

# 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.

# 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).



# Mutagenesis as 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**

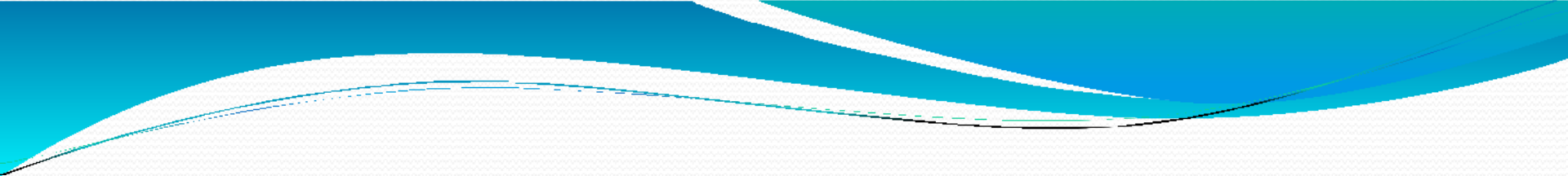
# Random mutagenesis

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.

# 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.



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- Oligonucleotide-directed mutagenesis is used to test the role of particular residues in the structure, catalytic activity, and ligand-binding capacity of a protein.
  - In the absence of a three-dimensional structure, this type of protein engineering relies on informed guesses concerning the structure of the protein and the contribution of individual residues to protein stability and function.

1981



Garry Ruvkun



Frederick M Ausubel

developed a general method for  
site-directed mutagenesis in prokaryotes

1987



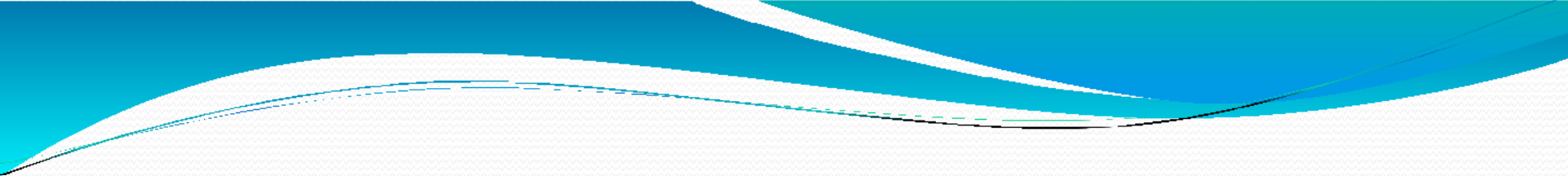
**Mario Renato Capecchi**  
(Oct 6, 1937- )



**Oliver Smithies**  
(Jun 23, 1925 - )

Nobel prize in Physiology or Medicine 2007

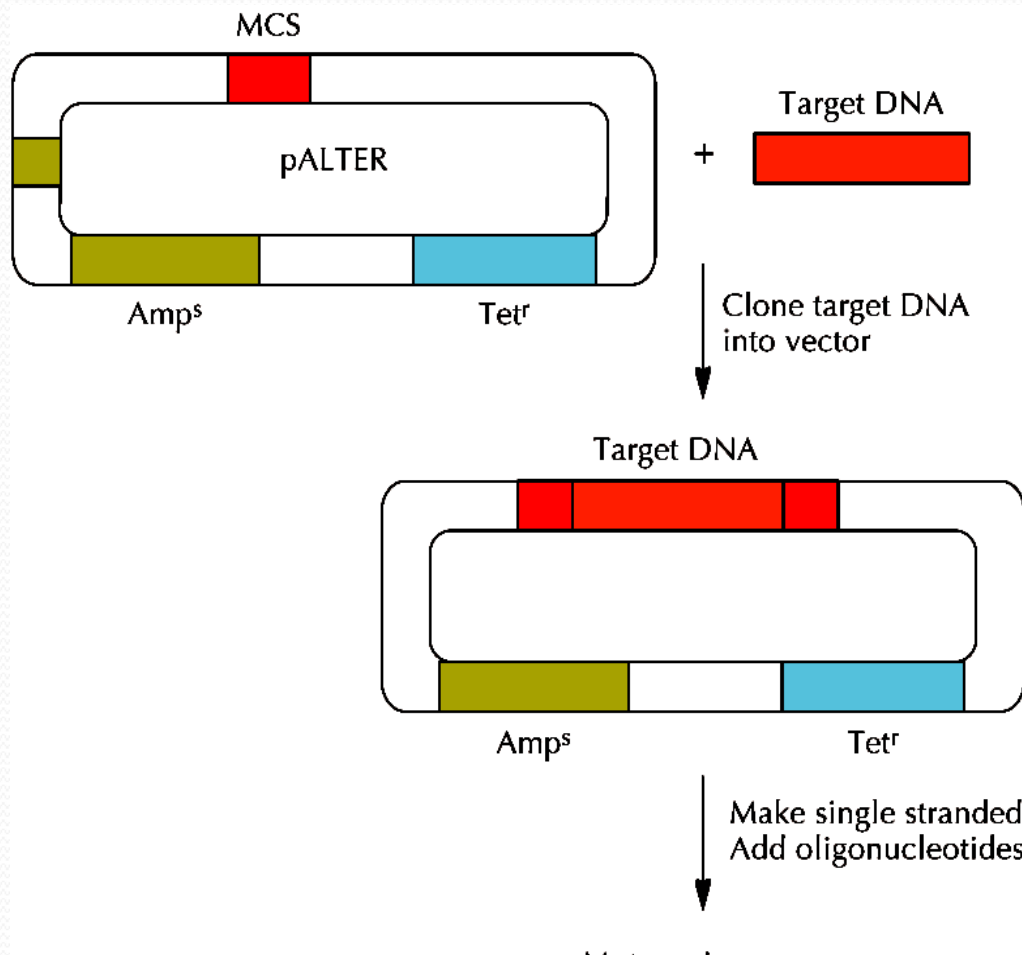
**Site directed mutagenesis** of the mouse genome



