What is Synthetic Biology? Differences from Other Disciplines

Since our first look at synthetic biology has deepened considerably, we have summarized the relationship between synthetic biology and other disciplines.

1. Relationship with other fields of biology

Synthetic biology is a new discipline, and Tom Knight of MIT is known as the father of synthetic biology. The basis of synthetic biology are molecular biology, biochemistry, and genetic engineering, however, there is a crucial difference between these disciplines and synthetic biology.

Difference 1: The scale of genes handled is large.

In genetic engineering, for example, the scale of genes handled is small, as in the case of CRISPR, which changes a base here and there in a gene. On the other hand, in synthetic biology, the scale of genes handled is large, as if the entire genome is changed (see BioBuilder). In other words, genetic manipulation using plasmids and the like will become more important. Although it is a difficult operation, the dynamic nature of synthetic biology can be seen as its appeal.

Difference 2: Engineering Perspective

Synthetic biology is often considered from an engineering perspective. It is premised on the act of “creating,” as the engineer Tom Knight has said. Other aspects of biology are strongly based on the assumption of “making”. In synthetic biology, the general flow is from wet experiments to dry engineering considerations. In engineering considerations, for example, there is the interpretation of biological reactions using differential equations. I would like to study this area in the future.

2. Relationship with Organic Chemistry

Organic chemistry is a branch of chemistry that explores carbon-containing compounds. There are several relationships and differences between organic chemistry and organic chemistry.

Difference 1: Synthesis by living organisms or synthesis in the laboratory

Synthetic biology deals with biosynthesis, while organic chemistry deals with total synthesis in the laboratory.
The details will be discussed in next week’s post!

Difference 2: Enzymes

In synthetic biology, the emphasis in synthetic biology is on “which molecules are converted to which molecules in the body,” but in organic chemistry, the emphasis seems to be on “how do enzymes work and by what mechanism?” In organic chemistry, however, the emphasis seems to be on “how do enzymes work?”.

I have summarized many of the above, but there are many similar perspectives to discuss.

Next week, I will summarize again biosynthesis and total synthesis.
(Shoya Inoue)

Minimal Genome Project [Challenge the smallest (least) genome!]

Hi, this is Inoue. This time, I would like to summarize a study on the “Minimal Genome Project” submitted to Science in 2016. It has little to do with the natto kinase project that Grand Tokyo is currently working on, but I thought it looked interesting so I summarized it here.
Please stay with us until the end!

Overview

Paper Title
Design and synthesis of a minimal bacterial genome
Doi 10.1126/science.aad6253
This study was designed **to create a bacterium with the minimal genome found in nature**.

Research Background and Objectives

There are many different organisms in nature, each with a different genome size.
The first complete cellular genome sequence was reported in 1995. The human genome was fully decoded in 2003. Many organisms have vast amounts of genetic information, and synthetic biologists were interested in the question, **“What are the minimum number of genes necessary to sustain life?”** This, they thought, would enable a clear understanding of gene function and the design of efficient biological systems.

Method

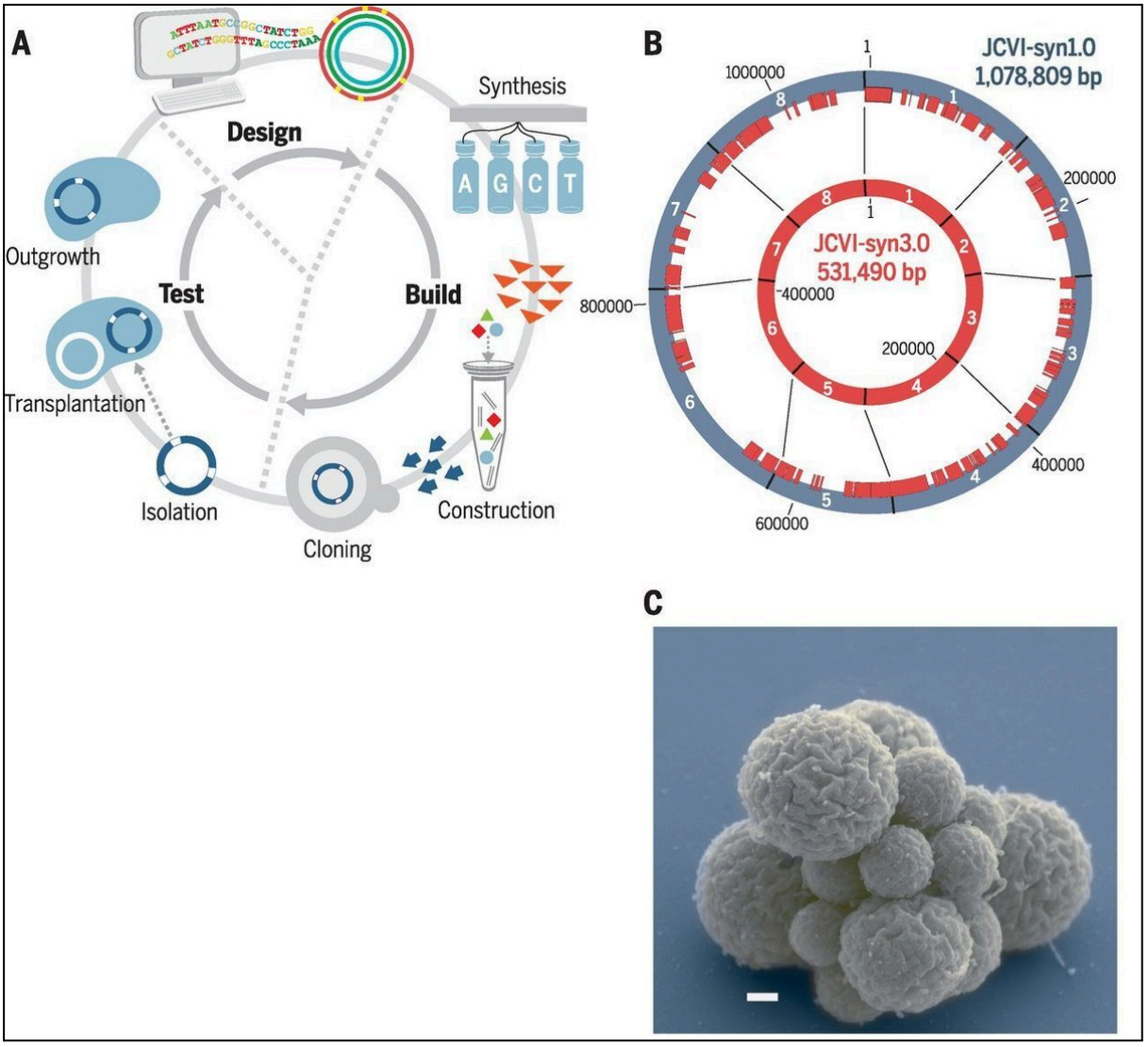
For this project, the authors first designed the minimum required genome based on the 525 genomes of an existing bacterium, **“Mycoplasma mycoides”**. In the process, they deleted genes from the existing genome that they considered unnecessary and observed the resulting effects. We also artificially synthesized bacteria with the minimum necessary genome and confirmed their viability.

