8

Microbiology

Doctor 2017 | Medicine | JU

Sheet Slides

Done By

Rawan Almujaibel

Contributed in the Scientific Correction

...

Contributed in the Grammatical Correction

...

Doctor

Anas Abu-Humaidan

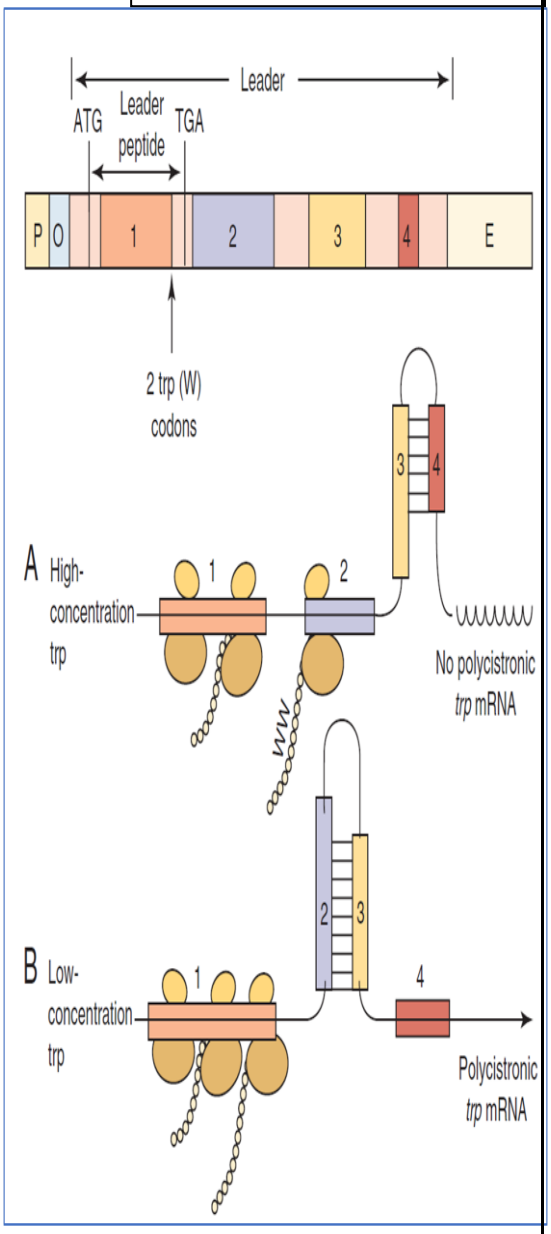
In the previous lecture the Dr. talked about DNA structure and their 4 types of nitrogen bases. Then he talked about bacterial DNA (chromosomes) and their replication process. Then, we ended up our lecture by talking about Models of DNA replication, DNA mutations and their repair mechanisms.

Note no.1: The Operon is (the whole structure "regulatory + coding gene)

Regulation of transcription

We will be continuing talk about attenuation, as we talked about it so briefly last lecture.

Attenuation is a regulatory mechanism of some biosynthetic pathway (eg. The tryptophan "trp" biosynthetic pathway) that controls the efficiency of transcription after transcription has been initiated but before mRNA synthesis of operon genes takes place, especially when the end product of the pathway is in short supply.



We will be taking tryptophan as an example to clarify the attenuation process. The *trp* operon contains structural genes for the synthesis of tryptophan when needed, and is under the control of an attenuation-antitermination mechanism. Upstream of the structural genes are the promoter, the operator, and a leader (shown in figure) which can be transcribed into a short peptide containing two tryptophans *(W),* near its distal end. The leader mRNA possesses four repeats (1, 2, 3, and 4), which can pair differently according to the tryptophan availability, leading to an early termination of transcription of the *trp* operon or its full transcription. In the presence of a high concentration of tryptophan, regions 3 and 4 of the leader mRNA can pair, forming a terminator hairpin, and no transcription of the *trp* operon occurs. However, in the presence of little or no tryptophan the ribosomes stall in region 1 when translating the leader peptide because of the tandem of tryptophan codons. Then regions 2 and 3 can pair, forming the antiterminator hairpin and leading to transcription of the *trp* genes.

