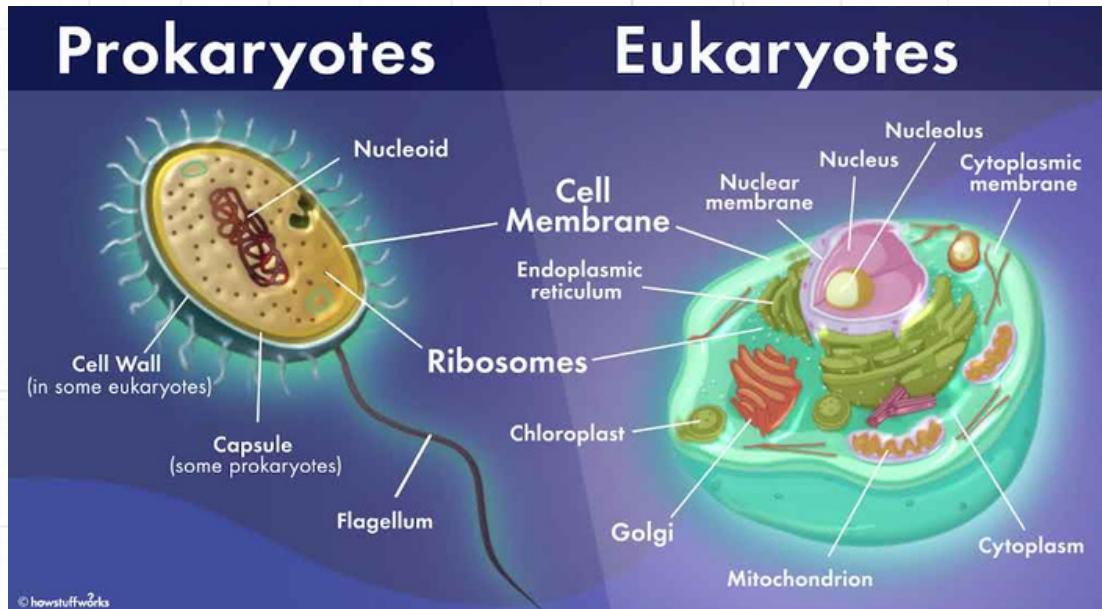


eukaryotes and prokaryotes



4 cytoplasmic compartments

Cytoplasmic Organelles

- Membrane bound structures
- Endoplasmic reticulum, golgi apparatus, mitochondria, lysosomes, peroxisomes, vesicles

Cytoskeleton

- 3 filament systems

Cytoplasmic "structures"

- Ribosomes
- DNA → mRNA → Protein
- Proteins

- Receptors, signaling, metabolism, structural
- viruses, bacteria, prions

Functional compartments

- occur in nucleus, cytoplasm, in organelles and outside organelles
- signaling, metabolic reactions, processing genetic information, cytoskeleton dynamics, vesicle dynamics

component replication fork

- DNA gyrase
- helicase
- SSBS

3 component nucleotides

- a phosphate group
- a sugar molecule
- the base

1. The supercoils are unwound by DNA gyrase (DNA topoisomerase). The gyrase cuts both strands of double stranded DNA to give a double stranded break.
2. Then, the double helix is unwound by the enzyme DNA helicase.