Results and Findings

The investigation revealed that the minimum required genome size consisted of approximately 473 genes; this number was even smaller than the existing bacteria, which have the smallest genome in nature, in the organism named JCVI-syn3.0. Interestingly, **some of the deleted genes were considered important by conventional science.**

Huh? You don't want important genes? What does it mean?

This has given us new insights into the basic mechanisms of life.
It is possible that the parts that have been considered important may not be so important.



Four DBTL cycles (design, build, test, learn) **produced one with this genome sequence.**

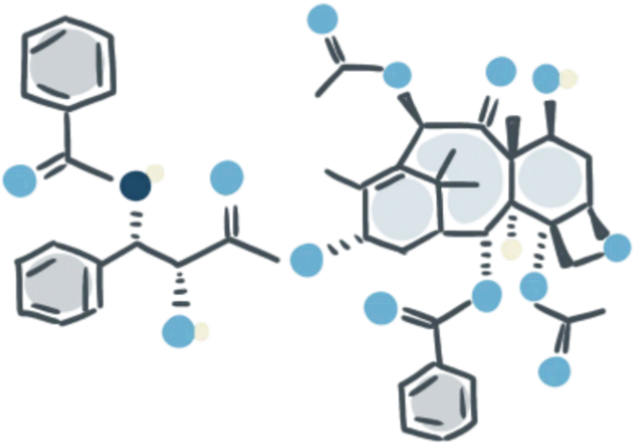
Application and Significance

This research is an important step in expanding the possibilities of synthetic biology. Organisms with minimal genomes are expected to have a variety of future applications. For example, they could be used to design biofactories with specific functions or as basic models in the development of new drugs in the medical field. This research may also provide new perspectives on the origin and evolution of life.

Summary

The Minimal Genome Project is an important study in understanding the basic building blocks of life and represents new possibilities for synthetic biology. The creation of bacteria with the minimum necessary genome answers fundamental questions in the life sciences and holds promise for future applications. This research will be positioned as an important milestone in the advancement of synthetic biology!
(Shoya Inoue)

What's the difference between total synthesis and biosynthesis?



This week, we will delve deeper into the chapter on the relationship between organic chemistry and synthetic biology, which I wrote about last week, whether to have organisms synthesize them or to synthesize them in the laboratory.
Please understand that this is a bit of a departure from synthetic biology.

1， What is total synthesis?

This may not be well known, so here it is in a nutshell.
Total synthesis is the synthesis of natural products and pharmaceuticals from commercially available raw materials (the cheaper the better). The goal is to synthesize large quantities of natural products that can only be obtained in trace amounts from nature. The research on total synthesis of Taxol, an anticancer drug ingredient, and TTX, a pufferfish poison, is particularly well-known.

2, Biosynthesis and total synthesis, and the advantages of each

In biosynthesis, natural products are obtained through biological reactions such as the metabolism of organisms. The structures of natural products are quite complex. Naturally, the total synthesis of these products requires large amounts of money, time, manpower, and chemicals. Biosynthesis can solve these problems. For living organisms, complex structures are a no-brainer! It reduces time, effort, and money. You don't need that many expensive chemicals. It is full of advantages. Furthermore, from the perspective of chirality (asymmetric synthesis), the advantages of biosynthesis are also significant.

On the other hand, the advantage of total synthesis is that **it can be used to make compounds other than those found in nature, depending on the method**. For example, it is possible to change this part of the structure. By doing so, we can discover unknown functions of natural products or create more beautiful structures of natural products. Another

key point is the rapid development of methodology! Total synthesis is a field that has seen tremendous development in the past few years, especially from a single paper.

3, Summary

Biosynthesis views living organisms as chemical factories, while total synthesis is produced from laboratory or actual factory plants.
It has been a long time since I last counted, and I am not sure that one of these two is better than the other, but I hope you recognize that both are necessary technologies to create the future.

Finally, allow me to advertise my field of expertise. Over the past 100 years, various organic chemical reactions and transformations have been developed. For example, the cross-coupling reaction developed by Kohei Tamao, Akira Suzuki, Eiichi Negishi, and many other researchers was used for the asymmetric synthesis of palitoxin and has since been used for the total synthesis of various natural products. In other words, total synthesis is the culmination of organic chemistry up to now. By studying total synthesis, one can learn organic chemistry more broadly.

