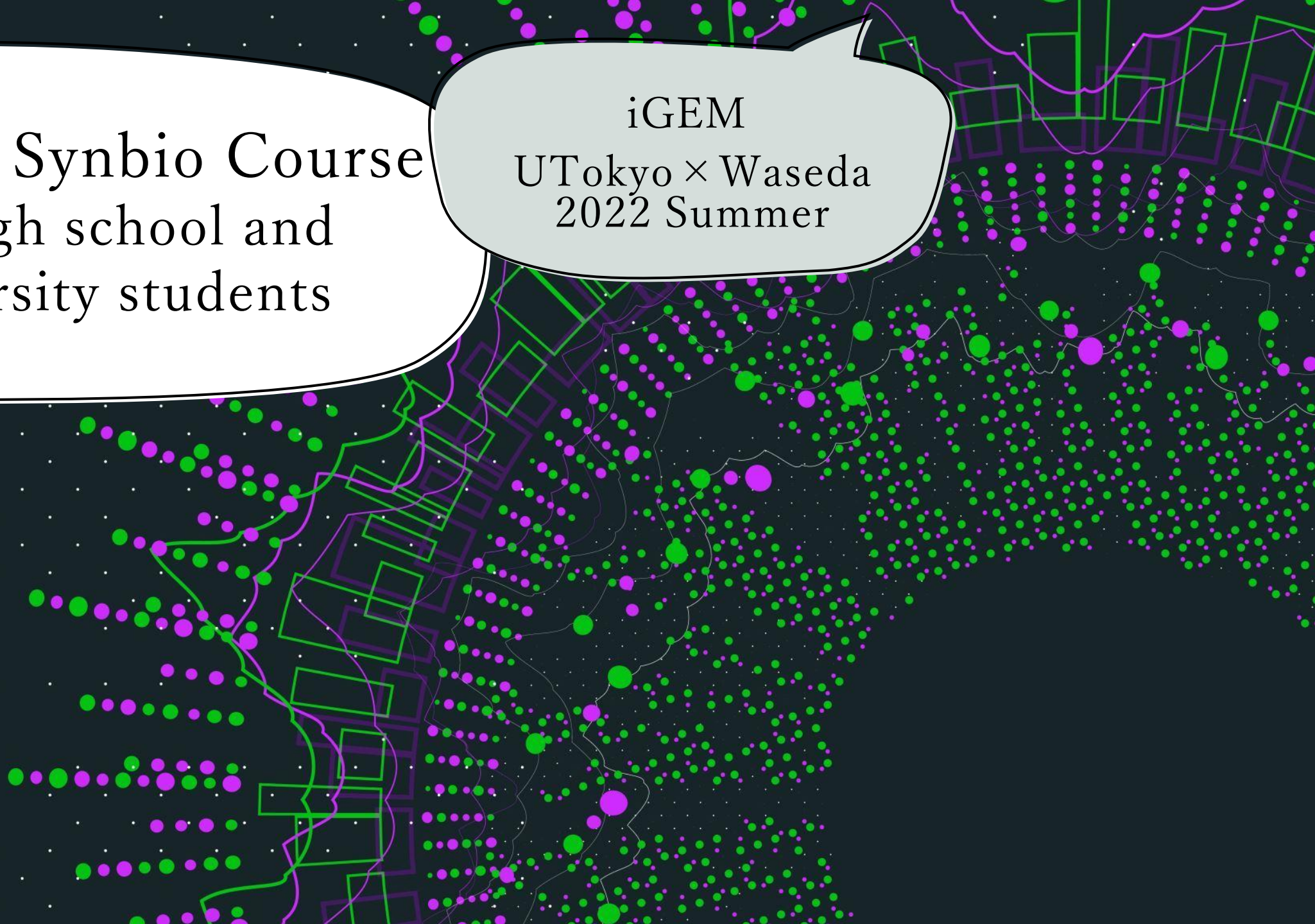


Summer Synbio Course  
for high school and  
university students

iGEM  
UTokyo × Waseda  
2022 Summer



# Program

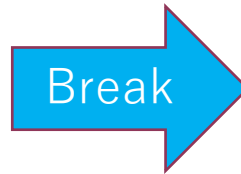
## Chapter 1

### Basic Part 1 :

- Self-introduction, What is iGEM
- Basic Biology
- What is Synthetic Biology

### Practice Part 1 :

Let's Program Organisms



## Chapter 2

### Basic Part 2 :

- This Year's Project of iGEM Waseda
- This Year's Project of iGEM UTokyo

### Practice Part 2 : Group Work

~Q&A~

# Final Goals

## Basic Part

- Understand what iGEM is and what synthetic biology is from the basics of biology
- Understand the contents of and how to proceed the iGEM project

## Practice Part

- Program organisms
- Consider an iGEM project to achieve the SDGs

# What is iGEM UTokyo

- A team from the University of Tokyo participating in the world competition of synthetic biology "iGEM".
- Mainly 1st and 2nd year students play a central role, and it have diverse members.



# What is iGEM

## Waseda

- A team from Waseda participating in the iGEM, a global synthetic biology competition.
- We are working with about 25 people!

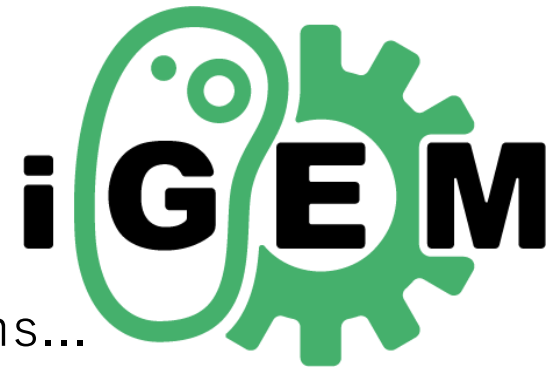


# What is iGEM

- A competition established by MIT for the purpose of developing and popularizing the academic field of synthetic biology.
- Emphasis on creating socially useful things by freely combining the abilities of different organisms
- Each team creates creatures with new abilities and competes, so it can be said **Biological Robot Competition**.

For example...

Microbes that make spider silk, that can be used instead of blood, that smell like bananas, that solve math problems...