# **METHODS OF OLIGONUCLEOTIDE- DIRECTED MUTAGENESIS**

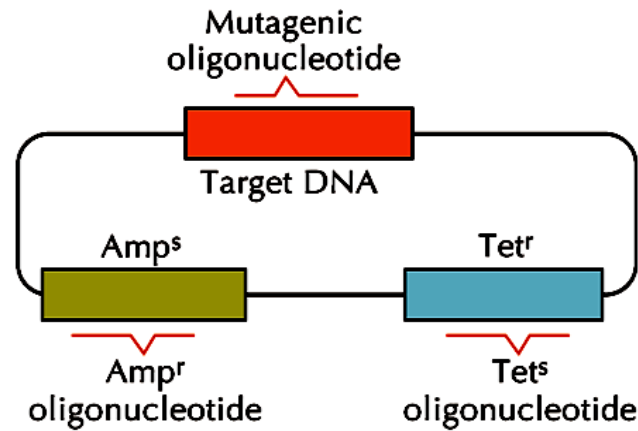
## BY USE OF A SYNTHETIC OLIGONUCLEOTIDE

- 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.

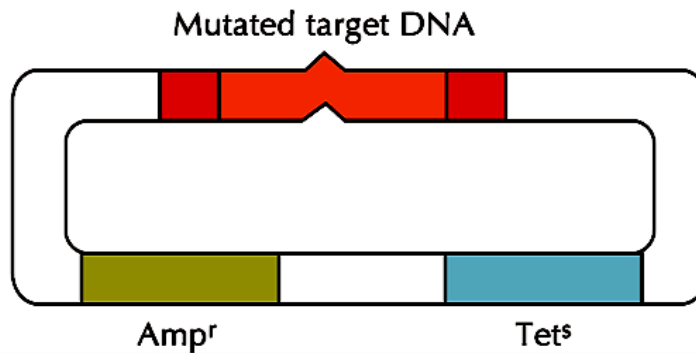


## Oligonucleotide-Directed Mutagenesis Using Plasmid DNA

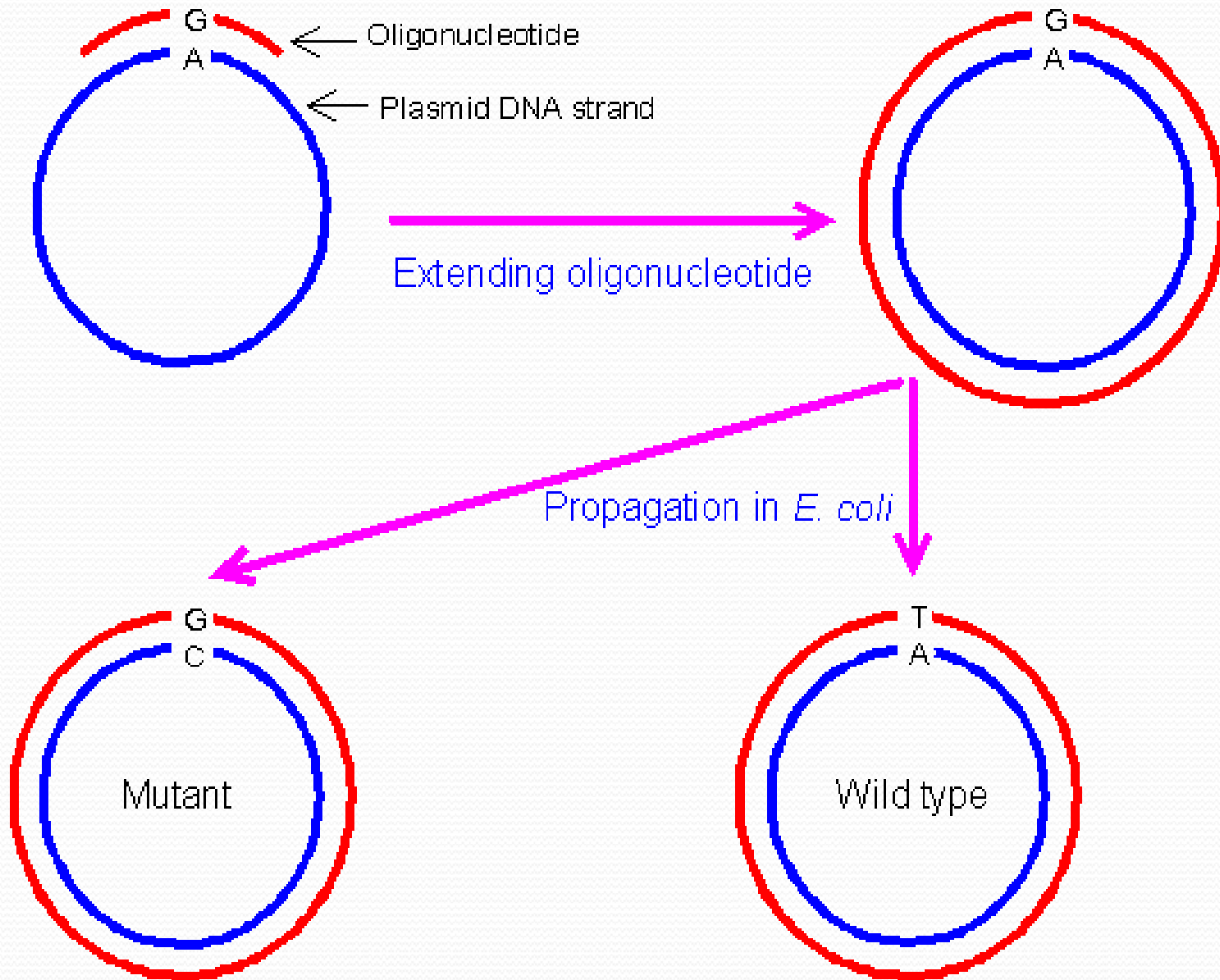
Make single stranded  
Add oligonucleotides



DNA synthesis  
transformation  
selection