If you are familiar with both total synthesis and biosynthesis, you can become a master of natural products, can't you?　(Text: Shoya Inoue)

Reference data

https://www.chem-station.com/blog/2014/02/-vs.html

Past nattokinase iGEM Projects

Top image: from the wiki of NYCU_Taipei (2021)

https://2021.igem.org/Team:NYCU-Taipei

This week marks the beginning of the Weekly iGEM serialization project that Inoue will be in charge of✨.

The first installment will be about past iGEM research on natto kinase.

Since we are focusing our research on Natto this year, we wanted to summarize one of our few past projects on natto, “Natto it Out.”

<Overview>

The NYCU-Taipei team for the 2021 competition focused on nattokinase, which may serve as a promising supplement to prevent cardiovascular disease. Since nattokinase is known as an excellent thrombolytic agent, this project aimed to eliminate deep vein thrombosis from society after studying the effects of nattokinase on deep vein thrombosis, among others.

<Learn more>

What is deep vein thrombosis?

→Thrombosis occurs when a blood clot forms in a vein in the lower extremity, causing pain, swelling, tenderness, redness, warmth, and impaired blood flow. It is a non-negligible disease that causes tissue necrosis, putting patients at risk of amputation.

Natto kinase is known to be a useful substance for preventing this deep vein thrombosis and has fewer side effects than other amino acid-based drugs!

Therefore, this team has developed,

① A compact system to detect D-dimer in saliva (a home-use test kit to test the absorbance intensity of d-dimer in saliva)

→ a product derived from the breakdown of blood clots and clinically used for early detection of the risk of deep vein thrombosis.

The device developed by their team is a system that calculates the risk of deep vein thrombosis via a smartphone app after testing D-timer levels from saliva and displays the patient's recommended nattokinase intake.

② E. coli bacteria that continue to produce nattokinase in the small intestine

Their team also used synthetic biology to design a gene-edited **E. coli Nissle 1917** that can produce improved production of nattokinase to help prevent the risk of deep vein thrombosis. Combined with the BphP1-QPAS1 light induction system, the production of nattokinase through the aforementioned application can be remote which can control the production of nattokinase remotely through the aforementioned application.

First, explaining the function and mechanism, E. coli Nissl 1917 is lyophilized to ensure long-term storage and delivered to the small intestine with an enteric-coated HPMC capsule. After the capsule dissolves, the E. coli Nissl 1917 product is released into the small intestine, where the E. coli product can form colonies in the small intestine and constantly release nattokinase. Finally, to ensure the biological safety of the product, a Mazeph toxin-antitoxin system is employed as a kill switch, which is designed to kill the E. coli in the body after a certain period.

How was it? It may have been somewhat difficult to talk about, but it is interesting to note that past iGEM team projects have solved social issues with such amazing ideas and technologies! (Shiori Kajikawa)

The basics of DNA engineering, Dip in toes in BioBrick・Gibson Assembly!

DNA engineering is an important technology that forms the basis of synthetic biology. In this article, I will summarize the basics of DNA engineering based on what I covered in my biology class last year.

1. Creating DNA?

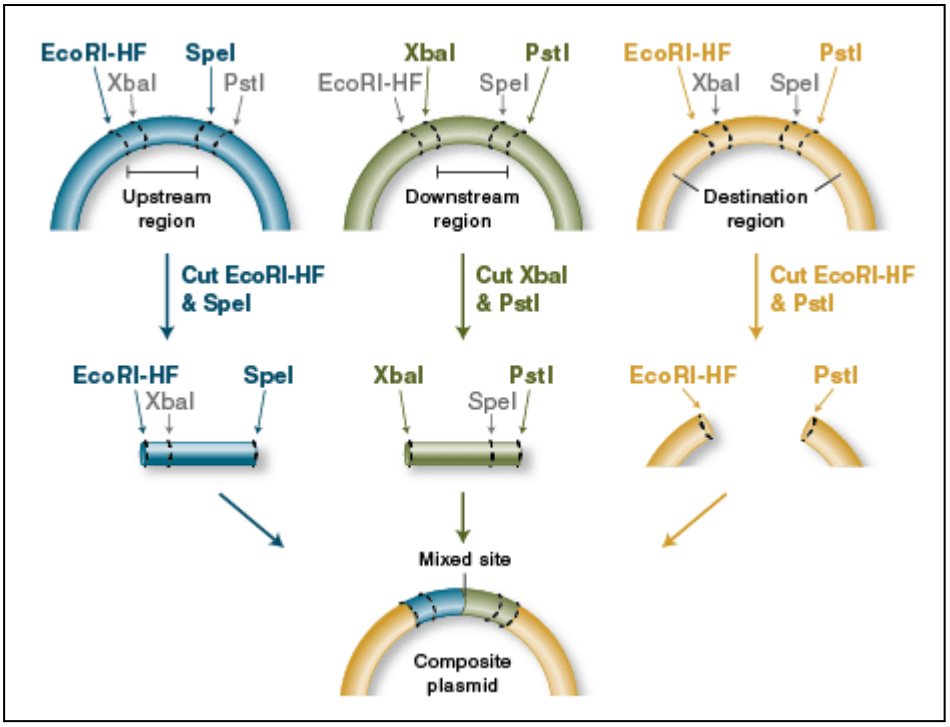
DNA is one of the most popular nucleic acids in biology, consisting of deoxyribose, phosphate, and four types of bases (A, T, C, and G), which form a double helix structure. Research on the creation of such DNA has been conducted for a long time. The technique is **a process called DNA assembly**, which is an approach to recombining genes. DNA created using this method is called rDNA (recombinant DNA).
The rDNA is then incorporated into the cell using a plasmid (cloning). The DNA fragments required for assembly are synthesized using **the PCR method**. Here, the temperature is varied from 95°C to 60°C to 72°C. The first step is to cleave the hydrogen bonds between the bases. First, hydrogen bonds between the bases are cleaved, then primers are attached to the nucleotides, and the nucleotides are synthesized.

