Biotechnological Applications of Recombinant DNA

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1. Introduction

What is mutation?

Mutation is defined as random and permanent change in a DNA sequence which caused by some mutagens that can be a viruses, mutagenic chemicals (ethyl methane sulfonate, methyl methane sulfonate,...), transposons, and radiation as well as errors that occur during meiosis or DNA replication. Many mutations can be neutral in effect that can cause a gene or a chromosome [13]*.

Thus, broadly mutation maybe:

- **Gene mutation** where the allele of a gene changes.
- **Chromosome mutation** where segments of chromosomes, whole chromosomes, or entire sets of chromosomes change.

There are various schemes for classification of different kind of mutations. Depending on :

A. The Type of Cell Involved and mode of origin

1. Somatic mutations

- Mutations that are in the somatic tissues of the body.
- Mutations are not transmitted to progeny.
- The extent of the phenotypic effect depends upon whether the mutation is dominant or recessive (dominant mutations generally have a greater effect).
- The extent of the phenotypic effect depends upon whether it occurs early or late in development (early arising mutations have a greater effect).

2. Germinal mutations

- Mutations that are in the germ tissues of the body.
- Mutations may be transmitted to progeny.
- Dominant mutations are seen in first generation after the mutation occurs.
- If a female gamete containing an X-linked mutation is fertilized, the males will show the mutant phenotype.
- Recessive mutations will only be seen upon the chance mating with an individual carrying the recessive allele too; thus, the recessive mutation may remain hidden for many generations.

3. Spontaneous mutations

- The spontaneous mutations occur suddenly in the nature and their origin is unknown. They are also called “background mutation” and have been reported in many organisms such as, Oenothera, maize, bread molds, microorganisms (bacteria and viruses), Drosophila, mice, man, etc.

4. Induced mutations

- Besides naturally occurring spontaneous mutations, the mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as radiation, certain physical conditions (i.e., temperature) and chemicals.

(cf. 1589181737.pdf)

***Exemple* : Sickle cell anemia**

Sickle cell anemia

● Hemoglobin is a protein in your red blood cells that helps you carry oxygen but in the disorder sickle cell anemia, the gene that codes for hemoglobin is mutated.

● If you inherit two copies of this gene (one from each parents), you can have this disorder.

● This disorder can make it difficult for your red blood cells to carry oxygen because the shape of red blood cell is affected from this mutation.

● It is worth to mention that if an individual only inherits one copy of the mutated gene from one parent, they are a carrier and do not officially have the disease.