# Oligonucleotide-Directed Mutagenesis Using Plasmid DNA





# Cassette mutagenesis

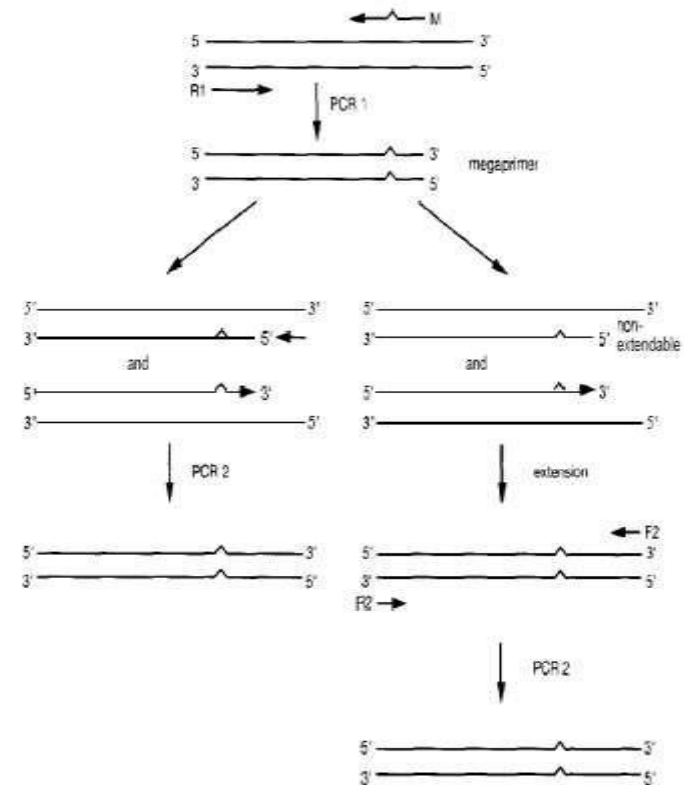
- Unlike other methods, cassette mutagenesis need not involve primer extension using DNA polymerase. In this method, a fragment of DNA is synthesized and inserted into a plasmid.
- It involves the cleavage by a restriction enzyme at a site in the plasmid and subsequent ligation of the synthesized DNA fragment.