If the tryptophan is severely lacking, the ribosome stalls when it encounters 2 trp codons in the mRNA, because tRNA with tryptophan are in short supply. The stalled ribosome allows the 2-3 stem loop to form. The 3-4 stem loop will not form and transcription continues. However, if we have high amount of tryptophan the 3-4 stem loop is formed so the transcription is stopped.

Initiation of tryptophan synthesis can also be controlled by a repressor. When tryptophan is present, it binds to repressor and together they bind in the operator. RNA polymerase can't move pass repressor molecule and no transcription occurs. In low tryptophan environment, the repressor does not bind to the operator and transcription begins (that is happening in normal situation).

This video might help you to more understand:

https://www.youtube.com/watch?v=RQrdY07JkFU

Horizontal Gene transfer (HGT)

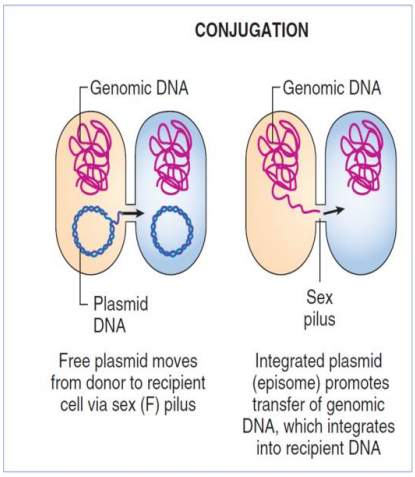
Horizontal gene transfer is transferring DNA from one organism to anther in a horizontal way and that DNA can be stably incorporated in the recipient permanently changing its genetic composition.

It is different from the vertical inheritance transfer as it is inheritance of parental gene, so it is passing genetic material from parent to daughter cells.

There is no parental relationship between the two organisms which are sharing the gene.

There are 3 broad mechanism mediating efficient movement of DNA between cells under (HGT):

1. **Conjugation:**
   It requires donor cell-to-recipient cell some physical contact to transfer only one strand of DNA. The recipient completes the structure of dsDNA by synthesizing the strand that completes that strand acquired from the donor.



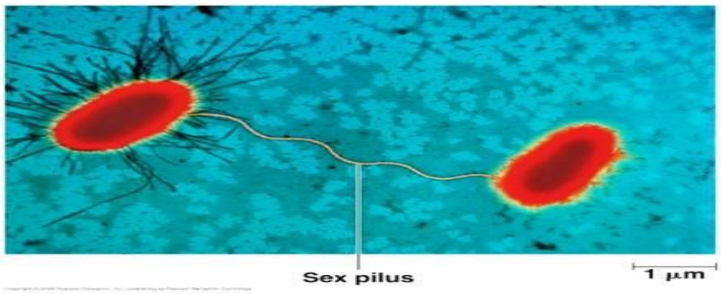
The Mechanism:

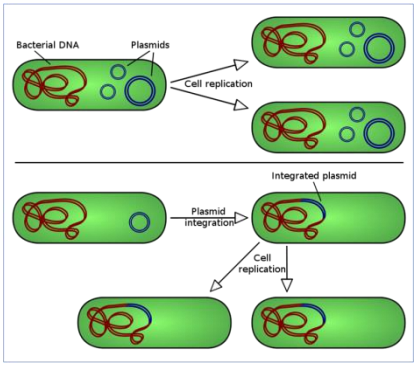
The donor cell produces sex pillus and extends their pillus to the recipient cell. Afterward, the donor cell retracts the pillus to bring the cells into close contact, and a pore forms in the adjoining cell membranes. Then, a single stranded of the plasmid (it is a circular DNA that is outside of the chromosomes DNA) is transferred to the recipient through the pore. In another form of conjugation, Part s of the genomic DNA is moving from donor to the recipient with the plasmid.

*the genetic material that can be replicate by itself called "replicon" and usually refers to the chromosome and the plasmid.

Plasmids:

It is a small circular DNA molecule (1-200 kbp, it is coding for at least one gene) within a cell that is physically separated from a chromosomal DNA and can replicate independently. Plasmids can integrate themselves in chromosomal DNA using enzymes that cut the DNA and integrate itself within bacterial DNA. Sometimes, a plasmid which can incorporate into a chromosome is referred to as an episome.