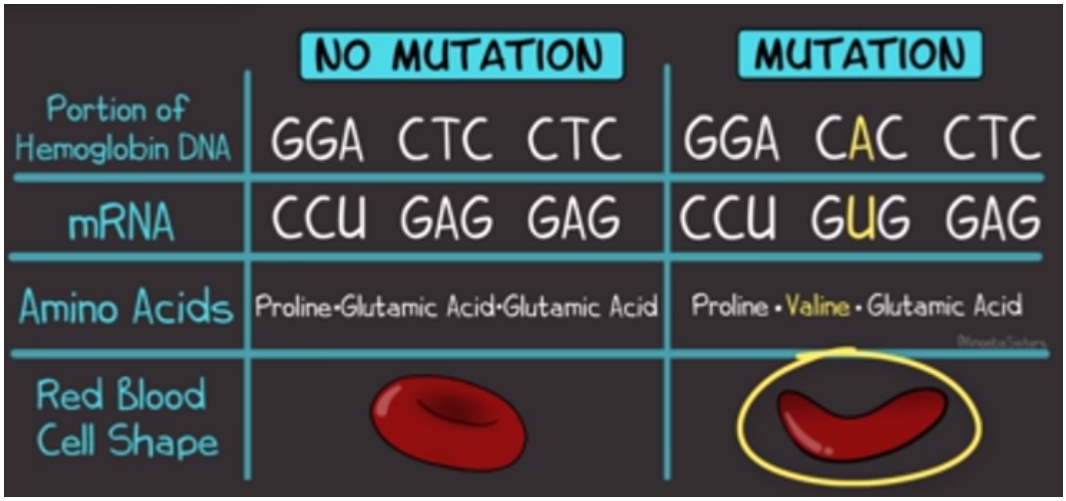


Image 1 Sickle cell anemia gene mutation

2. Objectives

- Explain how directed mutagenesis techniques, such as site-directed mutagenesis and random mutagenesis.
- Learn the mechanisms and tools used to create mutations at defined gene locations.
- Explore the applications in biological engineering.

3. What is mutagenesis?

Mutagenesis is defined as “to generate mutation” in a DNA for a particular cause. It gives us the capability of testing the role of any amino acid in a protein by replacing it with any of the other naturally occurring amino acids. We can do it in the lab through chemical irradiation or insertional methods.

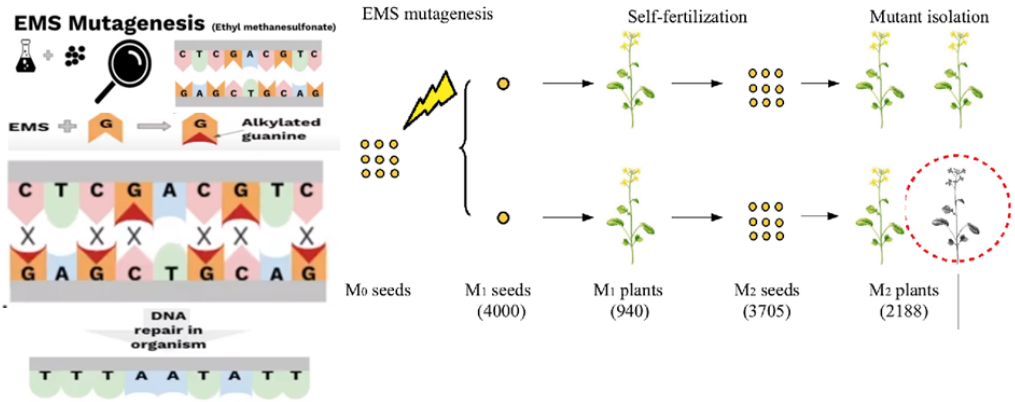
Mutagenesis is the process of an organism's deoxyribonucleic acids (DNA) change, resulting in a gene mutation. A mutation is a permanent and heritable change in genetic material, which can result in altered protein function and phenotypic changes. DNA consists of nucleotides that contain a phosphate backbone, a deoxyribose sugar, and1 of 4 nitrogen-containing bases (adenine [A], guanine [G], cytosine [C], and thymine [T]). DNA mutagenesis occurs spontaneously in nature or as a result of mutagens (agents predisposing to alter DNA). Furthermore, molecular genetic techniques, such as

polymerase chain reaction (PCR), have revolutionized how mutations are obtained and studied. Mutagenesis is the driving force of evolution; however, it can also lead to cancers and heritable diseases.

Mutagenesis is the driving force behind evolution and genetic variation results from mutations. Any change in genetic information may result in advantageous or disadvantageous phenotypic characteristics that impact an organism's fitness. When a mutation results in a higher fitness, natural selection favors these phenotypes, and these traits are more likely to be passed to offspring[14].

Exemple : Mutagenesis of Arabidopsis

Arabidopsis is one of the most popular model organism in the modern lab. It is characterized by a small size, rapid generation time, grows well in the lab, and very small plant genome that has been entirely sequenced. Mutagenesis of Arabidopsis involves the use of various mutagenic agents, such as ethyl methanesulfonate to the seeds, which alkylated all guanine base leading to not link with cytosine base. Then, screen the desired plants that contain the desired mutation. Grow the selected candidate to maturity and retest phenotype in the next generation.