2. how to assemble?

In this chapter, we will explain the assembly technique of how to incorporate the gene of your choice into the plasmid. The main methods are as follows.

α BioBrick Assembly
β Gibson Assembly

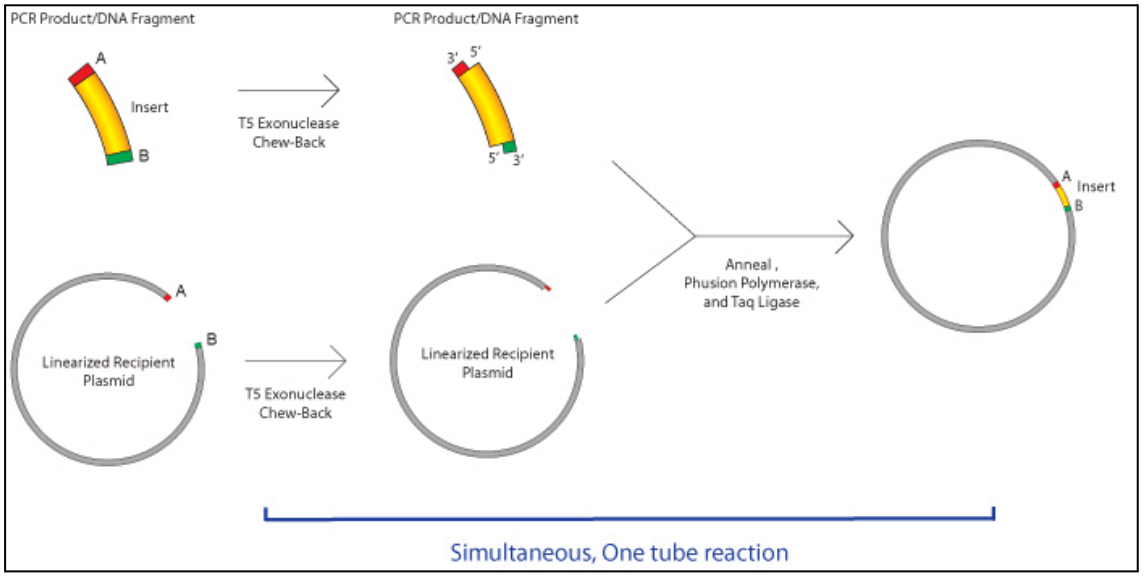
In α, a restriction enzyme is used to cut the gene in the region of the plasmid to be incorporated, and the desired DNA fragment is then incorporated into the gene using DNA ligase.



From the NEB website

However, the disadvantage of this method is that many DNA fragments cannot be assembled at once.

For those of you who are having trouble with this, I recommend Gibson Assembly! Well, this one uses an enzyme called exonuclease to shorten one of the DNA fragments in each DNA fragment and assemble them. One of the advantages is that you don't need restriction enzymes or DNA ligase.



From Addgene website

The price you are wondering about! What a surprise, it is cheaper than BioBrick Assembly. Many fragments indeed need to be made by PCR, but the amount of restriction enzymes floats away! Please consider introducing this system!

3. Summary

In this article, I have described how to do DNA assembly and the PCR method. I hope that you have gained some understanding of the basics of DNA engineering. (A bit of a joke, but…) In the next article, I will explain how to clone this plasmid into cells. (Shoya Inoue)

What is important for a Japanese team to win the Grand Prize?

～The Day Japan Becomes an iGEM Powerhouse～

It is no exaggeration to say that the Grand Prize is what all iGEMers are looking for. Last year, Japan United became the first Japanese team to win the Grand Prize. Our team is aiming to follow suit and win the Grand Prize, but what is important and necessary in the end? In this article, we will analyze the importance and necessity of the Grand Prize.

1. How does it contribute to the real world?

One of the characteristics of most biological research is that it contributes to society easily and quickly. In the case of chemistry, physics, and mathematics, depending on the field, direct application to society is not easy (proving Fermat's Last Theorem does not necessarily make society a better place), and applications to medicine, engineering, pharmacology, and agriculture take 20 to 30 years after some basic research is completed. In other words, the first thing to consider in synthetic biology research is how to contribute to society. This is the first thing to consider in synthetic biology research.

The research that won the Grand Prize last year was (supposedly) a study on drugs for depression. The research that won the Grand Prize last year was on drugs for depression (as it should have been), and the research before that was on early screening for coronary artery disease. However, teams above a certain level will choose a theme that makes a significant contribution to the real world. What other points will make the difference?

2. Is the research to some degree complete?

It may be obvious, but as long as the research is submitted to the public, a certain degree of depth of consideration and completeness is required. This point is easy to understand since many of you are involved in GSC (Global or private research outside of iGEM.

In other words, no matter how noble the theme, research by a team that has not completed its work is often not highly regarded. At the Jamboree a few years ago, a European team's theme was so good that I am sure the judges were astonished. However, the result was not complete, because the perfection of the theme was insufficient. (Sorry, this information is not complete, as I saw it a long time ago.)

This is why the level of perfection is important. I will try my best in my future experiments to achieve a high level of perfection in my research.

3, Strength of Chinese and Taiwanese Teams

The Chinese and Taiwanese teams are very strong overall. Why is that? Putting aside the question of money, I would like to mention the strength of the community. In Japan, there is the iGEM Community, but compared to communities in other countries, I think it is weak in some areas. That would be the number of people. There are not as many iGEM teams in Japan as there are in China and Taiwan. So how should we increase the number of iGEM teams?