Logo of iGEM  
From iGEM blog

# Prepare Basic Biological Knowledge

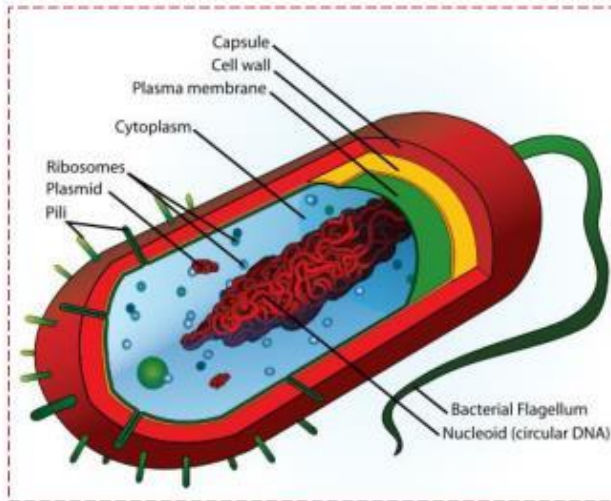
## Contents

- 1, Basic Structure of Organisms
- 2, Gene and its Expression
- 3, Biotechnology

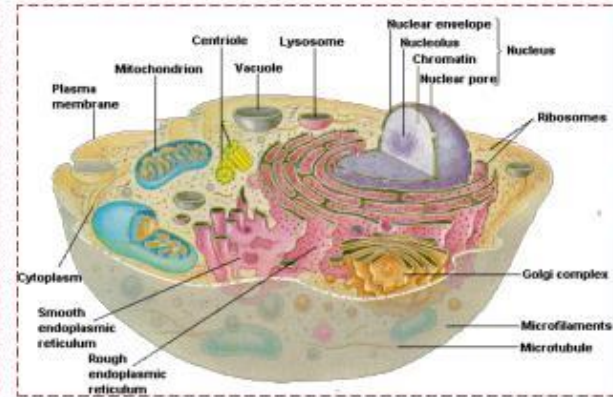
First, for those who are unfamiliar with biology (even though are interested in it), we will explain the basics of biology necessary to understand this lecture.

## Types of Cells

### Prokaryotic cell



### Eukaryotic



Courtesy: [http://en.wikipedia.org/wiki/Cell\\_\(biology\)](http://en.wikipedia.org/wiki/Cell_(biology))

# 1, Basic Structure of Organisms

- The basic unit of organisms is the **cell**.
- Organisms are divided into **prokaryotes** and **eukaryotes** according to cell structure.

# 1-1 Prokaryotes and Eukaryotes

## Prokaryotes

- DNA is **circular** and in the **cytoplasm**
- Underdeveloped organelles
- Generally smaller than eukaryotic cells

**Bacteria**, Archaea

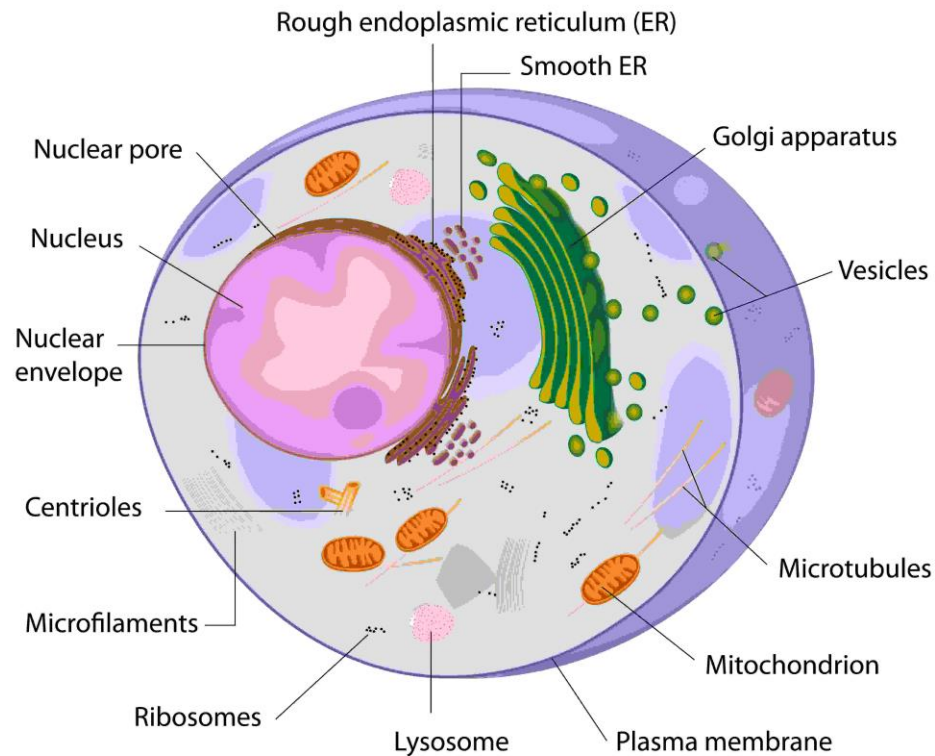
## Eukaryotes

- DNA is **linear** with **chromatin** structure and surrounded by a **nuclear membrane**
- Developed organelles
- Generally bigger than prokaryotic cells

Animals, Plants, **Fungi**, Protists

# Note:Organelle

Structure of a Typical Animal Cell



From [Cell Structure \(biology-questions-and-answers.com\)](http://biology-questions-and-answers.com)

Intracellular membrane-compartmental structures

**Mitochondrion** : Energy(ATP) production

**Rough-surfaced endoplasmic reticulum(Ribosome)** : Protein synthesis

**Smooth-surfaced endoplasmic reticulum** : Lipid synthesis,  $\text{Ca}^{2+}$  storage

**Golgi apparatus** : Protein modification

**Lysosome** : Molecular hydrolysis

# Note:Chromatin structure

- A structure in which DNA is wrapped around a protein called a **histone** is called a **nucleosome**.
- A nucleosome aggregated into a fibrous form is called a **chromatin fibre**.
- Deeply involved in transcriptional control of genes(described later) that do not involve changes in the DNA sequence, called **epigenetics**.

# 1-2 Model Organism

- A **model organism** is an organism frequently used in biological research.
- Favorable conditions for research, such as easy rearing, breeding, and observation, rapid generation change, advanced analysis of genetic information, etc.

# 1-2 Model Organism

Among model organisms, give one example each from prokaryotes and eukaryotes

## *Escherichia coli*

Prokaryotes

2~6  $\mu\text{m}$

Living in the human large intestine, etc.

A representative example of a prokaryotic model organism that is easily propagated.