Mutagenesis of Arabidopsis

4. Exercice : Mutation is .....

[solution n°1 p. 15]

Define the mutation

- ☐ A deliberate change in DNA sequence
- ☐ A process of creating new genes
- ☐ A random and permanent change in DNA sequence
- ☐ A temporary change in protein structure

5. Exercice : Which of the following is NOT a type of mutagenesis ?

[solution n°2 p. 15]

Type of mutagenesis

- ○ Reverse mutagenesis
- ○ Random mutagenesis
- ○ Insertional mutagenesis
- ○ Site-directed mutagenesis

6. Exercice : Mutation vs Mutagenesis

[solution n°3 p. 15]

1. in
2. the DNA sequence
3. while
4. change
5. typically
6. mutagenesis
7. artificially
8. is
9. is
10. induced
11. a permanent
12. A mutation

Réponse : __ __ __ __ __ __ __ __ __ __ __ __

7. Mechanism of mutagenesis

Mutagenesis occurs due to DNA replication errors, damage, and lab techniques. Here, we break mutagenesis down into endogenous and exogenous causes.*14*[14]*

7.1. Endogenous mutagenesis

Endogenous mutagenesis refers to the process by which mutations occur naturally within an organism's own cells, without the influence of external factors like radiation or chemicals. These mutations arise due to internal cellular processes that can damage DNA or lead to errors during DNA replication or repair. Here are some of the key sources of endogenous mutagenesis:

a) Errors in DNA replication

Our body possesses high and low-fidelity DNA polymerases. Although the error rate is minimal, high-fidelity polymerases and mismatch repair (MMR) mechanisms still make 1 in 10^6 to 10^8 base substitution per cell per generation. Furthermore, some errors occur due to replication slippage at repetitive sequences, which can lead to insertions and deletions. A mutation results if these errors are not repaired before the next round of DNA replication.

b) Errors in DNA repair mechanisms

The DNA damage response (DDR) is a group of mechanisms that sense DNA —errors and promote repair. Errors in repair or mutations affecting the DDR network cause different cancers. There are multiple DNA repair mechanisms, including MMR, base excision repair (BER), nucleotide excision repair (NER), translesion synthesis (TLS), homologous recombination (HR), and nonhomologous end-joining (NHEJ) pathways. A cell is predisposed to DNA damage if these repair mechanisms disappear. For example, xeroderma pigmentosum (a rare autosomal recessive skin disorder that makes a person highly prone to developing skin cancer) is caused by a mutation in the NER pathway, resulting in a build-up of UV-associated damage. The TLS repair system and NHEJ are of interest to endogenous mutagenesis. During DNA replication, high-fidelity polymerases have difficulty passing damaged bases (eg, pyrimidine dimers or crosslinked DNA), which stalls DNA replication. Failure to restart replication can result in double-stranded breaks, chromosomal rearrangements, and cell death. Therefore, it is often beneficial to circumvent these replicative arrests to promote cell survival. One mechanism to accomplish this is the TLS system. TLS involves DNA polymerases with larger active sites that allow them to tolerate and bypass DNA lesions. However, it comes at the expense of lower replication fidelity and high error rates, resulting in a greater likelihood of base substitutions.[2]Otherwise, NHEJ is a repair mechanism for double-stranded DNA breaks. It brings 2 ends of DNA fragments together and doesn't require homologous sequences. Therefore, this can result in deletions and insertions.[3]

c) Spontaneous base deamination

Base deamination is when a nucleotide base loses an amine group, effectively changing the nucleotide. The following are major deamination reactions: cytosine to uracil (U), adenine to hypoxanthine, guanine to xanthine, and 5-methyl cytosine (5mC) to thymine. If these alterations are not repaired, there can be a change in the DNA sequence. For example, if cytosine deaminates to uracils, there are new A:T mutations in 2 replication events. This G:C to A:T transition accounts for 33% of the single-site mutations resulting in human hereditary diseases.