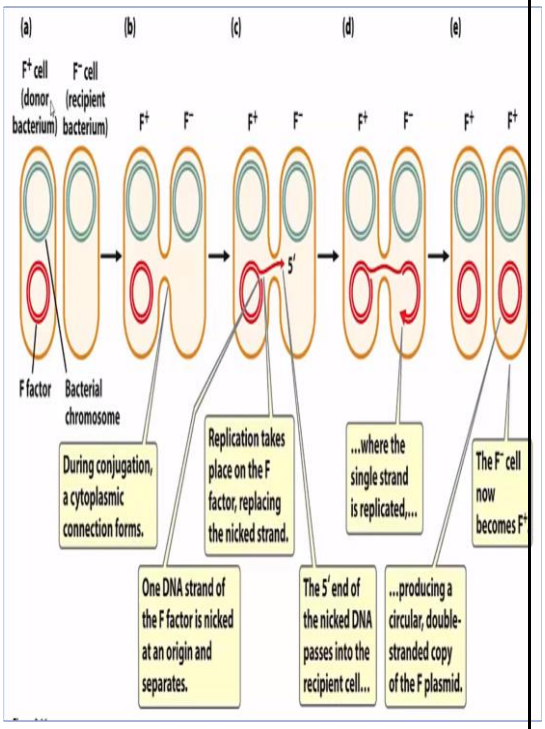
Plasmid are not essential for bacterial life, however they are usually beneficial for the bacterial cell. For example, they can carry genes that code for antibiotic resistance or virulence factor (increase the ability of bacteria to cause disease).

Plasmids can be classified by:

- Their ability to initiate conjugation: either it contains genes important to initiate conjugation (e.g. sex pilus formation) or not.
- Their compatibility (if they can exist and propagate in the same cell, they are in the same compatibility group, some plasmids can not be in the same cell as another plasmids, In the other word, if the introduction of a second plasmid negatively affects the inheritance of the first, the two are considered to be incompatible.

- Their functions: if they code for antibiotic resistance, or virulence factors.

Examples

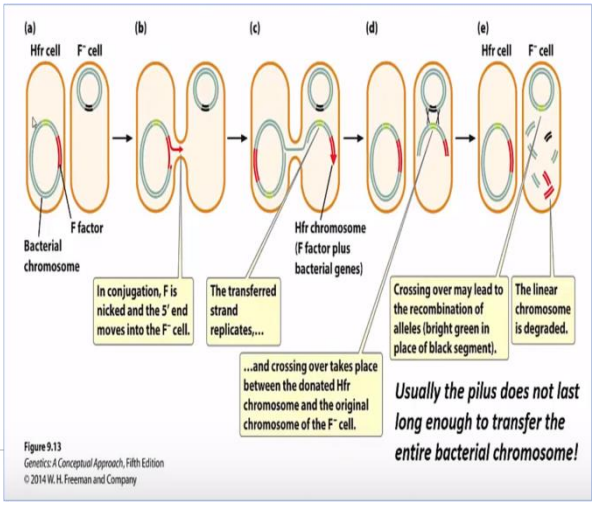


As you can see in the picture is the mechanism of plasmid mobilization, there is a donor bacteria that have F factor (stand for fertility factor) we call it "F+ plasmid" and the recipient cell called "f- plasmid" as it must not have F factor inside it.

1- During conjugation a cytoplasmic membrane connection is formed.
2- Then F factor is cut or nicked at a specific region called origin of transfer by a protein assembly and separates.
3- Afterwards, replication is taking place on the F factor, so it replaces the nicked strand.
4- The 5' end of the nicked DNA passes into the F- cell.
5- In the F- cell the single strand is replicated producing a circle, double strand copy of the F plasmid.
6- Now the F- becomes F+.