## *Saccharomyces cerevisiae*

Eukaryotes(Fungi)

5~8  $\mu\text{m}$

Used in various fermented foods

Single-celled representative eukaryotic model organism

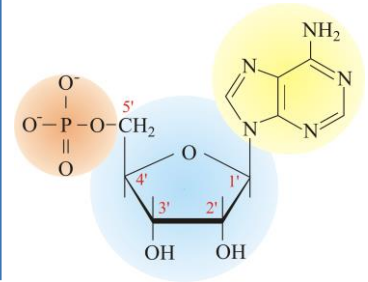
## 2, Gene and its Expression

- Organisms have **genes** to pass on their genetic information to the next generation
- The substance of many genes is a molecule called **DNA**
- Products based on gene information work in cells, and this is called **gene expression**

# 2-1 Nucleic Acid and Protein

## Nucleotide

A pentose with a nucleobase and a phosphate attached



E.Generalic, <https://glossary.periodni.com/glossary.php?en=nucleotide>

## DNA

- The pentose is **deoxyribose** and is divided into four types of nucleotides: **adenine (A)**, **thymine (T)**, **guanine (G)**, and **cytosine (C)**
- DNAs are linked by phosphodiester bonds, and **A and T**, **G and C** of the DNA strand which mainly form a **double helix** structure become a pair
- Mainly in the **nucleus** in cells

## RNA

- The pentose is **ribose** and is divided into four types of nucleotides: **adenine (A)**, **uracil (U)**, **guanine (G)**, and **cytosine (C)**
- RNA strands mainly have a **single-stranded** structure
- Exists everywhere in the cell, including mRNA, tRNA, rRNA

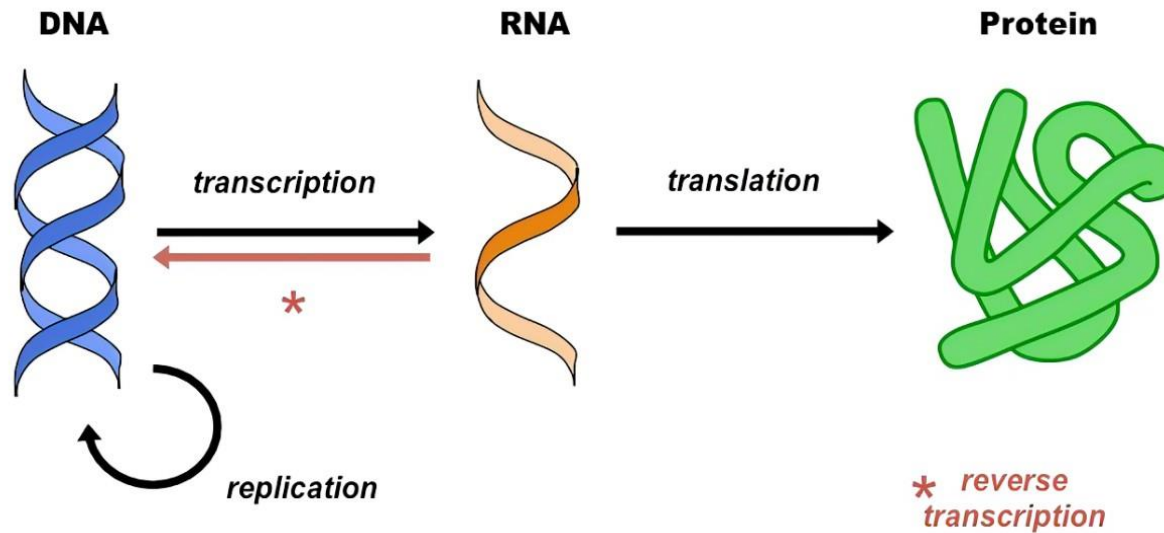
# 2-1 Nucleic Acid and Protein

## Protein

- A major component of living organisms formed by folding, modifying, and combining polypeptide chains in which 20 kinds of **amino acids** (there are various theories) are connected by **peptide bonds**
- There are a variety of proteins, each with a specific function, such as constructing living organisms (structural proteins) or catalyzing chemical reactions (**enzymes**)

Glycine Gly G	Alanine Ala A	Valine Val V	Leucine Leu L	Isoleucine Ile I	Proline Pro P
Methionine Met M	Phenylalanine Phe F	Tryptophan Trp W			
Serine Ser S	Threonine Thr T	Asparagine Asp N	Glutamine Glu Q	Tyrosine Tyr Y	Cysteine Cys C
Aspartic acid Asn D	Glutamic acid Gln E	Histidine His H	Lysine Lys K	Arginine Arg R	

## Diagram of Central Dogma



From City University of Hong Kong Computational Soft Matter and Biophysics Group  
[Central dogma, translation, transcription \[with exercise questions\]](#) | Dai Liang Group ([cityu.edu.hk](http://cityu.edu.hk))

## 2-2 Central Dogma

- The process by which genetic information is transmitted through DNA, RNA, and proteins
- DNA is **replicated** and distributed to each of the two divided cells
- Replication is catalyzed by an enzyme (protein) called **DNA polymerase** and requires short RNAs called **primers** for initiation

## 2-2 Central Dogma -Transcription-

- **Transcription** is the making of mRNA from DNA
- The part of DNA where transcription starts is called the **promoter**, and where it ends is called the **terminator**
- The transcription reaction is catalyzed by an enzyme (protein) called **RNA polymerase**, which is regulated by **general transcription factor (GTF)** and **transcription-regulating factors**
- Among transcription factors, those that promote transcription are called **activators**, and those that suppress transcription are called **repressors**
- Only eukaryotes have GTF

## 2-2 Central Dogma -Translation-

- **Translation** is the making of protein from the base sequence of mRNA
- One amino acid in a protein corresponds to three bases, and this set of three bases is called a **codon**
- Translation occurs at the **ribosome** and **tRNA** transports amino acids to the ribosome
- tRNAs have **anticodons**, pairs of bases corresponding to codons
- Translation begins at the **start codon** and ends at the **stop codon** of the mRNA

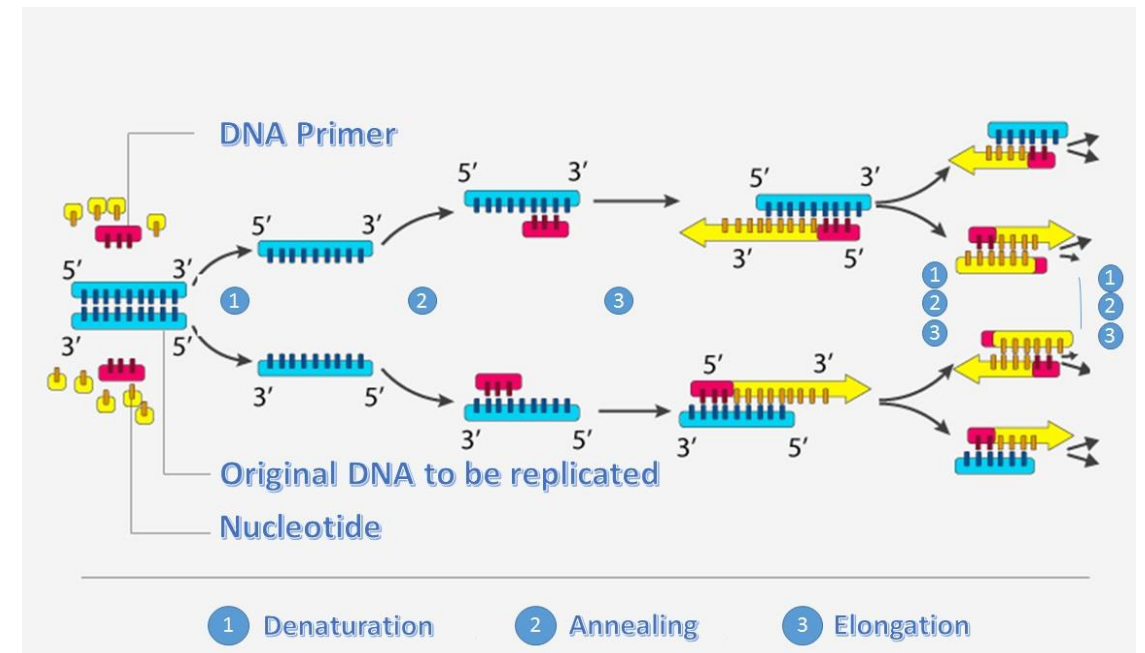


## 3, Biotechnology

- We will explain biology-related science and technology that are commonly used in biological research