Here, we hope that one team in a country will achieve results that “junior members will admire and follow in their footsteps,” and that many people will get involved with iGEM in a way that will be followed by others. The secret to the strength of China and Taiwan is money and many universities, but I think the number one secret lies here. Fortunately, Japan now has an opportunity. The success of Japan United last year has certainly increased the iGEM fever. If we can make the most of this opportunity and produce results that will further increase the iGEM enthusiasm of the entire Japanese team, Japan will finally become an iGEM powerhouse.　(Shoya Inoue)

Explanation of A User's Guide to Golden Gate Cloning Methods and Standards

In this issue, I will summarize the contents of the article **“A User's Guide to Golden Gate Cloning Methods and Standards”** published in ACS Synthetic Biology, a journal on synthetic biology. The following is a summary of the contents of the paper.

Information on the paper
Doi: ACS Synth. Biol. 2022, 11, 11, 3551-3563
First author Andrea Giachino
Currently, this is the most-read article in the journal (article views are an astonishing 43,000!)
https://pubmed.ncbi.nlm.nih.gov/36322003/

Overview

This is like the applied version of DNA engineering and DNA assembly summarized in the last issue.
The technique of fiddling with DNA fragments with restriction enzymes is modular and standardized and includes a subfamily of different methods, but the most widely adopted are the MoClo and Golden Braid standards.
This paper is a beginner's guide to Golden Gate Assembly, comparing the various standards available. It also describes the latest information on this Golden Gate Assembly.

What is the Golden Gate method?

(This is where we come in.)
・Restriction Enzyme-mediated Assembly Method Using Type ⅡS Endonuclease
What is an endonuclease?
→Enzyme that cleaves the phosphodiester bond of a specific nucleotide chain
→The restriction enzymes we have seen so far are one type of endonuclease
→Type IIS does not use ATP in the degradation process (EcoR Ⅰ is an example of Type II)
・Onepot can assemble a large number of DNA fragments.
(similar to the Gibson assembly summarized before)

・Both linear DNA and cyclic DNA can be used → Information can be stored in plasmids.
・It can be used for various experiments and research from CRISPR to protein localization.

However, it is a method that is currently not very widespread (unfortunately 🥺)

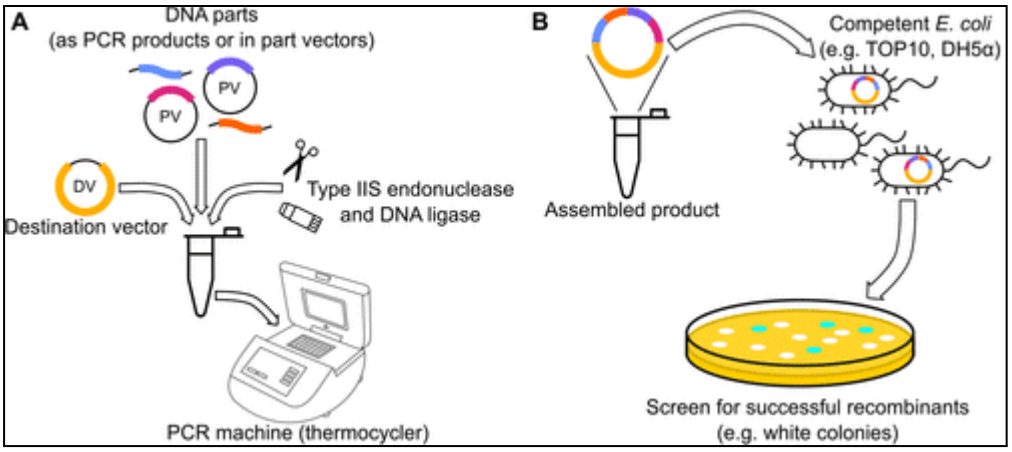
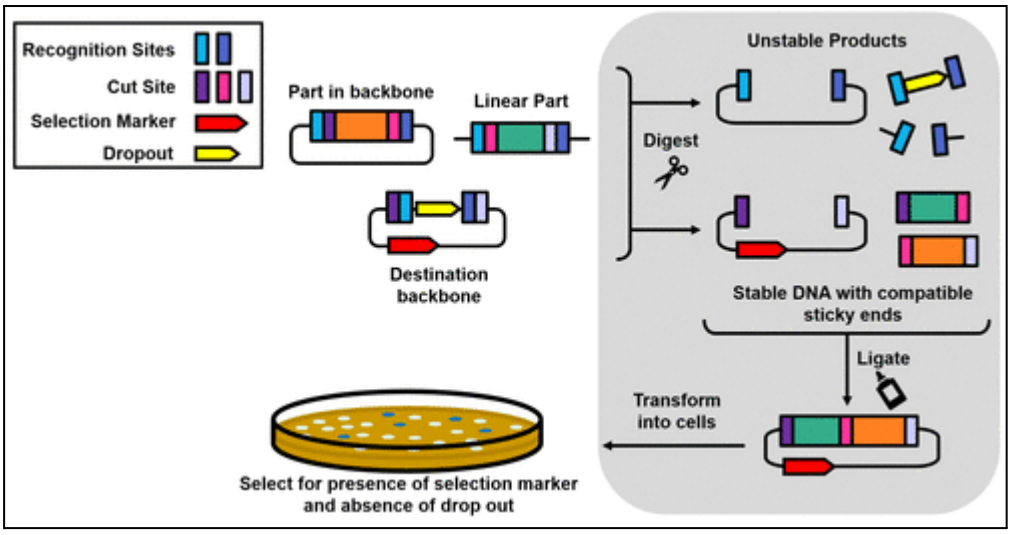


Figure 1: Two ways of doing the Golden Assembly (taken from the paper)

Role of restriction enzymes

Restriction enzymes cut DNA at a certain distance to their recognition sequences. In other words, its recognition sequence only determines where the endonuclease cleaves DNA, not which bases it cleaves. A single IIS-type endonuclease can produce a DNA sticky end of any base sequence simply by placing the endonuclease recognition sequence at the appropriate distance from the target cleavage site, and proper design of the position and orientation of the cleavage site will also ensure that the recognition sequence is not retained in the final structure The DNA sticky end can be generated by simply placing the DNA sticky end at the appropriate distance.

