Transformation

Goals

1. Get to know what plasmids are as well as their characteristics
2. Get to know the GFP gene and its uses as a reporter gene in research
3. Get to know that Lac and Arabinose operons as examples of inducible operons
4. Get to know transformation strategies

Keywords

1. Plasmid
2. ORI- origin of replication
3. Polylinker
4. Antibiotic resistance
5. GFP
6. Reporter gene
7. Induction
8. Transformation
9. Competent cells
10. Selective growth mediums
11. Plating bacteria

Summary

GFP is a green fluorescent protein when exposed to light with wavelengths from blue to ultraviolet. The gene for GFP was isolated from a type of jellyfish. The protein is used a lot in genetic engineering, medical research, and more. GFP can be used as a reporter gene by attaching it to a sequence for a certain protein, in the DNA, so that the GFP will be translated attached to the desired protein, enabling us to follow its location easily.

An operon is a DNA sequence which contains a few genes, one after the under, under the control of a promoter. A promoter is a regulatory sequence usually upstream from the gene, which defines the conditions under which the gene will be expressed. GFP can be cloned under promoters of certain operons in order to help us characterize their regulation characteristics.

A plasmid is a circular sequence of DNA which bacteria can pass from one to another. In genetic engineering, desired genes are placed on a plasmid and then inserted into bacteria. Transformation is the process of inserting a plasmid into an organism. Two main transformation procedures are used: electroporation and heat shock.

Transformation- Technion iGEM HS

In the lab, we will insert a plasmid containing GFP into bacteria by performing transformation be heat shock.

Lesson Plan

1. Review of the topic "genetic engineering" from last week
2. Plasmid characteristics
3. GFP in genetic engineering
4. GFP as a reporter gene
5. GFP in research
6. Gene regulation- Lac and Ara operons
7. Gene expression using the pGLO plasmid
8. Transformation- introduction, techniques, steps

Supplementary Material for the Teacher

Slide 2- In all living things, the information coding their traits is found in the same material- DNA. In living cells, there are proteins, and the information for the creation of those proteins is found in the DNA.

Slide 3- How do we do genetic engineering? There are many tools which can help us genetically engineer organisms. Here are some examples:

- Viruses: viruses don't only cause sickness. Viruses effectively inject their DNA into cells. In genetic engineering, we can use viruses to inject the desired genes into cells.
- Genetic Enhancement: PCR- by using special enzymes, DNA can be replicated many times, giving us a large amount of the desired gene.
- Restriction Enzymes: enzymes which can cut DNA at specific points, based on the base pair sequence at those points. This allows us to cut certain genes.
- Plasmids: bacteria have circular DNA called plasmids, which they can copy and transfer amongst themselves. In genetic engineering, genes can be places on plasmids which can then be inserted into bacteria.

Transformation- Technion iGEM HS

Slide 4: A plasmid is a double stranded sequence of circular DNA which replicates independently of the cell. There are 3 important characteristic of plasmids which are required in order to use them for genetic engineering.

- ORI (origin of replication)- an area which gives the start signal for the replication mechanism of the bacteria.
- Polylinker- an area which contains many restriction sites which enables the desired gene to be inserted there. Restriction sites are DNA sequences which restriction enzymes can identify and cut. In order to insert our gene into a plasmid, we cut the plasmid using restriction enzymes, and "past" our gene into the site using ligation enzymes.
- Antibiotic resistance gene or another gene that allows us to easily identify the bacteria which receive our plasmid.
- In nature, plasmids contain genes which are not always crucial for the survival of the bacteria under regular condition. However, they may help the bacteria survive in unusual conditions, as does a plasmid containing an antibiotic resistance gene.
- When pathogenic bacteria become resistant to antibiotics, medical challenges can be posed since the bacteria cannot be eliminated with the help of regular antibiotics.
- With genetic engineering, we take advantage of the antibiotic resistance in order to select only bacteria which received our desired plasmid. The gene of interest is placed on a plasmid containing antibiotic resistance, and then the plasmid is transformed into the bacteria. We will grow the bacteria on a medium with the appropriate antibiotics. If they grow, we know they received the plasmid and the target gene.

Slide 6:

In our lab, we will practice this process by inserting the GFP- green fluorescent protein- gene into a plasmid, and transforming the plasmid into bacteria. The gene fluoresces in green when exposed to blue to ultraviolet wavelengths. The protein was isolated from a type of jellyfish. It is used in much biological research such as in bacteria, plants, fish, rats, and more.

Transformation- Technion iGEM HS

Slide 7:

Usually, a gene which codes a protein ends with a STOP codon which symbolizes to the translation mechanism to stop making the protein. A scientist named Douglas Prasher thought of the idea of using GFP as a reporter gene. The sequence of bases which codes for GFP can be added immediately after the gene of a protein of interest, but before the STOP codon in the DNA. As a result, the translation mechanism will translate the protein attached to GFP before it stops the translation. Since the GFP fluoresces, we can use it to see what happens to proteins within a cell.