# 3-1 PCR

- Method to make millions to billions of copies of DNA
- Repeat the cycle of heating to **dissociate the DNA double strands**, lowering the temperature to **bind the primer** (annealing), and slightly raising the temperature to **elongate the DNA strands**
- PCR that visualizes the copying process is called **Real-Time PCR (RT-PCR)**, and it is also used to test for coronavirus



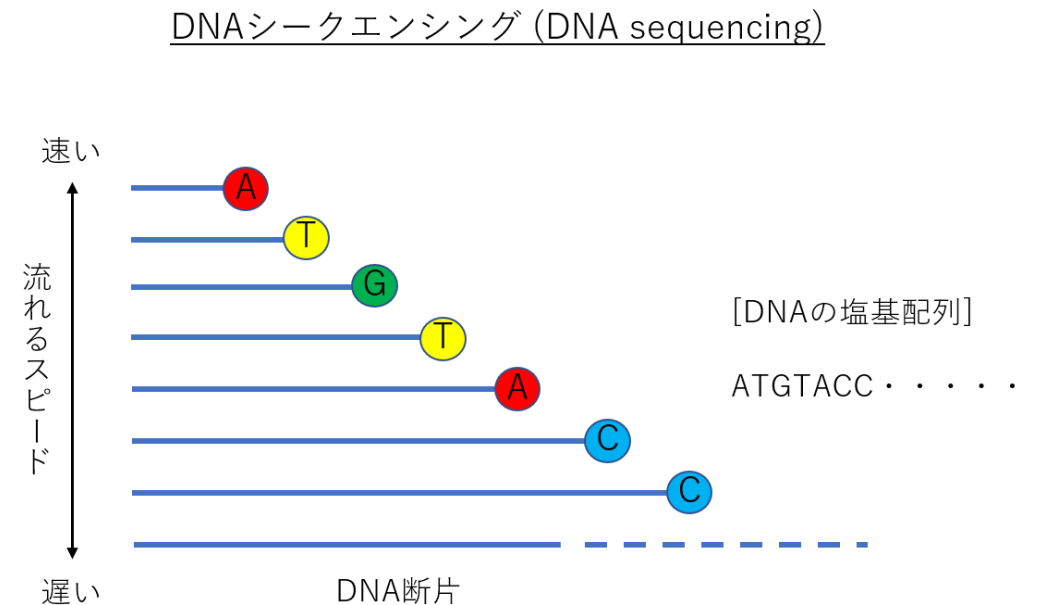
From [Conventional PCR, One-step RT PCR or Two-step real-time RT PCR: Which one is the best option? – Wnt Proteins](#)

## 3-2 Electrophoresis

- A method to examine the length of DNA strands by moving them in an **agarose gel**
- Since DNA is **negatively charged** by ionizing the hydrogen of the phosphate group, it moves **from - to +** when an electric current is applied
- The shorter the DNA, the easier it is to move in the agarose gel

# 3-3 DNA Sequencing

- Technology to analyze DNA sequence
- One of the methods, the **Sanger method**, attaches fluorescence to the dideoxy NTP that stops DNA elongation and analyzes it by electrophoresis
- Faster **next-generation sequencing** analyzes multiple DNA fragments in parallel and then aligns them for sequence analysis



From [DNAシーケンシング \(DNA sequencing\) - reverse-aging-research ページ ! \(jimdofree.com\)](#)

## 3-4 Reporter Gene

- A foreign gene that **visualizes** gene expression
- transcribed together with the objective gene or bound to the objective protein
- **Drug resistance genes** and **fluorescent proteins** such as GFP are frequently used

# 3-4 Gene Transfer Technology

- **Transformation**, the target gene is put into a **plasmid** and put into the cell, have various method:

Electroporation method: open holes with electric pulses

Microinjection method: Inject with a thin needle

Viral vector method: insert with virus

Calcium chloride method: Create a state (competent cells) that can easily incorporate foreign DNA

Particle gun method: DNA attached to fine metal particles and injected

and so on.