Type II has two sites: a recognition site and a cleavage site. (see figure below for reference)



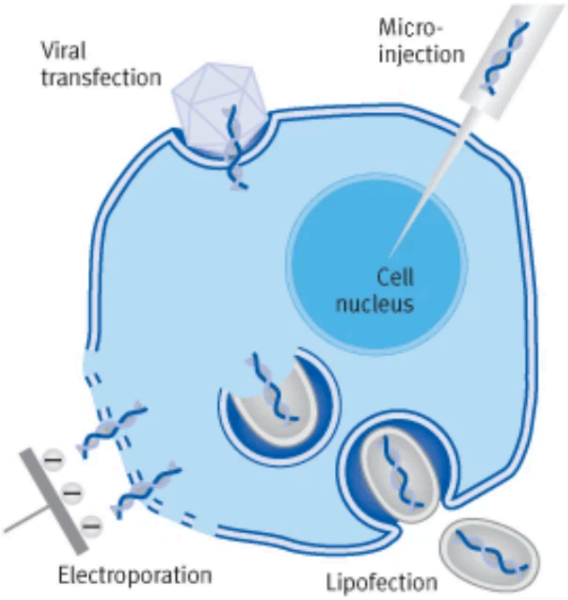
taken from the paper

Selection of target vectors
・Supply the backbone of the acceptor.
I Plasmid replicator
II Selectable marker
III Dropout markers between endonuclease recognition sequences
These are replaced by the assembled gene upon successful assembly.
Recognition sequences are outward-facing.
The assembly's target vector must have different markers compared to all the part vectors in the assembly.
(Important~~~!!!!!)

Performing Hierarchical Assembly
Golden Assembly is a hierarchical assembly, i.e., the assembled Product can be reused as DNA parts in subsequent assembly processes.
The procedure is to assemble each transcription unit separately and then integrate the separate units into a single assembly.
The details are cut here because of the complexity of the mechanism.

SUMMARY
There is no universal method for DNA assembly.
An active community of tool makers and users (synthetic biologists) is important to tackle this problem. (Shoya Inoue)

Dip in toes in the basics of DNA engineering and cloning!



DNA engineering is an important technology that forms the basis of synthetic biology. In this article, I will summarize the basics of DNA engineering based on what I covered in my biology class last year. In this second part, I will cover cloning.

1. Introduction to Gene Cloning Methods

There are various methods for gene cloning. Let us summarize them one by one.

A. Microinjection Method

This uses fertilized eggs of mammals. A fragment of a gene is introduced into the nucleus of a sperm-derived egg via a tube. The fertilized egg then develops into a child, and the child is introduced to the target gene.

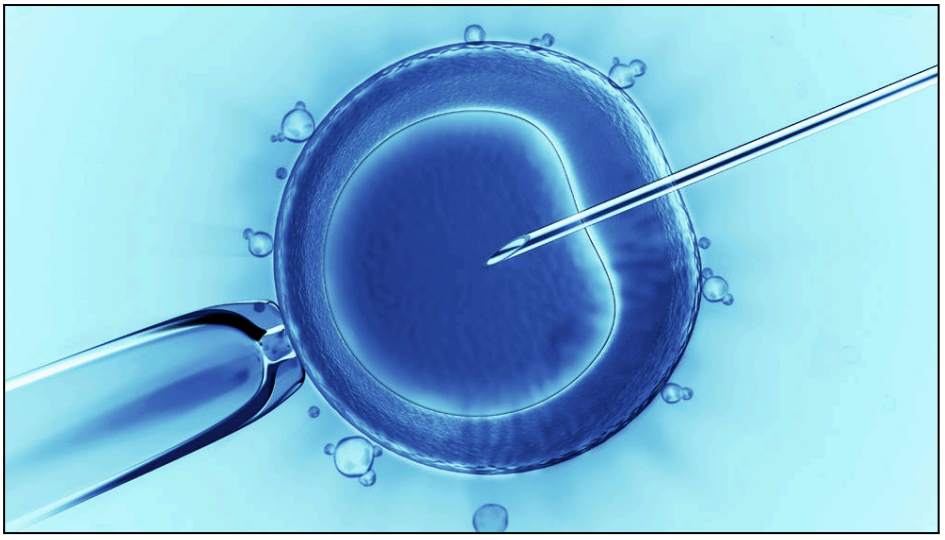


Figure from The Jackson Laboratory

B. Electroporation

This is a technique to incorporate DNA by applying electric current to cells for a certain period to form pores in the cell membrane.
(Incidentally, electroporation is also used in cosmetology as a technique to introduce beauty ingredients such as vitamin C and hyaluronic acid into cells 👶 by a passing editor).