TOPIC 1&2

steps in DNA replication

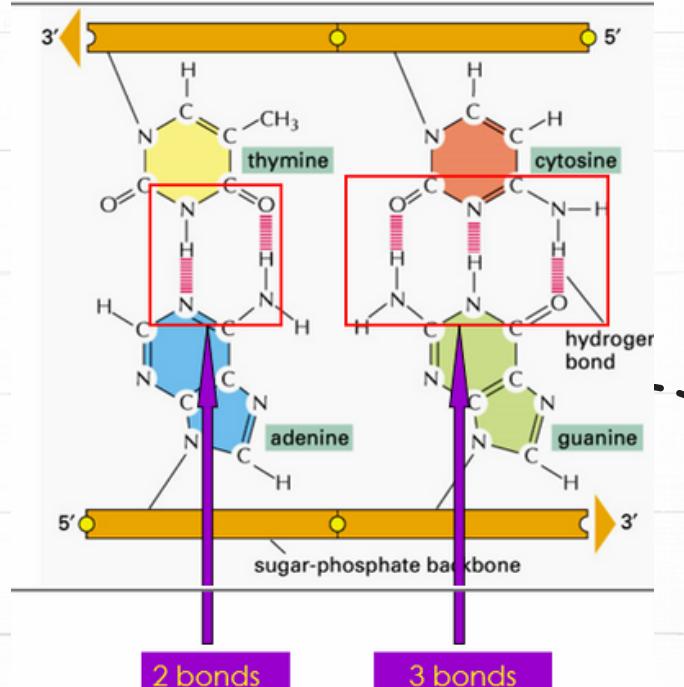
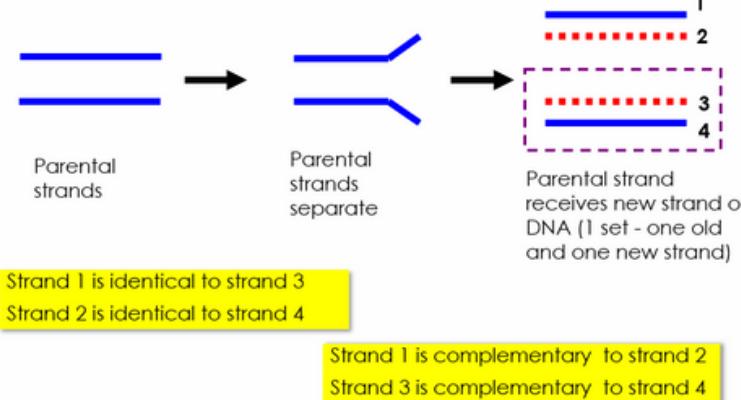
- (1) Separation of the two strands of the DNA double helix.
- (2) Building of a complementary strand on each of the two original strands.

DNA complementary strand

Since A only pairs with T, and G only pairs with C, the sequence of each strand dictates the sequence of its complementary strand.

Two double stranded DNA molecules are produced, both with sequences identical to the original one

one of these daughter molecules has the original left strand and the other daughter has the original right strand



GENETICALLY ENGINEERING *

3 FACTORS TO CHOOSE A VECTOR

1. DNA Extraction:

The first step is to extract the DNA from the organism of interest. This is done by breaking open the cells and isolating the DNA using mechanical or chemical methods.

2. Gene Manipulation:

Once the DNA is extracted, specific genes or gene fragments are cut out using enzymes called restriction enzymes. These enzymes recognize specific sequences of DNA and cut the DNA at those sites. The desired gene or fragment can then be inserted into a vector, such as a plasmid.

3. Gene Transfer:

The vector carrying the desired gene is then introduced into the target organism or cell. This can be done through various methods, such as transformation, where the vector is taken up by the cells, or viral vectors, where a modified virus is used to deliver the gene. Once inside the target organism or cell, the vector integrates the gene into the host genome, allowing the organism to express the desired trait or produce the desired protein.

X TWO CLASSES OF RESTRICTION ENZYME

1. Type I Restriction Enzymes:

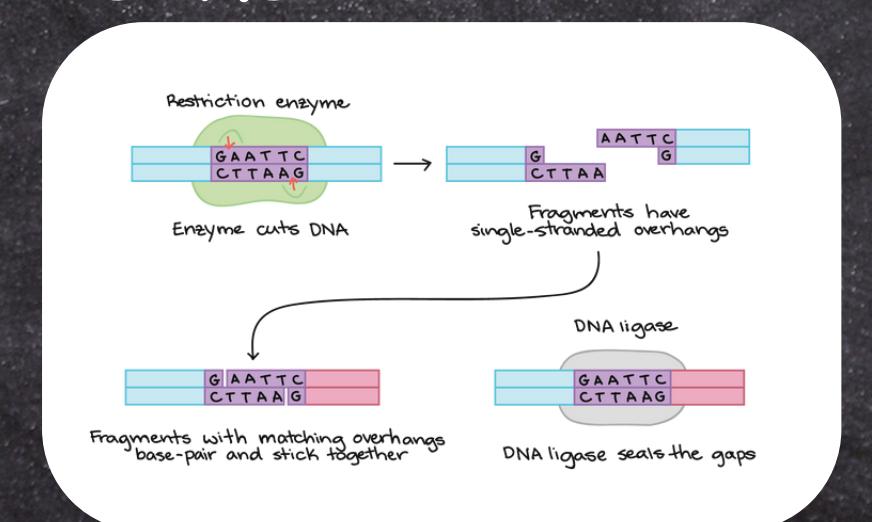
These enzymes cut DNA far away from their target sites and are not commonly used in genetic engineering.