d) Oxidative DNA damage

Reactive oxygen species (ROS) are a byproduct of the electron transport chain (ETC) and other cellular processes in normal cell physiology. ROS serves essential cellular roles, including redox signaling and immune defense. However, in high quantities, ROS damages a cell and its DNA. There have been 100 different oxidative base lesions and 2-deoxyribose modifications described. One example is the oxidation of carbon #8 of guanine, forming 8-oxoguanine (8-OG). 8-OG incorrectly pairs with adenine (instead of cytosine), resulting in a G:C to A:T transition. Excess ROS are known to be associated with many diseases, including Alzheimer's disease, cancer, and heart failure.

e) Base methylation

S-adenosylmethionine (SAM) is used as a methyl donor during physiologic DNA methylation. At a concentration of 4x10^-5M, SAM can generate over 4,000 methylated base changes per cell per day. The significance of this can be depicted in the methylated products O6-methylguanine and O4-methylamine. These highly mutagenic products result in G:C to A:T and T:A to C:G transition mutations.

2. https://www.ncbi.nlm.nih.gov/books/NBK560519/#
3. https://www.ncbi.nlm.nih.gov/books/NBK560519/#

f) Abasic sites (ie, apurinic and apyrimidinic sites)

Approximately 10,000 abasic sites are made daily by spontaneous hydrolysis or DNA glycosylase cleavage. Abasic sites are unstable and commonly removed by endonucleases. In other cases, they are repaired by TLS polymerases. If these damage sites are not corrected, they may result in mutagenesis.

7.2. Exogenous mutagenesis

Exogenous mutagenesis refers to the process by which mutations are induced in an organism's DNA by external environmental factors, rather than arising from internal cellular mechanisms. These external agents are referred to as **mutagens** and can include physical, chemical, or biological factors that cause DNA damage, leading to mutations. Here are the major categories and mechanisms of exogenous mutagenesis:

a) Ionizing radiation (IR)

IR comes from the soil, radon, medical devices, and cosmic radiation, among others. It can damage DNA directly (eg, DNA strand breaks) or indirectly (eg, radiolysis of water molecules producing ROS). Ionizing radiation can generate a range of nucleotide base lesions, similar to that discussed for ROS, resulting in mutagenesis.

b) Ultraviolet (UV) radiation

UV light falls between 100 to 400 nm, with the most harmful radiation at lower wavelengths—UV light damages DNA through direct and indirect energy transfer (to nearby molecules. The 2 main products of UV damage are pyrimidine dimers and pyrimidine pyrimidone photoproducts. These byproducts distort the DNA helix, requiring the NER system or the error-prone TLS polymerases to bypass them. Pyrimidine dimers cause C:G to T:A, T:A to C:G, and tandem CC to TT transition mutations.

c) Alkylating agents & Aromatic amines

Alkylating agents (eg, nitrogen mustard gas, methyl methanesulfonate [MMS], ethyl methanesulfonate [EMS], N-ethyl-N-nitrosourea [ENU]) have a high affinity for nitrogens on nucleotide bases, mainly N3 of adenine and N7 of guanine. MMS, for example, reacts with adenine and guanine to produce N3-methyladenine and N7-methylguanine, respectively. These methyl products are susceptible to N-glycosidic bond cleavage that can create abasic sites. Aromatic amines (eg, 2-aminofluorene, previously used in insecticides) are metabolized by the CYP450 system and converted into alkylating agents. These products primarily cause lesions to the C8 position of guanine. C8-guanine lesions are known to give rise to base substitutions and frameshift mutations.

d) Polycyclic aromatic hydrocarbon (PAH)

AHs (eg, dibenzo[a,l]pyrene, naphthalene, anthracene, and pyrene) are carbon compounds with 2 or more aromatic rings. They are commonly present in tobacco smoke, automobile exhaust, charred food, and combustion products of fossil fuels and organic matter. The CYP450 enzymes convert PAHs into reactive DNA intermediates that intercalate into DNA, ultimately forming a DNA adduct (a segment of DNA bound to a cancer-causing chemical). This results in DNA damage and, thus, can cause mutagenesis.