## Plasmid

Circular DNA that exists in cells separately from genomic DNA

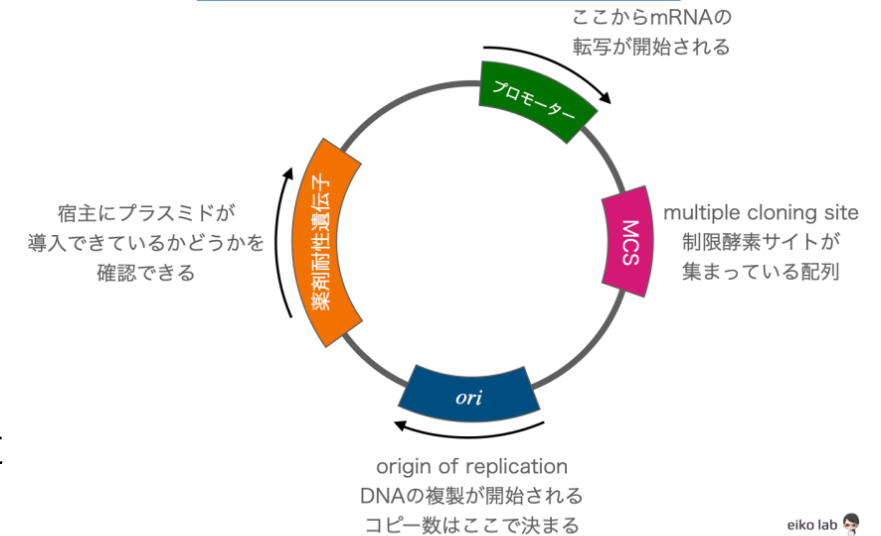


図1. プラスミドベクターの基本骨格

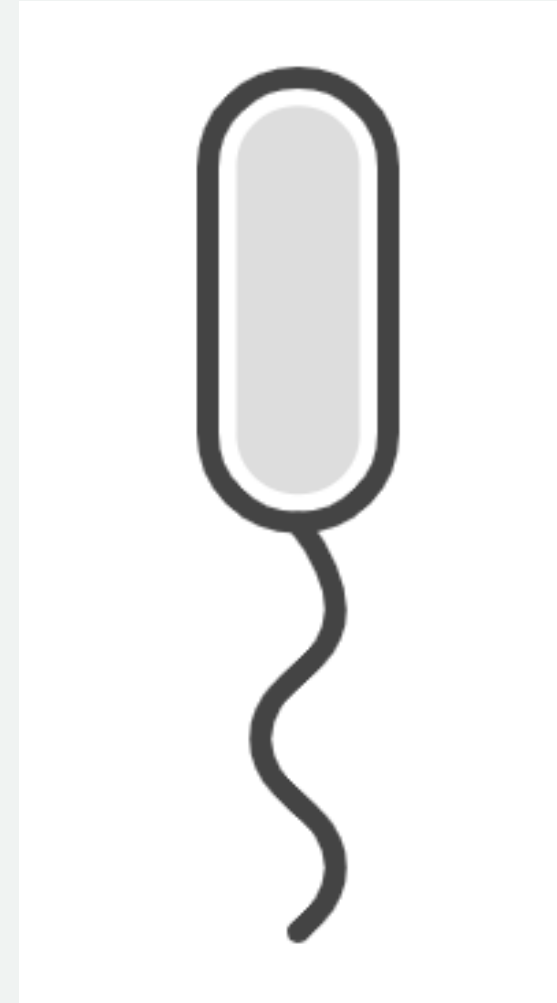
From [https://eiko-lab.com/2021/05/20/plasmid\\_map/](https://eiko-lab.com/2021/05/20/plasmid_map/)

# Note: Restriction Enzyme

- An enzyme that recognizes a specific base sequence in DNA and **cuts** that part
- Many of the recognition sequences are **palindromic**, reading the sequence of one DNA strand in one direction matches the reading of the complementary strand of that DNA strand in the opposite direction
- DNA fragments with the same restriction enzyme cleavage plane can be joined together using an enzyme called **DNA ligase**

## Practice Part 1: Let's Program Organisms

- Let's create a gene circuit by combining genes using the synthetic biology experience tool "genochemy"
- We will propose the organism we want to create, so let's design the genetic circuit of the organism with the desired function



Microbes to help you  
experience synthetic  
biology  
[Genomy](#)

## Recommended environment

More than Google Chrome 104

More than Microsoft Edge 104

More than Mozilla Firefox 104

(Safari and Internet Explorer are not supported)

Touch not recommended, operation on PC recommended

<https://genochemistry.netlify.app>

<https://genochemistry.vercel.app>

accessible from either

# Tutorial: Let's make a creature that glows red



Q1. Let's make a creature that glows green only when there is blue light

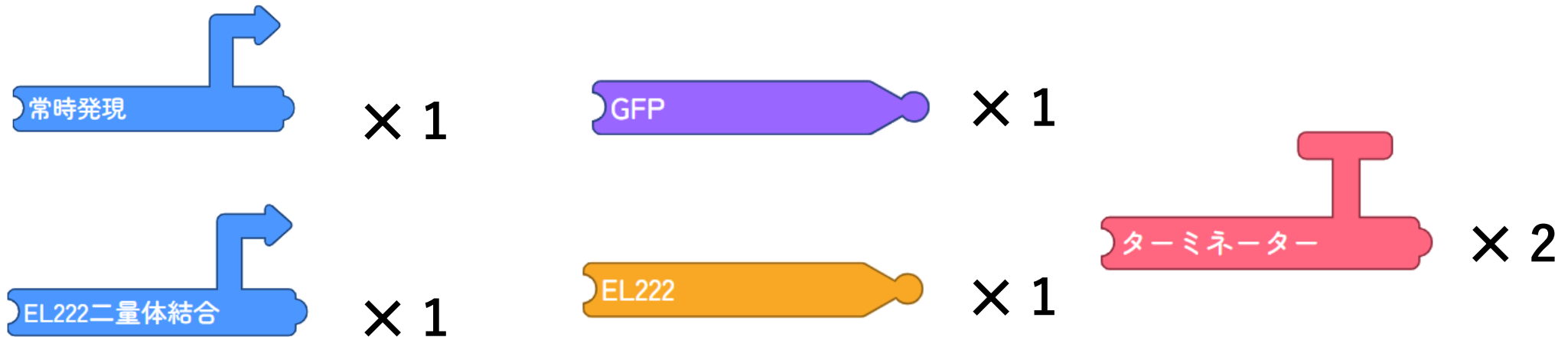
Hint 💡

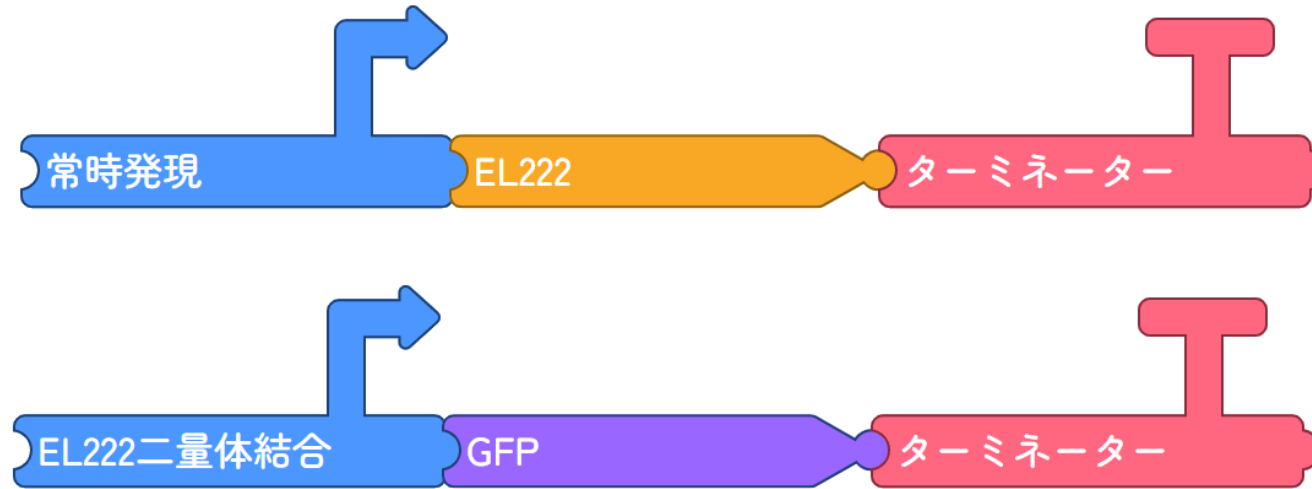
Parts list:

# Q1. Let's make a creature that glows green only when there is blue light

Hint 💡

Parts list:





A1. Creature that glows green only when there is blue light

Q2. Let's make an organism that does  
not glow red only when blue light and  
drug A is present

Hint 💡

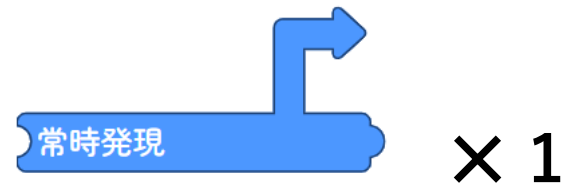
Parts list:

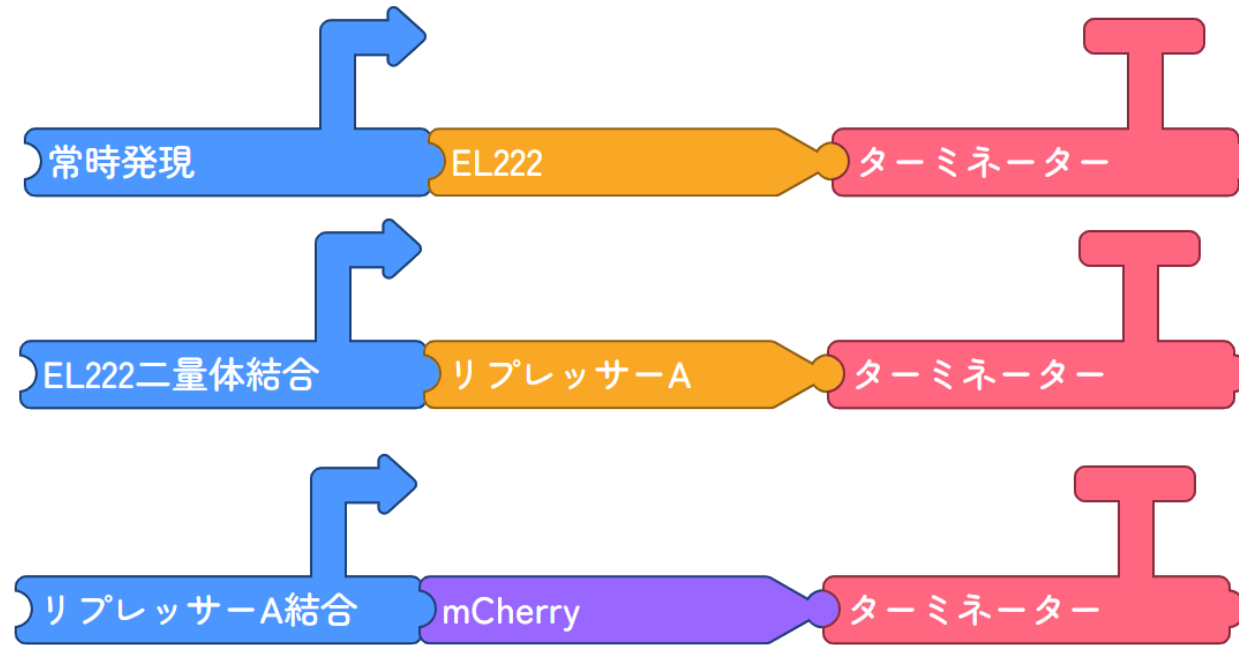
Drug A	Blue light	mCherry
○	○	×
○	×	○
×	○	○
×	×	○

Q2. Let's make an organism that does  
not glow red only when blue light and  
drug A is present.

Hint 💡

Parts list:





A2.organism that does not glow red only when blue light and drug A is present

# Q3. Let's make a creature that glows green all the time after exposure of blue light

Once exposed to blue light, it will glow green, and will continue to glow green even after the blue light is stopped

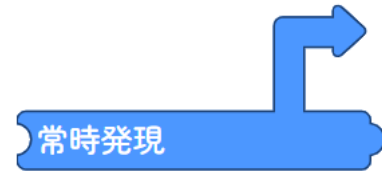
Hint 

Parts list:

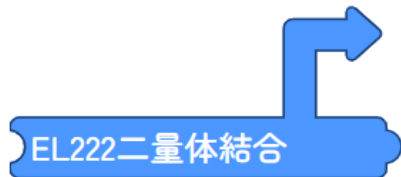
Q3. Let's make a creature that glows green all the time after exposure of blue light

Hint 

Parts list:



× 2



× 1



× 1



× 1



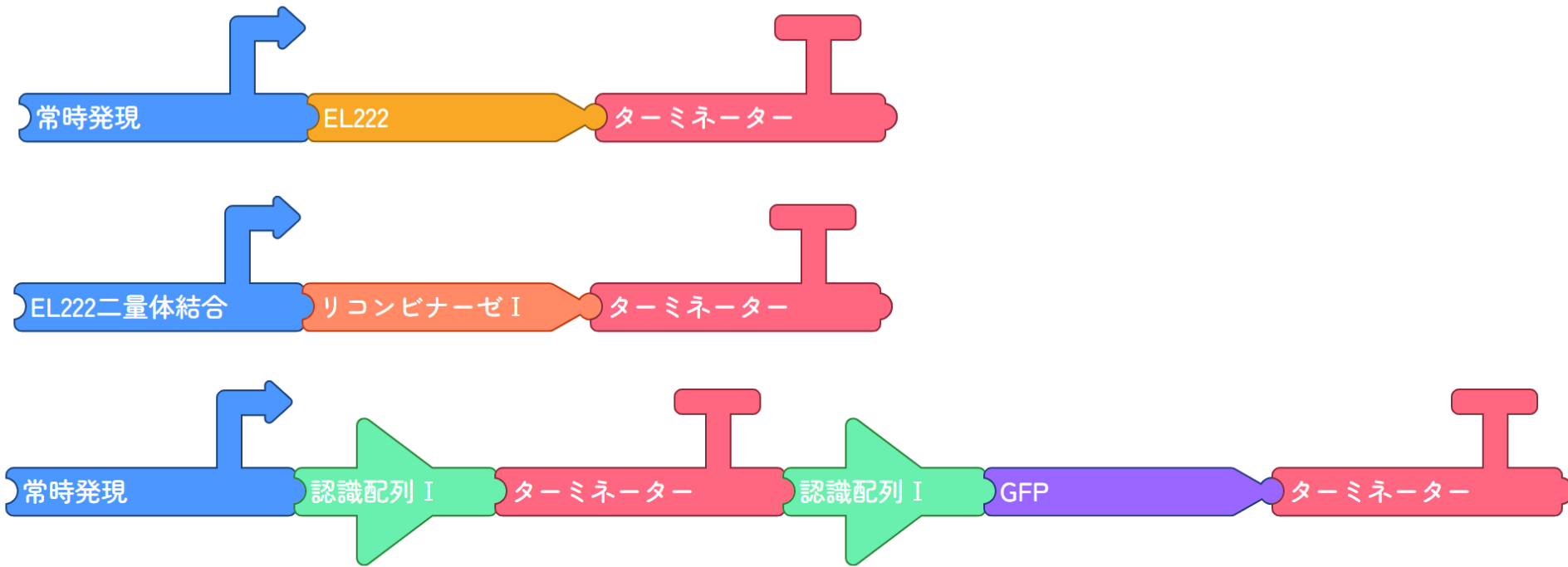
× 1



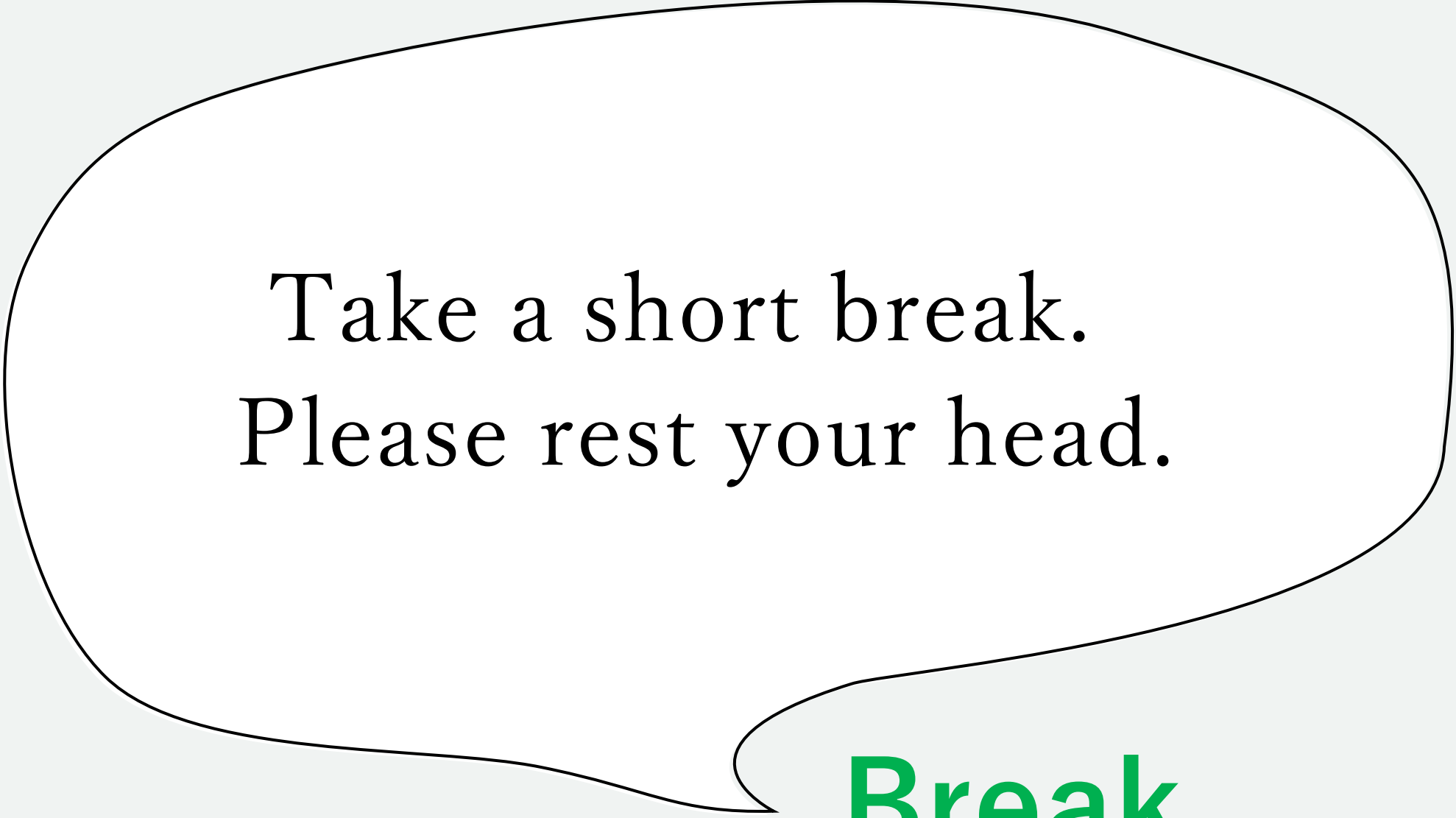
× 2



× 4



A3. creature that glows green all the time  
after exposure of blue light



Take a short break.  
Please rest your head.

**Break**

# iGEM UTokyo 2022 "Optopass"

~Security for yeast substance production~

# iGEM UTokyo 2022 "Optopass"

Optogenetics × Syntheticbiology

## Security for yeast substance production

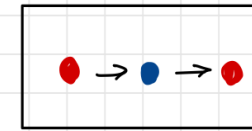
When the yeast is exposed to light in a certain order,

if it matches a pre-defined order, it produces the desired substance,

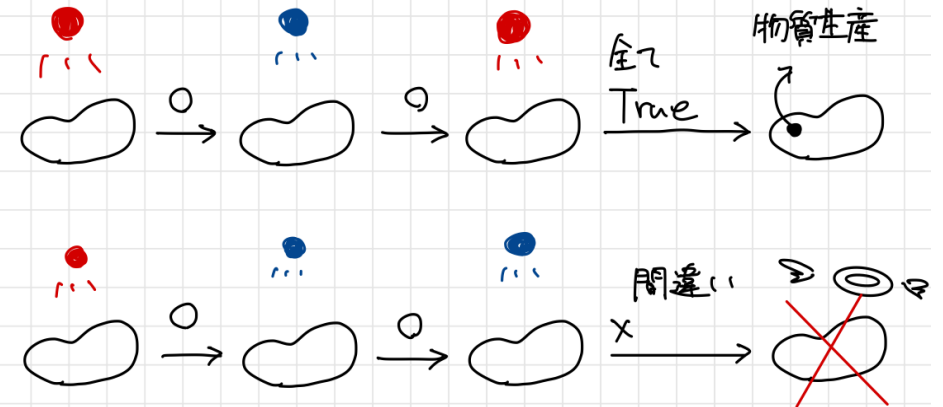
if not, a kill switch is triggered and it dies.

Making light order the 'key' to substance production!

暗号



生産



# Before introducing the project ...

## How to proceed with the iGEM project

At iGEM, each team works on its own defined project over a period of approximately one year. Within this, there are three broadly defined roles.

- Wet: actually introduce the designed gene into the organism to realise the project.
- Dry: Thinking about the project from the simulation.
- Human practice: Developing the project by talking to companies and laboratories in related fields and learning about the technical aspects and social implementation.

# Before introducing the project ...

## What is optogenetics?

Around 2005, an attempt was made to manipulate cellular activity by light stimulation using a protein expressed on the plasma membrane of green algae in animal neurons.

The field of optogenetics, which is a combination of optics and genetics, is currently developing, particularly in the field of brain science, to elucidate cellular functions by using light stimulation with high temporal and spatial precision.

Our project combines this idea with the synthetic biology theme of material production in yeast.

# Project background

The range of material production using synthetic biology is increasing year by year.

ex) Condiments, ingredients, pharmaceuticals,...

Strict security and quality control are important for some substances.

→ There may be a demand for a mechanism whereby the yeast is exposed to blue, red and  $\alpha$  (a new third color) light in the correct order to release security, activate the desired metabolic pathways and produce substances.