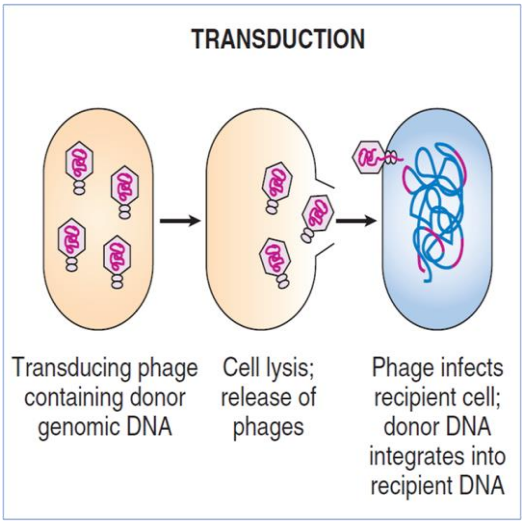
When the microbiologists did those experiments between donor and recipient bacterial, they found that in the recipient bacteria were not all of them became F+ and at the same time they still have some recombination in their genes, if they didn't get the F factor how they did get their recombination?.

The microbiologist found that the F factor can work in a different way from the above mechanism. So, the other way is it's integrated within the bacterial chromosomes we will get another type called High frequency recombination. In this way the F factor still is coding for the same things as for the pervious mechanism, however the mechanism is slightly different.



The mechanism is:

1- After being integrated in the bacterial chromosomes the cytoplasm membrane connection is formed with F- cell.
2- Then, one DNA strand is cut at the F origin the free 5' end moves through the transfer pore, while the chromosome replicates, the donor strand is replicated as it enters the recipient (Hfr).
3- When the cells separate the donor cell chromosome is restored in the recipient integration events may lead to recombination of gene alleles (by crossing over) and Linear DNA is eventually degraded.
4- When many HFR and F- cell are mixed, conjugating pairs can form quickly.



NOTE: usually the pilus does not last long enough to transfer the entire bacterial chromosome.

Coming back to the 2nd mechanism of DNA transfer is transduction …

1- **Transduction:**
It is a phage –mediated genetic recombination in bacteria. In the simplest terms, a transducing phage is containing donor genomic DNA inside the cell. When the cell lyses, the phages is released, then the phages insert or integrates the genes into the recipient cell DNA.

An example is bacteriophages (are viruses that infect bacteria). They have 2 life cycles are called lytic cycle and lysogenic cycle:

First of all:

1- The phage attaches to the bacterial cell.
2- Then, the phage is injected the cell with viral DNA.
3- The phage DNA inside the bacteria cell is circulated

"After injecting the DNA the cell can go 2 ways either to lytic or lysogenic cycle".

Lytic cycle:

4- Biosynthesis of viral nucleic acid and protein ocuurs.
5- Viral component are assembled into virions.

6- The plasma membrane breaks (lyses) and the virions is released from the host cell.

Lysogenic cycle:

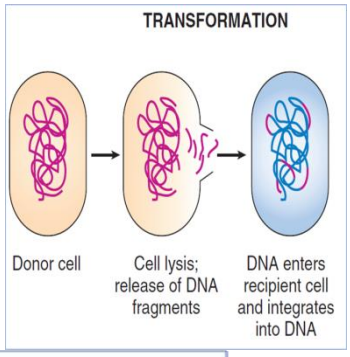
4-When the phage DNA integrated into the bacterial chromosome, it becomes a prophage.

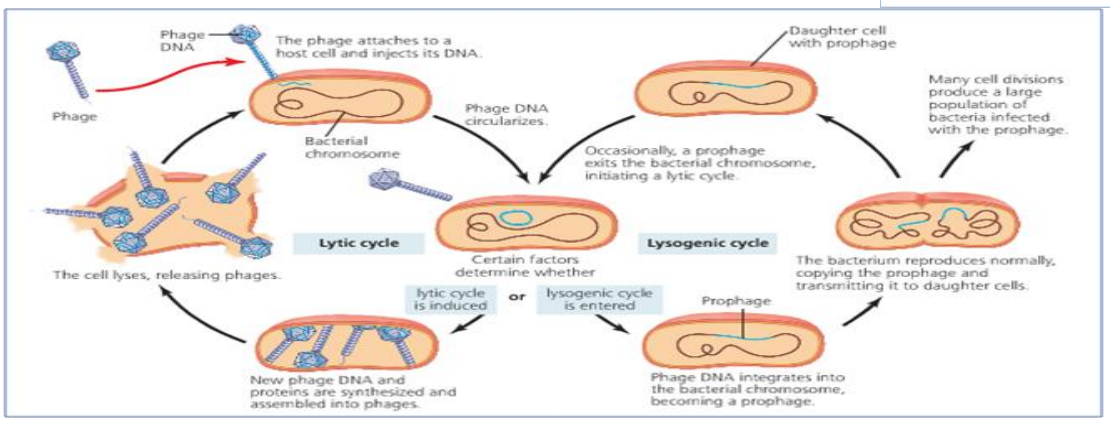
5-The bacterium reproduces normally copying the prophage and transmitting it to daughter cells.