e) Crosslinking

Crosslinking occurs when 2 nucleotides form a covalent link. Agents commonly associated with crosslinking include cyclophosphamide, cisplatin, and psoralens. Interstrand crosslinking blocks DNA replication, and this requires repair or bypassing. TLS is one of these repair mechanisms and is associated with high substitution rates.

f) Insertional mutagenesis

This is the process by which exogenous DNA integrates into host DNA. Insertional mutagenesis can be natural, mediated by transposons or viruses, or accomplished in a laboratory. Given there is an addition of nucleotides, insertional mutagenesis commonly results in frameshift mutations.

g) Other toxins

Aflatoxin is a naturally occurring toxin from *Aspergillus*. The CYP450 system metabolizes aflatoxin into an active form that adducts with N7 of guanine, which results in depurination. Aflatoxin is a well-established liver carcinogen that is associated with hepatocellular carcinoma. N-nitrosamines are organic compounds commonly found in tobacco smoke, preserved meats, and the environment. The CYP450 system metabolizes them to form DNA alkylating agents. N-nitrosamines have been implicated in nasopharyngeal, esophageal, and gastric.

h) Laboratory techniques

Different laboratory techniques, including PCR, non-PCR, and gene-editing tools, induce mutagenesis.

8. Mutagen and its types

(cf. Mutagen and its types)

***Complément* : Types of Mutagenesis**

- **Random Mutagenesis:** Mutation can be generated randomly at any region of DNA sequence using a variety of mutagens like UV-irradiation, X-ray, chemicals.
- **Sited-directed Mutagenesis:** induces site specific changes in DNA. It is a method that target a specific gene or specific nucleotide.

9. Difference between mutation and mutagenesis

***Fondamental* :**

Mutation	Mutagenesis
A mutation is a permanent change in the DNA sequence of an organism.	Mutagenesis is the process of deliberately inducing mutations in an organism's DNA for research or practical purposes.
Mutations can occur naturally as a result of DNA replication errors, environmental factors, or genetic recombination.	Mutagenesis is typically induced artificially, although it can also occur naturally due to exposure to mutagenic agents.
Mutations can result from various factors, including spontaneous errors in DNA replication, radiation, and chemical agents.	Mutagenesis is primarily caused by intentionally introducing mutagenic agents, such as chemicals or radiation.
Mutations can occur without human intervention, and they are not necessarily deliberate or controlled.	Mutagenesis is a controlled and deliberate process, often used in research or breeding programs.
Mutations can lead to genetic diversity and evolution; they may also be responsible for genetic diseases.	Mutagenesis is used to create specific mutations for scientific studies, to study gene function, or to develop organisms with desired traits.
Mutations can be beneficial, harmful, or neutral in their effects on an organism's traits or survival.	Mutagenesis is carried out with specific goals, such as studying gene function or creating organisms with desired characteristics.

Tableau 1 Table 4.Difference between mutation and mutagenesis

10. Site-Directed Mutagenesis

Also known as Site specific Mutagenesis. SDM is a molecular biology technique in which a mutation is created at a defined site in a DNA molecule known as Plasmid.

Or

Powerful technique where site specific changes in DNA sequences are produced in vitro for instance to change an amino acid residue into another by changing the codon sequence within the gene sequence *15*[15]*.

There are many reasons to make specific DNA alterations (insertions, deletions and substitutions), including:

- To study changes in protein activity that occur as a result of the DNA manipulation.
- To select or screen for mutations (at the DNA, RNA or protein level) that have a desired property.
- To introduce or remove restriction endonuclease sites or tags.

***Méthode* : Requirements**

- Require knowledge of the DNA sequence.
- Require ability to synthesize Oligonucleotides (primer).
    - **Conditions require for primer**
        - Primer must contain the mutation.
        - Mutation should be in middle of the primer.
        - Primer should be **24-25** nucleotides long and have GC contents of at least **40 %**.
        - The melting temperature should be >**78°C**.
        - The 3'-end of the primer has to end on a G or C.