# CLASSICAL SITE-DIRECTED MUTAGENESIS

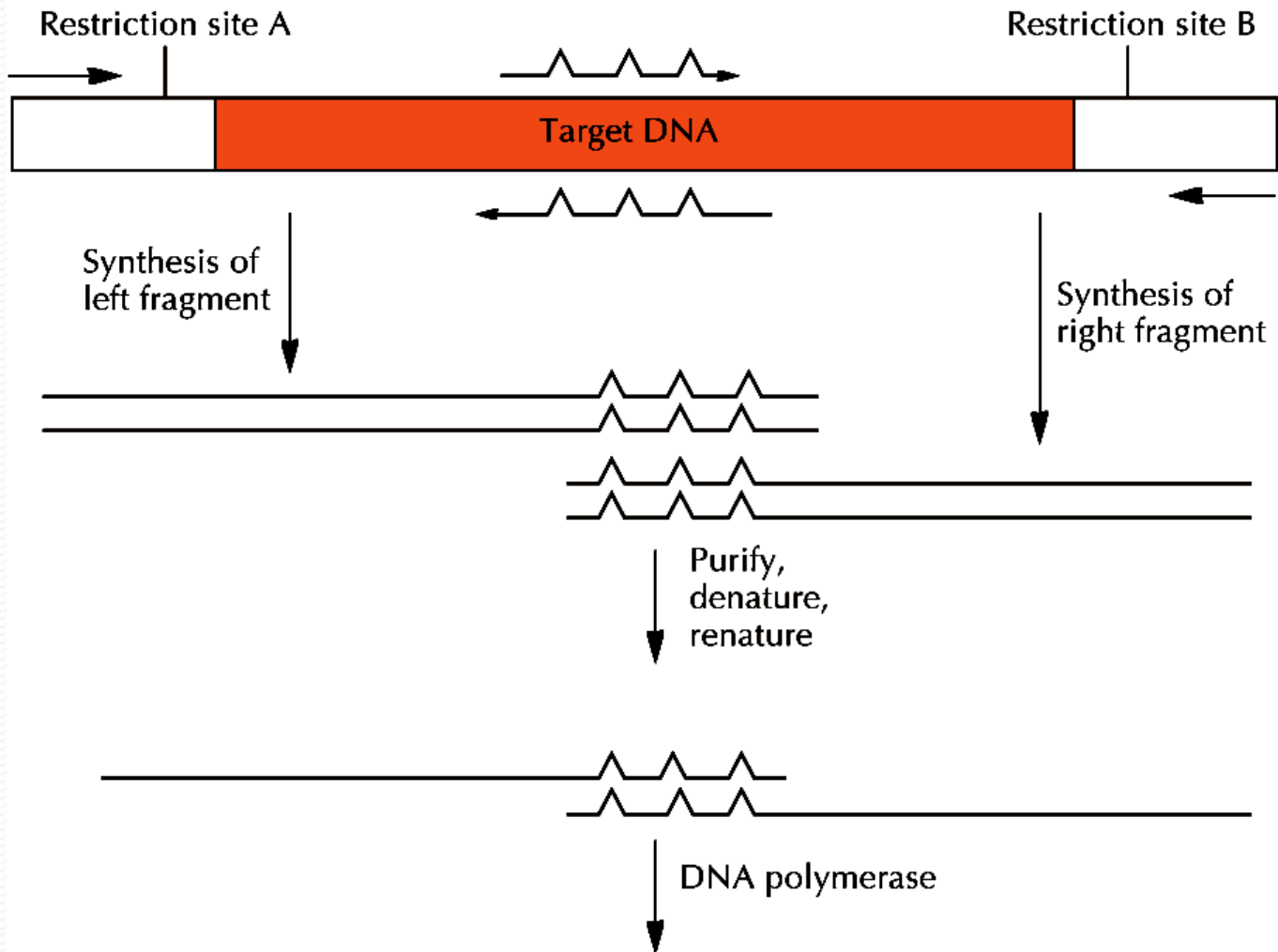
- The feasibility of introducing specific changes at defined locations in DNA was first recognized in the early 1970s from work aimed at mapping the locations of mutations on the single-stranded genome of the small bacteriophage  $\Phi$ 174.

# PCR-based Oligonucleotide Directed Mutagenesis

- PCR in site-directed mutagenesis accomplishes strand separation by using a denaturing step to separate the complementary strands and allowing efficient polymerization of the PCR primers.
- PCR site-directed methods thus allow site-specific mutations to be incorporated in any double stranded plasmid, eliminating the need for M13 vectors or single stranded rescue.



# PCR-amplified Oligonucleotide Directed Mutagenesis



# PCR-amplified Oligonucleotide Directed Mutagenesis

