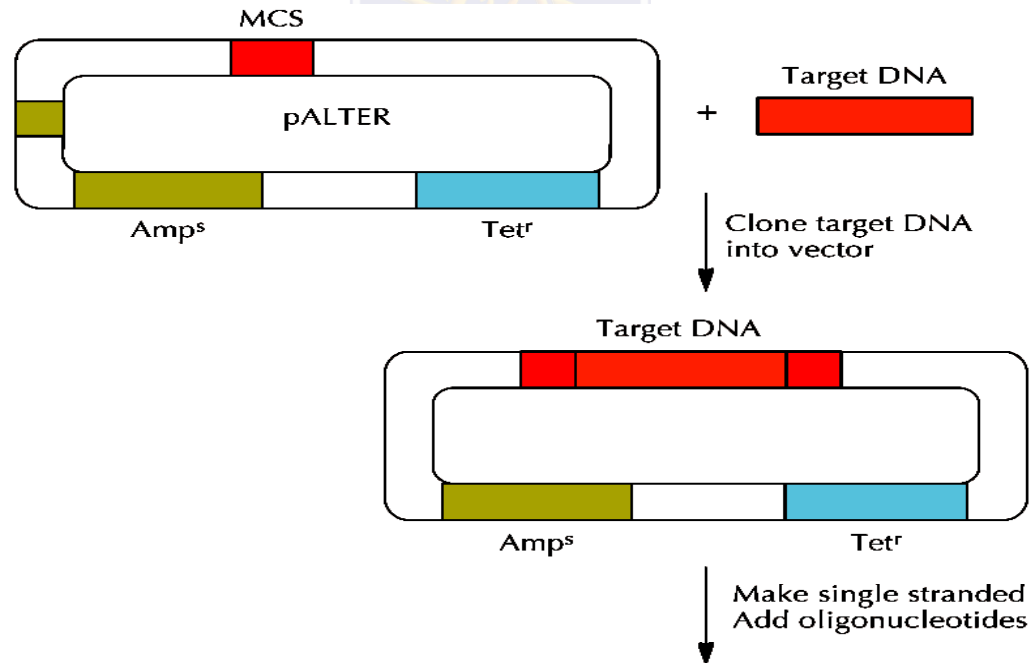
Random Mutagenesis

When an organism is exposed to a physical or chemical mutagen, mutations are induced randomly in all genes of the organism. Hence, this process of generating mutations is known as random mutagenesis. The desired mutant is selected from the mutagenised population.

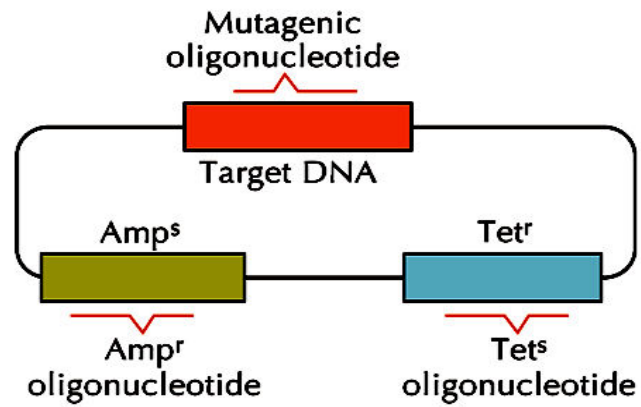


METHODS (OLIGONUCLEOTIDE MUTAGENESIS)

- ❖ A synthetic oligonucleotide encoding the desired mutation is annealed to the target region of the wild-type template DNA where it serves as a primer for initiation of DNA synthesis *in vitro*.
- ❖ Extension of the oligonucleotide by a DNA polymerase generates a double-stranded DNA that carries the desired mutation.
- ❖ The mutated DNA is then inserted at the appropriate location of the target gene, and the mutant protein is expressed.



Make single stranded
Add oligonucleotides



DNA synthesis
transformation
selection