***Complément* : Site-Directed Mutagenesis**

(cf. Site-Directed Mutagenesis)

11. Types of Site-Directed Mutagenesis

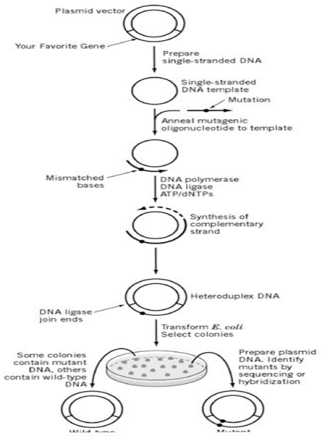
Various types of site-directed mutagenesis exist, each suited for specific applications. Here are the main types:

11.1. Oligonucleotide SDM

The synthetic primer contain the desired mutation and is complementary to the template DNA around the mutation site so it can hybridize with the DNA in the gene of interest. The single strand primer is them extended using the DNA polymerase, which copies rest of gene. Taking in note that the primer is a chemically synthesized oligonucleotide (7-20 nucleotides long). It is complementary to a position of a gene around the site to be mutated; however, it contains mismatch of the base to be mutated. The gene thus copied contains the mutated site, and is then introduce into a host cell as a vector (plasmid or phage) and cloned.

Hybridization (despite a single base mismatch) is possible by mixing at low temperature with excess of primer, and in the presence of high salt concentration. The addition of 4-deoxyribonucleoside triphosphates and DNA polymerase to occur replication followed by sealed the ends by DNA ligase. The double-stranded DNA containing the mismatched

introduced by nucleotide into host cell transformation. It is expected that half of the plasmid or M13 phage should carry wild type sequence while the other half mutant sequence. Finally mutant are selected by Screening and selection method.



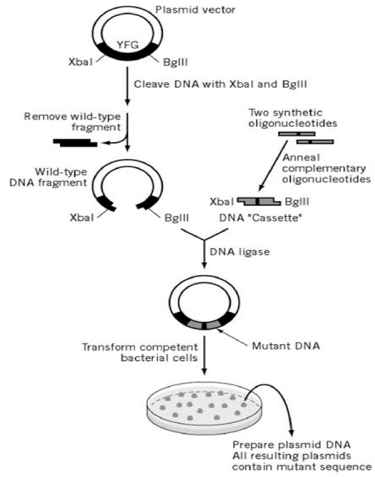
Oligonucleotide SDM

Drawbacks of using the M13 DNA for carrying out SDM

- There is a need to subclone a target gene from a plasmid into M13 and then, after mutagenesis, clone it back into a plasmid.
- Additional step of transforming enzyme defective E. coli necessary to enrich the yield.
- Lengthy process involving multiple steps.

11.2. Cassette mutagenesis

Cassette mutagenesis that uses a short double stranded oligonucleotide (gene cassette) to replace the fragment of target DNA. Technique for altering a protein sequence at DNA level by replacing section of genetic information with alternative sequence. It differs from the single oligonucleotide in that a single gene cassette can contain multiple mutations and also does not involve Primer extension by DNA polymerase. Cassette mutagenesis is possible if the fragment of the gene to be mutated lies between two restriction enzyme cleavage sites (The target DNA is cut with restriction enzymes, such as EcorRI and Hind111). It is complementary to a position of a gene around the site to be mutated; however, it contains mismatch of the base to be mutated.