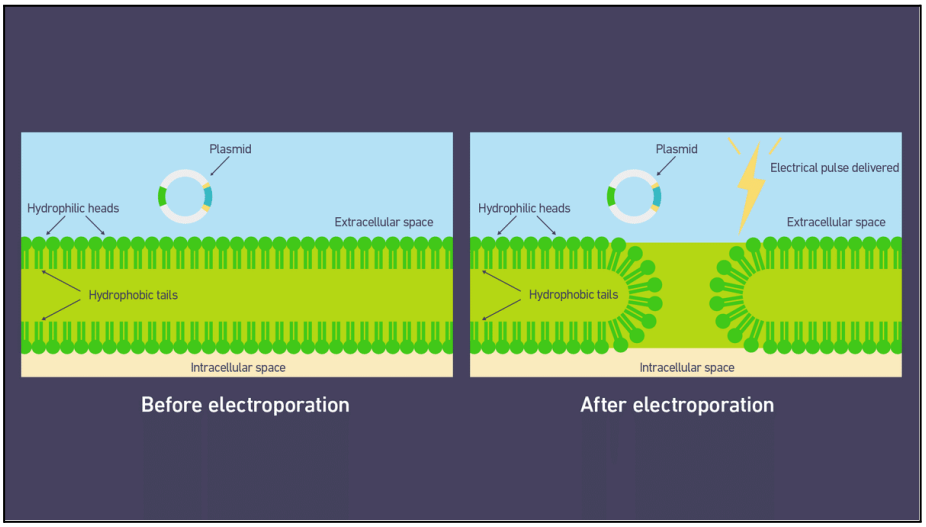


Figure from Technology Networks.

C. Lipofection Method

This method involves wrapping a fragment of a gene in a ribosome and fusing it with the cell membrane to introduce the gene. It is mainly used for cloning into animal cells.

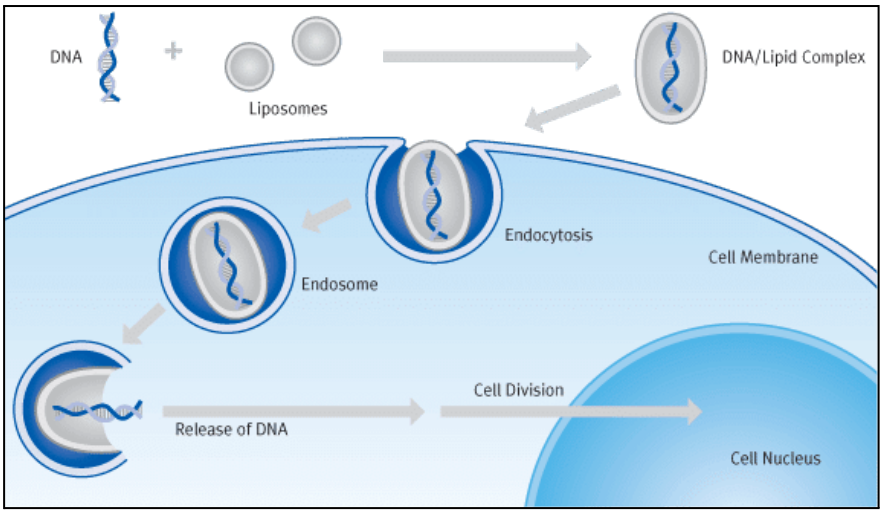


Figure from Biontex Laboratories GmbH

D. Particle Gun Method

In the last particle gun method, genes are introduced into cells by attaching metal particles such as Au to the plasmid and exposing them to high-pressure gas.
Gold Au is used in this method because

・To create a bullet with high specific gravity to increase penetration into the cell.
・The use of a metal that is chemically inert and less likely to harm the living body.

The reasons for this are as follows.

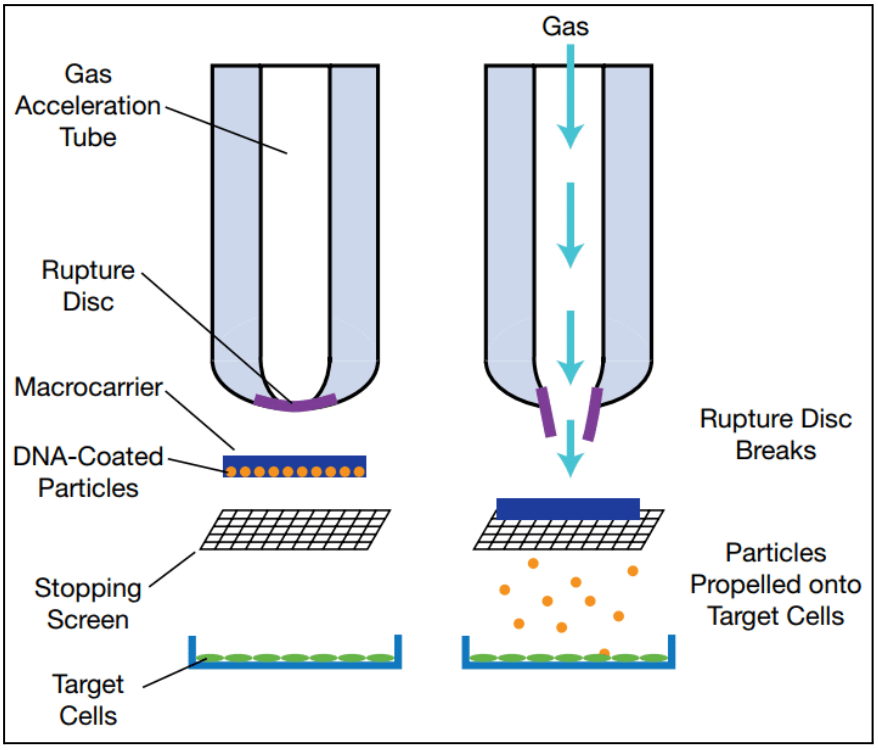


Figure from Creative Biolabs

Summary

It is common practice to use different cloning methods depending on the target cell type and other factors.
An organism that has been genetically modified to carry a foreign gene is called a transgenic organism. (example: golden rice, herbicide-tolerant soybeans)