Slide 8:

All living creature contain information for traits coded in their DNA. The GFP is easy to see when lit with light between blue and UV wavelengths, and therefore we can know where the protein is located in the cell and follow it. Since the GFP protein is small, it usually doesn't interfere with the activity of a protein of interest when attached to it. GFP fluoresces without needing the addition of any other materials. Other fluorescent molecules may require the addition of a chemical in order to see them. Because of these advantages, GFP is an ideal reporter protein.

Slide 12:

Before the DNA sequence coding a gene, there is a promoter- as short sequence of DNA found before the gene. The replication mechanism of the cell identifies the promoter and begins transcription of the gene. The mechanism begins the transcription process under certain conditions defined by the promoter. The promoter therefore regulates the expression of the gene.

There are a few ways a gene can be expressed:

- Constitutive- the promoter defines that the gene will always be expressed
- Inducible- the promoter defines that the gene will be expressed when there is a certain environmental signal
- Silenced- the gene will stop being expressed when there is a certain environmental signal.

Every cell in our body contains DNA which codes for all our genes, but each cell has a different purpose and not every protein is expressed in every cell and at all times. The promoters define if and when cells will express each gene. We can create DNA

sequences containing GFP under certain promoters, and use them to learn about the natural gene expression under these promoters. The GFP will be expressed instead of the original gene, and we will be able to identify where and when the original gene is expressed.

Slide 13:

An operon is a DNA sequence which contains a number of genes, one after the other. Usually, these are genes which are found in a common biochemical process. These genes are regulated under one promoter.

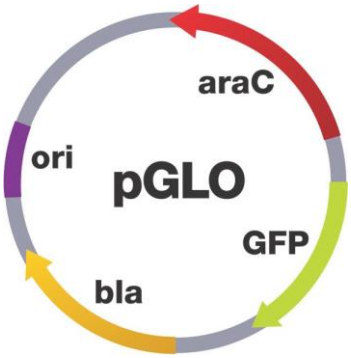
In the slide, there is an example of an operon and its regulation- the lactose operon (Lac operon). This was the first operon to be discovered. Bacteria can use lactose as a food source. In order to use the lactose as an energy source, however, they must synthesize certain enzymes. The gene for these enzymes are found on the Lac operon in the genome, under the Plac promoter. In the absence of lactose, a protein called a "repressor" blocks the DNA from being transcribed, and the genes on the operon are not expressed. In the presence of lactose, the lactose removes the repressor protein and enables the transcription of the genes which code the enzymes which enable the bacteria to break down lactose.

Slide 14:

In this system, the operon is the arabinose operon which works in a similar way as the Lac operon. The arabinose operon contains genes which code enzymes which help bacteria use arabinose as an energy souce. The operon is found under a promoter which is activated in the presence of arabinose. In the absence of arabinose, the repressor protein araC attaches to the promoter pBAD and prevents the RNA polymerase (the transcription enzyme) from identifying the pBAD, and therefore prevents the transcription of the enzymes which enable the use of arabinose. In the presence of arabinose, the arabinose attaches to the repressor and causes a conformation change so that it no longer covers the promoter. The RNA polymerase can then attach to the pBAD promoter and cause the expression of the enzymes encoded in the arabinose operon.

Slide 15:

We can take the GFP gene and place in under the pBAD promoter. This synthetic operon does not exist naturally. If we transform this synthetic operon into bacteria, they will produce GFP only in the presence of arabinose. Since the pBAD promoter causes a lot of transcription of the gene following it, we will get a lot of GFP.



Slide 17:

In our experiment in the lab, we will transfor a pGLO plasmid containing the following into bacteria:

- ORI- the origin of replication of the plasmid
- An Ampicillin antibiotic resistance gene
- The GFP gene under the pBAD promoter
- The araC gene for the pBAD repressor

Slide 18:

The transformation process: It should be noted that transformation is a process which exists naturally among bacteria. Bacteria can "share" their plasmids. However, the frequency of this process is rather low. People take advantage of this phenomenon in order to insert genes of interest into bacteria by building a synthetic plasmid containing the desired genes. In the transformation process, we take competent bacteria (bacteria which underwent a treatment which causes their membranes to be temporarily permeable to DNA). We add the plasmid DNA carrying the desired genes, and plate the bacteria on a selective medium. Selective mediums are used in order to ensure growth of only the desired bacteria (which received the plasmid during the transformation). Other bacteria will die because of the antibiotics. In our case, the selective medium will contain Ampicillin antibiotics since the pGLO plasmid will contain the Ampicillin resistance gene.

Question for the students: What other material will be needed in the growth medium?

Answer: Arabinose.

Transformation- Technion iGEM HS

Slide 20:

There are a few ways to cause bacterial membranes to be permeable to plasmids.

Electroporation- an electric shock which cause temporary, small holes in the bacterial membrane. These holes close naturally after the shock by natural mechanisms of membrane repair.

Calcium chloride and heat shock- the bacteria are surrounded by calcium chloride and the plasmid DNA and rotate from cold to hot conditions.