Cassette mutagenesis

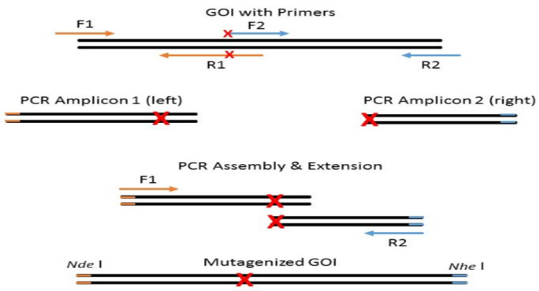
11.3. PCR-SDM

PCR and non-PCR techniques are in vitro methods for performing SDM. In vitro synthesis has 4 essential components: DNA template, modified primers, deoxyribonucleic nucleoside triphosphates (dNTPs [ie, dATP, dCTP, dGTP, dTTP]), and DNA polymerase (thermostable or thermolabile). The polymerase chain reaction technique utilizes thermostable DNA polymerases (eg, Taq, Pfu, and Vent), at least 2 primers, and multiple heating and cooling cycles (average 30 cycles). Each cycle has 3 phases: denaturation (approximately 95 degrees Celsius), annealing (about 55 degrees Celsius), and extension (about 72 degrees Celsius). The denaturation phase separates the template DNA molecule into 2 single-stranded molecules. The annealing phase is when the modified primer base pairs at the sequence of interest.

For PCR-based mutagenesis point mutations, nucleotide changes are introduced in the middle of the primer sequence.

- To create deletion mutations, primers must border the region of target DNA to be deleted on both sides and be perfectly matched to their annealing (or template) sequences.
- To create mutations with long insertions, a stretch of mismatched nucleotides is added to the 5' end of one or both primers, while for mutations with short insertions, a stretch of nucleotides is designed in the middle of one of the primers.

Lastly, the extension phase extends the annealed primers according to the template strand. The advantage of PCR is that it works more favorably with different DNA templates (ie, single or double-stranded DNA and GC-rich regions) and produces millions of copies of a target gene. The disadvantage is there is less sequence fidelity (ie, thermostable polymerases are more error-prone than thermolabile polymerases). The non-polymerase chain reaction technique utilizes thermolabile DNA polymerases, 1 primer, and a constant reaction temperature (eg, 37 degrees Celsius). The DNA template is denatured with an alkali solution or heat in this strategy. The template is annealed with the modified primer, and DNA synthesis occurs at 37 degrees Celsius. Though single or double-stranded DNA templates can be used, more favorable outcomes occur if a single-stranded DNA template is used (this increases the success of annealing). This process produces a hybrid DNA molecule (ie,1 template strand and 1 newly synthesized mutant strand), which can be transformed or infected into *E. coli*. Once in *E.coli*, the mutant DNA and wild-type DNA can be segregated. Non-PCR has greater sequence fidelity than the PCR method; however, only 1 DNA strand is generated (rather than millions).*16*[16]*



PCR-SDM

11.4. TALENS vs. Zinc Finger Nucleases

Various tools have emerged to facilitate precise genetic editing, including **TALENs** (Transcription Activator-Like Effector Nucleases) and **ZFNs** (Zinc Finger Nucleases), which operate using similar principles but differ in design and specificity. Both **TALENs** and **ZFNs** are engineered proteins designed to target specific DNA sequences, creating **double-stranded DNA breaks (DSBs)** at precise locations in the genome. These breaks are then

repaired by the cell's natural repair mechanisms, which can result in either **non-homologous end joining (NHEJ)** or **homologous recombination** (HR). NHEJ may lead to small insertions or deletions (indels), potentially disrupting a gene's function, while HR allows for the introduction of new DNA sequences.

Both TALENs and ZFNs consist of two major parts:

1. **DNA Binding Domain**: This part is responsible for recognizing and binding to the specific target DNA sequence.
2. **DNA Cleavage Domain**: This part, usually the FokI nuclease, cuts the DNA. FokI needs to dimerize (pair with another FokI molecule) to create a break in the DNA, meaning that two TALEN or ZFN proteins must bind on either side of the target sequence for DNA cleavage to occur *17*[17]*.