2. The Future of Genetic Engineering

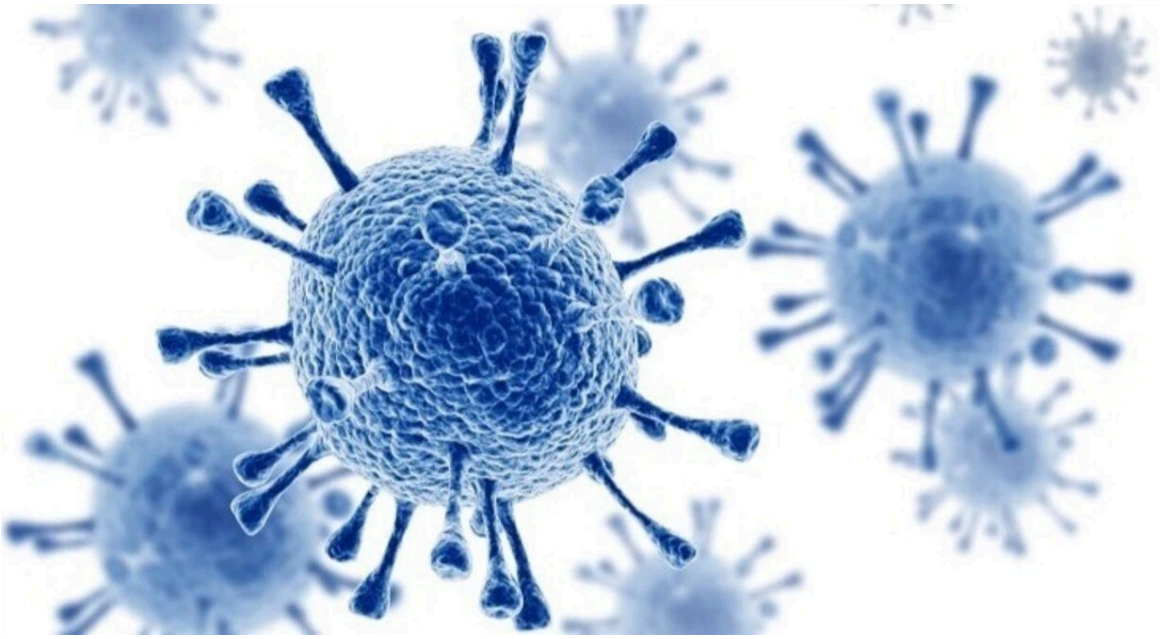
The field of genetic engineering is continuing to make technological progress. However, there are still many issues to be addressed, such as the safety of genetically modified foods and the impact of transgenic organisms on the ecosystem. Can those of us working in synthetic biology create a solution to these issues from our own unique perspective? Perhaps it is our mission to do so.

3. References

BioBuilder: Synthetic Biology in the Lab
Square Latest Illustrated Guide to Biology (Daiichigakusha)
High School Textbook of Biology (Daiichi Gakushu-sha)

(Text: Shoya Inoue)

From Operons to HIV! Special feature on research that originated in France



Hi, I'm Shoya Inoue. How is everyone doing now that summer is almost over? I wish the heat would ease up, but it looks like it will continue.
Well, the iGEM Grand Jamboree is only two months away. That means... I've been doing iGEM since February...has it been half a year already? Time flies by so fast, doesn't it?

That's why this issue of Jamboree is dedicated to biological research originating from France, the host country of Jamboree. I hope you will read this article to the end, even though some parts of it are a little far from synthetic biology.

1. the Pasteur effect ~ Louis Pasteur ~

Have you ever heard of the Pasteur effect? It means that **"in the presence of oxygen, respiration is activated and fermentation is suppressed"**. This effect was discovered when the fermentation of a certain yeast, which was active under anaerobic conditions, became inactive under aerobic conditions. The discoverer of the Pasteur effect, Dr. Pasteur, is a French biologist. Although he was a biologist, he also **discovered optical isomers** in my field of organic chemistry (systematic laws were not discovered until the 20th century), and his achievements are such that if there had been a Nobel Prize at that time, I am sure he would have won it. Incidentally, he also said, **“Science has no borders, but scientists have their own country"**.

2. operon theory ~François Jacob & Jacques Lucien Monod~

The proponents of the operon theory, Dr. Jacob and Dr. Mono, are also French researchers. What is the operon theory: When DNA is transcribed, the region that is transcribed at one time is called the transcription unit. Transcription is initiated by the attachment of basic transcription factors and RNA polymerase to a promoter, but in fact, a protein called regulatory protein controls transcription in each transcription unit. The region to which the regulatory protein is attached is called an **operator** (operators are often located between promoters and gene clusters), and the gene clusters regulated by one operator are called **operons**.

Dr. Jacob Mono and Dr. Mono **thought that this operon regulates transcription in prokaryotes.** In 1961, and four years later he was awarded the Nobel Prize in Physiology and Medicine. There are different types of operons, such as the lactose operon and the tryptophan operon. If you like biology, please look them up because they are studied in high school biology!

3. discovery of the HIV virus ~Françoise Barré-Sinoussi & Luc Antoine Montagnier~

Dr. Barre-Sinoussi and Dr. Montagnier are French biologists who discovered human HIV, which infects and destroys helper T cells, thus reducing acquired immunity. It is a highly mutable virus, and it is difficult to create a vaccine because there are so many different types of the virus, each with different surface antigens.

Dr. Barre-Sinoussi and Dr. Montagnier were awarded the Nobel Prize in Physiology and Medicine in 2008.

4. Summary

How was it? I have summarized the biological research that originated in France. I realized once again that a lot of wonderful research is being done in France now and in the past.

I am looking forward to going to Paris in two months. I have to do a lot of work for that. ....