2. Type II Restriction Enzymes:

These enzymes are widely used in genetic engineering. They cut DNA at specific target sites, creating either blunt ends or sticky ends. Sticky ends are more useful because they can easily be joined with other DNA fragments.



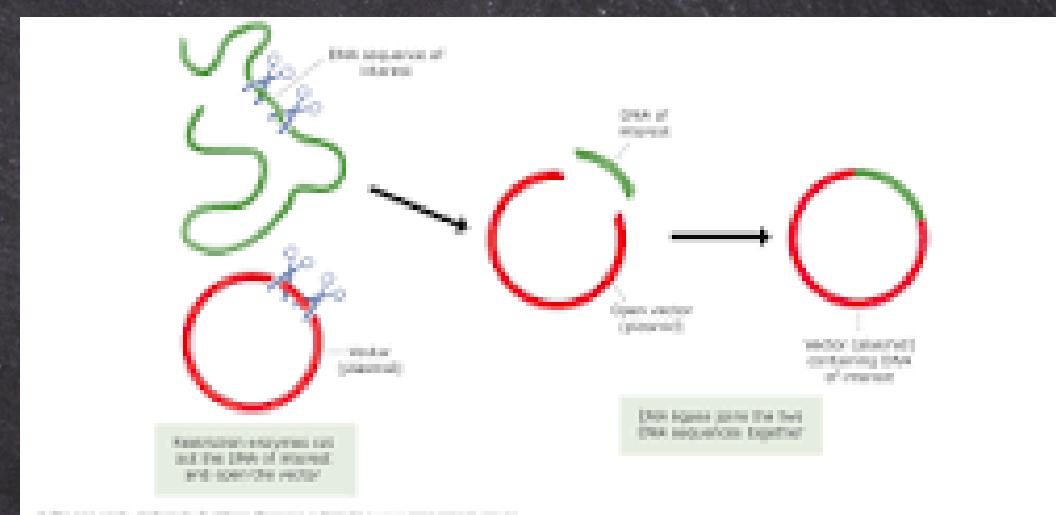
FRAGMENT OF DNA JOINED TOGETHER



INSERT GENE IN VECTOR

- Cut the vector and the gene of interest with the same restriction enzyme to create matching ends.
- Mix the cut vector and gene together.
- Use an enzyme called DNA ligase to join the ends of the vector and the gene.

By cutting the vector and gene with the same enzyme, their ends will match up, allowing them to be joined together using DNA ligase. This process inserts the gene into the vector, creating a modified vector that can be used for genetic engineering purposes



SILENT MUTATION

A mutation in the DNA sequence that has no effect on cell operation. Silent mutations do not alter phenotype.

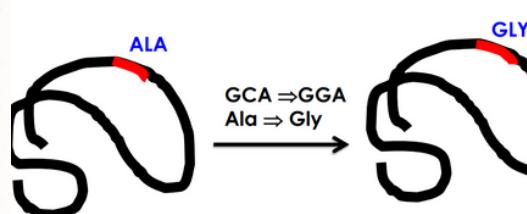


MISSENSE MUTATION

When the change in the base sequence alters a codon, so one amino acid in a protein is replaced with a different amino acid.