***Fondamental* : Differences in DNA Binding**

The key difference between TALENs and ZFNs lies in how they recognize their DNA target:

- **TALENs**: The DNA-binding domain in TALENs consists of **TALE (Transcription Activator-Like Effector) proteins**. Each TALE protein recognizes a single base pair in the DNA. This single-base recognition gives TALENs a high level of specificity because each protein can be designed to bind precisely to its target sequence.
- **ZFNs**: In ZFNs, the DNA-binding domain is composed of **zinc finger proteins**, with each zinc finger recognizing **3-4 base pairs**. Because zinc fingers recognize multiple bases at once, ZFNs can be less specific than TALENs, especially when recognizing sequences in which zinc fingers target overlapping base pairs.

12. Bioengineering applications

Directed and Site Directed mutagenesis is a powerful technique used in biological engineering to make specific, intentional changes to the genetic material of an organism. This method allows scientists to understand gene function, modify metabolic pathways, create novel proteins, or enhance desirable traits in microbes, plants, or animals. The precision of directed mutagenesis makes it indispensable in fields like synthetic biology, biopharmaceuticals, and agriculture.

(cf. 5-Site%20directed%20mutagenesis.pdf)

(cf. A_practical_guide_to_DNA_extraction_final_.8183727.pdf)

13. Exercice : Site-Directed Mutagenesis (SDM) is .....

[solution n°4 p. 15]

Define SDM

- ☐ A technique to create random mutations in DNA
- ☐ A method to create specific changes in DNA sequences
- ☐ A process to introduce mutations at defined sites in a DNA molecule
- ☐ A technique to remove all mutations from a DNA sequence

14. Exercice : Cassette mutagenesis characterized by

[solution n°5 p. 16]

Proprieties of CM

- ☐ using a short double-stranded oligonucleotide
- ☐ using multiple mutations in a single gene cassette
- ☐ requiring primer extension by DNA polymerase
- ☐ replacing a fragment of target DNA

15. Exercice : What are the requirements for primers in SDM?

[solution n°6 p. 16]

SDM experiment

- ☐ The melting temperature should be >78°C
- ☐ The mutation should be at the end of the primer
- ☐ The primer must contain the mutation
- ☐ The primer should be 24-25 nucleotides long

Solutions des exercices

Solution n°1

[exercice p. 5]

Define the mutation

- ☐ A deliberate change in DNA sequence
- ☐ A process of creating new genes
- ☑ A random and permanent change in DNA sequence
- ☐ A temporary change in protein structure

Solution n°2

[exercice p. 5]

Type of mutagenesis

- ◉ Reverse mutagenesis
- ○ Random mutagenesis
- ○ Insertional mutagenesis
- ○ Site-directed mutagenesis

Solution n°3

[exercice p. 6]

A mutation | mutagenesis | is | is | induced | a permanent | artificially | while | in | change | typically | the DNA sequence

A mutation is a permanent change in the DNA sequence while mutagenesis is typically induced artificially.

Solution n°4

[exercice p. 13]

Define SDM

- ☐ A technique to create random mutations in DNA
- ☑ A method to create specific changes in DNA sequences
- ☑ A process to introduce mutations at defined sites in a DNA molecule
- ☐ A technique to remove all mutations from a DNA sequence

Solution n°5

[exercice p. 14]

Proprieties of CM

- ☑ using a short double-stranded oligonucleotide
- ☑ using multiple mutations in a single gene cassette
- ☐ requiring primer extension by DNA polymerase
- ☐ replacing a fragment of target DNA

Solution n°6

[exercice p. 14]

SDM experiment

- ☐ The melting temperature should be >78°C
- ☐ The mutation should be at the end of the primer
- ☑ The primer must contain the mutation
- ☑ The primer should be 24-25 nucleotides long

Glossaire

Polymerase chain reaction (PCR)

A method widely used to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to amplify a very small sample of DNA (or a part of it) sufficiently to enable detailed study.

Abréviations

DNA : Deoxyribonucleic acid ; is the molecule that carries genetic information for the development and functioning of an organism.

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