6-Many cell divisions produce a large population of bacteria infected with the prophage.

7-daughter cell with prophage is produced now, Occasionally, a prophage exits the bacterial chromosome, initiating a lytic cycle.

In real life and in researches transduction is a helpful tool to use to get a specific product or DNA.





2- Transformation:

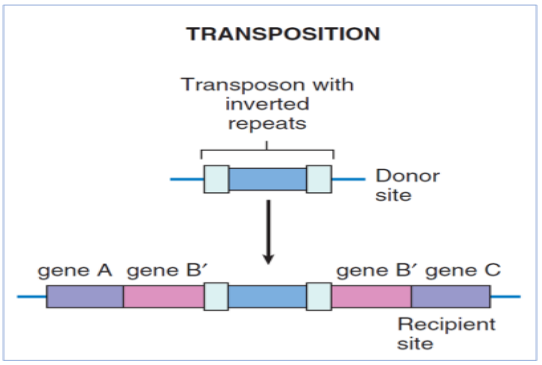
It is a genetic alteration of a naked cell resulting from the direct uptake or integrates and incorporation of exogeouns genetic material from it surrounding through the cell membrane into DNA.

Some bacteria are competent to uptake genetic material without any help by nature. However, other bacteria need to be induced to do so, like using some certain chemicals or heats in experimental environment (in genetic engineering).

Discovery of transformation transfer of DNA:

In 1928, an experiment has been done by a researcher called Griffith. He observed that virulent (causes a disease) Streptococcus bacteria in mice, when heat-inactivated and mixed with a non-virulent strain, could "transform" the non-virulent strain and make it virulent.

What he did is he brought 2 strains of streptococcus bacteria one of them virulent and the other is non- virulent strain.

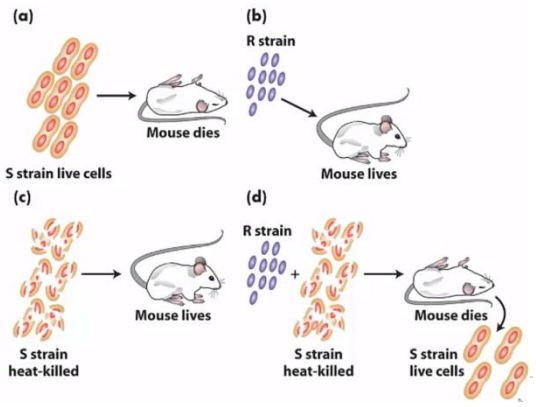


So, when he injected the mice with virulence strain they would die and when he injected them with other type they survive. Afterwards, he heated the virulent bacteria (thereby killing the bacteria) and injected again into the mice and the mice survived. However, when he mixed the heat killed virulent bacteria with normal strain the mice died. And the bacteria recovered from the dead mice was virulent. From this experiment he deduced that DNA released from dead virulent bacteria, was up taken by the non-virulent bacteria and transformed it.

3- Transposition:

It is transfer of a segment of DNA from one site to another site in the genome. The DNA segments that can move are called transposons. Transposons code for transposases that can cut the transposon from its place in the DNA, and ligate it in another place in the genome.

Transposons are found in eukaryotes as well and not only in bacteria.



The implication of HGT:

As previously discussed, transfer of antibiotic resistance genes and virulence factors can take place through HGT, creating bacteria that can be resistant to several antibiotics, and can cause severe disease or epidemics. HGT can take place between different bacterial species.