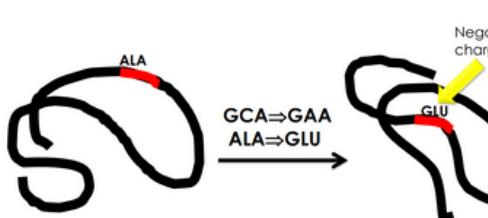
TYPES OF MISSENSE

CONSERVATIVE SUBSTITUTION



TYPE 1

RADICAL REPLACEMENT



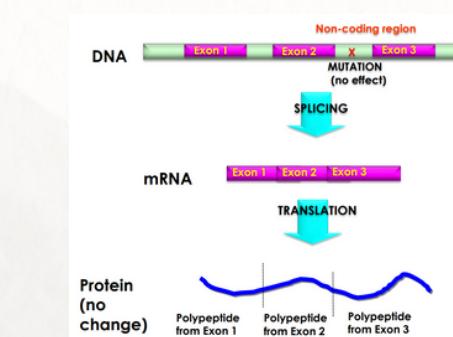
TYPE 2

8: Mutation

TYPES OF SILENT

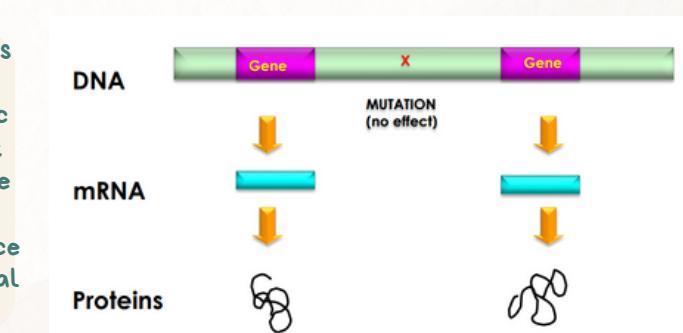
When the base change occurs in the non-coding DNA between genes. Therefore no genes are damaged and no proteins are altered.

When the change occurs in the introns within many of the eukaryotic genes. Intron is cut out and discarded when the mRNA is made, so an alteration in its sequence will not affect the final protein.



EFFECTS OF ALTERING DNA

1. When DNA replicates, any changes due to mutations of the original DNA base sequence will be duplicated and passed on to the next generation. Mutations are inherited.
2. DNA is used as a template in transcription to make RNA. Mutation in the DNA sequence will be passed on to mRNA. mRNA is translated to give protein. An altered RNA sequence may be translated into altered protein. This may cause defects in vital reactions catalysed by the protein.



REVERSION

Reversion refers to the observable outward characteristics of our organism (a phenotypic term).

TRUE REVERTANTS

The likelihood that exactly the one base out of millions that was previously mutated, will be the very one to mutate again is extremely low. · Those rarities where the original base sequence is exactly restored

MUTATION

REVERSION AND SUPPRESSION



DNA: GAG - G.C - AATC - GAA - TGT - GCA - GTG - TTG - GCA

Grouped as : GAG - GCA - ATC - GAA - TGT - GCA

Protein : Glu - Ala - Ile- Glu - Cys - Leu

REVERTANT

- Although the DNA sequence is not identical to its original state, the protein has been exactly restored.
- Similarly, an insertion mutation can be corrected by a second-site deletion.
- The key to success when reverting is to restore activity to the protein, not to get the exact actual DNA sequence back
- A less obvious but more frequent case is where the original mutation as a base change.

HOW REVERSION HAPPEN

occurs when a mutant organism or cell undergoes a change, typically a genetic mutation, that restores the original, wild-type phenotype. This means that the characteristics or traits that were altered by the initial mutation are now reverted to their normal state.

REVERTANT

- Consider a protein with 100 amino acids whose correct 3-D structure depends on the interaction between a positively charged amino acid at position 25 to a negatively charged one at position 50.
- Suppose the original mutation changes codon no. 50 from GAA for Glu (negatively charged) to AAA which encodes Lys, a positively charged amino acid. The protein's folding is now disrupted
- A true revertant could be made by replacing AAA with GAA. However, suppose instead codon No. 25 is mutated to give a negatively charged amino acid. We now have a negative charge at position No. 25 and a positive charge at No. 50.
- We have now restored the reaction between these two regions and the protein will fold O.K. again.