HGT can be a useful tool in the lab as well, because genetic engineering allows for manipulation of plasmids and then insertion of such plasmids into cells to carry out

An example of bacterial resistance:

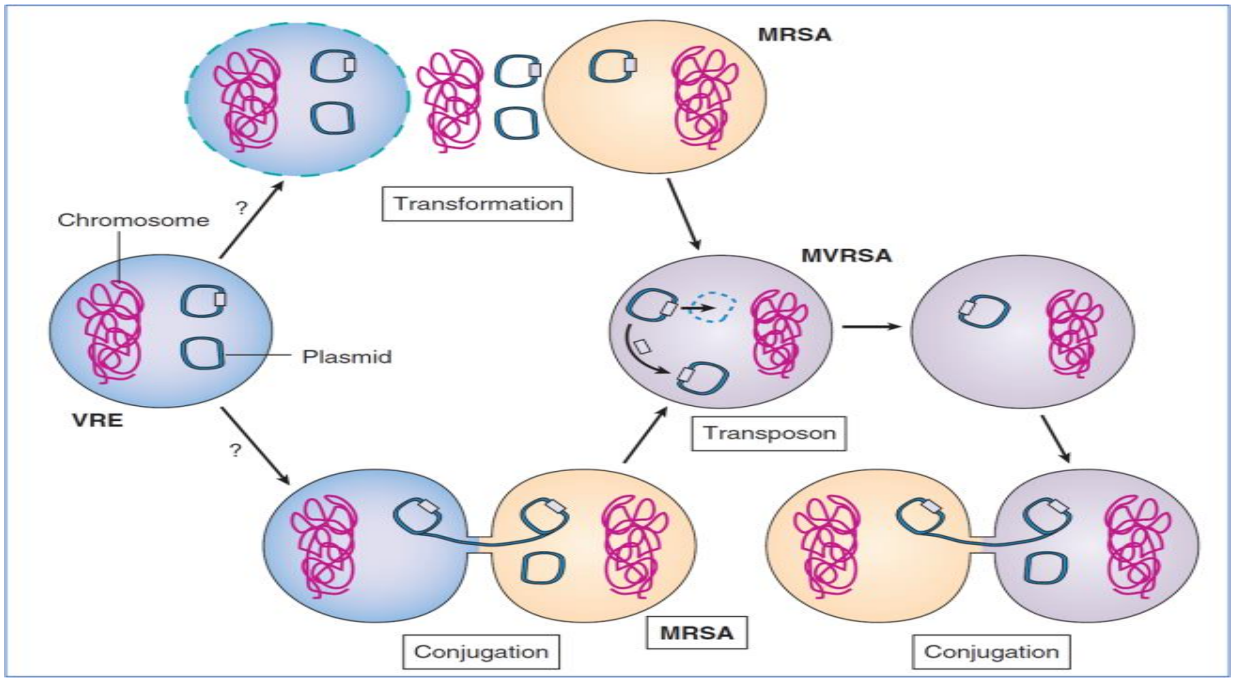
Methicillin-resistant Staphylococcus Aureus (MRSA) it is resistance to Methicillin. It has been a big scare in the medical field; especially it is transmitted very rapidly in the hospital environment. So that, they discovered Vancomycin is a certain antibiotic that is used against MRSA. Recently the researchers have noticed that MRSA has developed a resistance against Vancomycin as well.

The researchers found that's the reasons behind MRSA is continuing developing resistant to antibiotics are conjugation and transformation transfer for the genetic material.

Explanation:

In a case of co-infection with MRSA and VRE, they co-infected some host and then either through transformation which means that VRE died and it released its DNA, one of its plasmid that contains the antibiotics resistance (the multi-drug antibiotics resistance), was up taken by MRSA, and because it has transposons (the jumping gene), the genes responsible for vancomycin resistance moved into another plasmid in MRSA. The new version of MRSA now in called VRSA (Methicilin and Vancomycine resistance S. Aureus).

There is another mechanism can be used, which is happening through conjugation where simply the plasmid moves from VRE into MRSA, it inserts itself in the MRSA plasmid to make new type of bacterial resistance called VRSA. The cycle will continue, the resistant bacteria is conjugating with other bacteria and so on. Leading to spread of antibiotic resistance.



Another implication:

It is used as a tool in the laboratory so we manipulate it to use for our own purposes, for example:

In patients with diabetes type 1, they need to take insulin from quite young ages to regulate glucose rate.

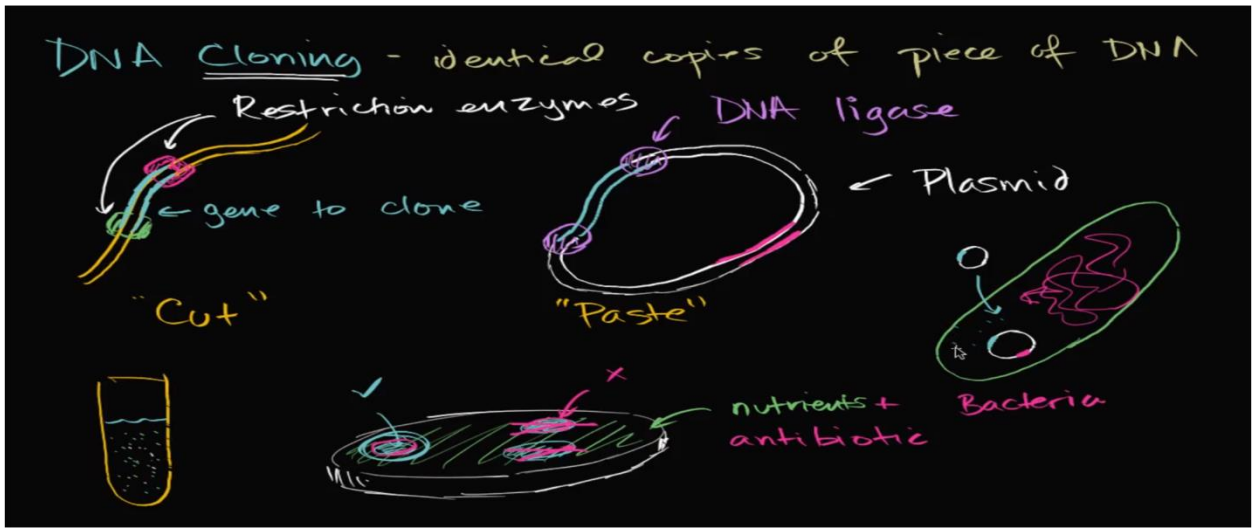
In the past, the only source to get insulin to treat patients is from animal pancreases (excrete insulin form secretary cells) and give it to patient as a treatment medication. It is not ideal and good way especially when we have a quite big population.

The researchers thought about another mechanism to produce more insulin by DNA cloning (getting 2 new identical copies of DNA from the old DNA). It is a mechanism of using HGT and by this we will be able to get big amount of insulin within cheap coast.

How does that happen?

Since we have a gene and we know their sequence to clone. So, we use an enzyme (restriction endonuclease enzyme) that helps us to cut the specific sequence that we need. Afterward, we paste the piece that we cut in another

location (plasmid) by ligase enzyme. Then, we have the plasmid with the gene that we replicate and inserted in a bacteria (e.g. E. coli).
We grow these bacteria in a plate with nutrients. Then we put it in a growth. Then, we extracted the insulin and use it for our purposes.



How do we know colonies make insulin we want, because as we know not all bacteria can take up the plasmid?

While we prepare the plasmid we added another type of gene to the plasmid (antibiotic resistance gene), because when we plate the bacteria in an antibiotic containing medium, only the one that is resistant to the antibiotics are the same one is contain the insulin gene.

BEST OF LUCK….

Some extra useful videos:

Lytic and lysogenic cycles of bacteriophages:
https://www.youtube.com/watch?v=hFwA0aBX5bE
Bacterial genetics part 1 (DR. recommended this video):
https://www.youtube.com/watch?v=DWUxB7oxwTE
Bacterial genetics part 2 (DR recommended this video):
https://www.youtube.com/watch?v=vqe6wVcr0vg