# 1 . Photoreception

The iGEM team at NUS (National University of Singapore) has developed a system of yeast that responds to light in 2021.

Blue → aggregation of  $\beta$ -defensins

Red → yeast produces  $\beta$ -defensins

Blue + Red → kill switch



In iGEM UTokyo 2022. For further improvement and application!

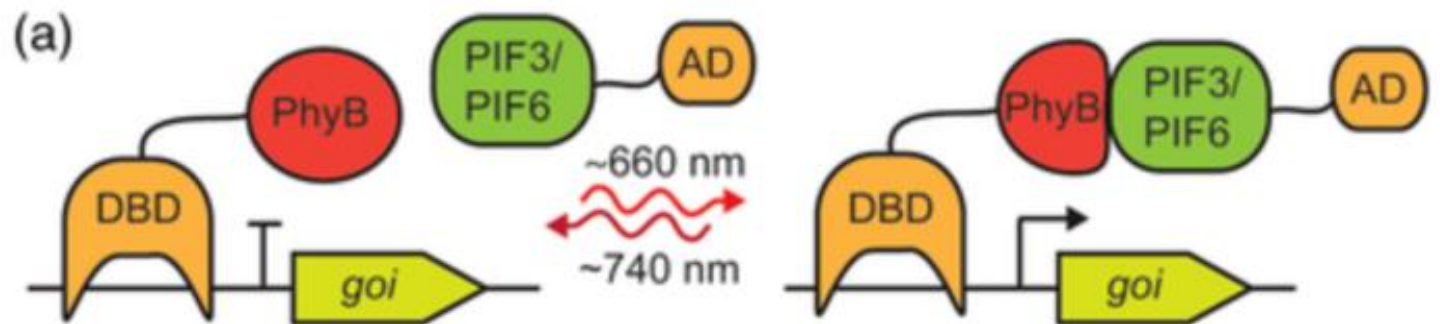
# 1-1. Acceptance of red light: Phirex System

When exposed to red light ...

Protein (SynTALE-PhyB) bound upstream of promoter and free protein (PIF3-VP64) with transcriptional activation domain

→ Transcription is promoted by the proximity of VP64 to the promoter.

- SynTALE(DBD): Recognises and binds to specific DNA sequences.
- PhyB/PIF3: Forms heterodimers in response to red light.
- VP64: four transcription activator VP16s linked together for enhanced effect.



We also tried to utilise the Zinc finger domain, which works in a similar way to SynTALE.

# 1-2. Acceptance of blue light: EL222 system

When exposed to blue light ...

EL222 protein dimerizes

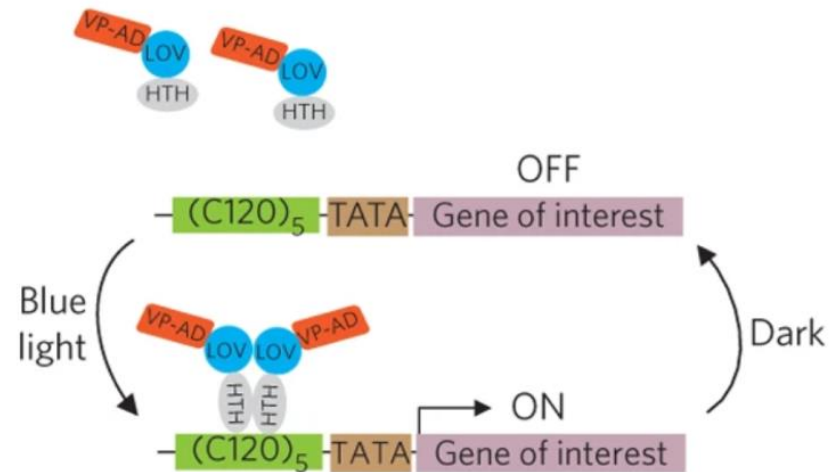
→ LOV and helix-turn-helix domains of EL222 are exposed

→ binds to the DNA sequence C120




DNA transcription is promoted by the approaching AD (activation domain)

- EL222: Protein consisting of a photoreceptor domain and a DNA-binding domain.
- EL222 promoter: EL222 binding site inserted upstream of part of the promoter.
- Activation domain: contains the transcription activator VP16.



# 1-3. Exploring the use of a new third colour

Current candidate.

Green: ? 

UV: UVR8 

(Photoreceptor proteins from plants.)

The construction of a third light acceptance system is being worked on, exploring its feasibility through experiments and other means.

## 2. Control of substance production by the order of light

Target:

When light is input in the correct order → the substance is produced (reporter expression).

When light is input in the wrong order → the yeast dies (kill switch activated).

In order to fulfil the function of the required gene circuit without over-complicating it, several ideas were considered through repeated computer simulations.

This time we use the 'Cre/loxP system'.

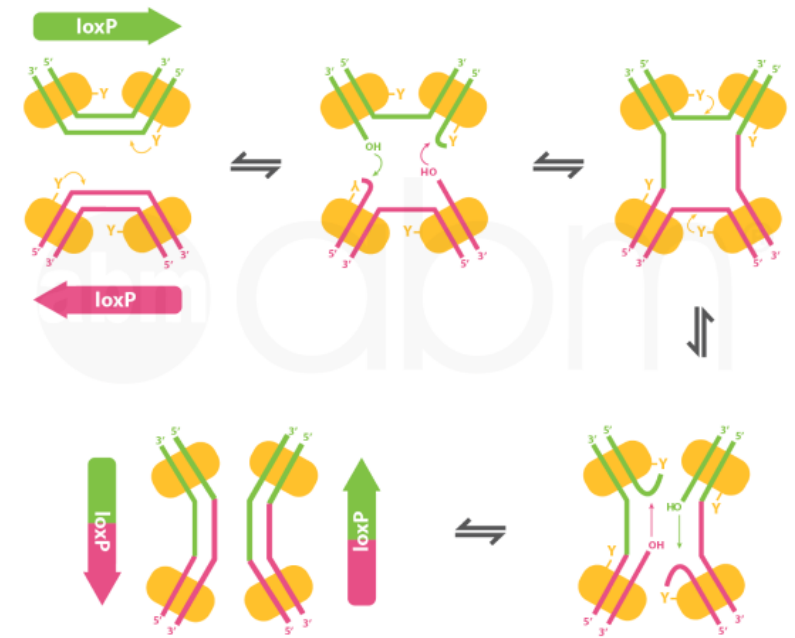
## 2-1. Cre/loxP System

What is Cre/loxP system ?

Recombination reaction consisting of a DNA sequence called 'loxP' and a DNA recombinase called 'Cre' from bacteriophage P1.

The DNA sequence between the two loxP sequences is cut into rings when the loxPs are in the same direction, and the sequence is inverted when they are in different directions.

Mechanism of Cre-Lox Recombination



## 2-2. Cre/loxP System

What can the Cre/loxP system do?

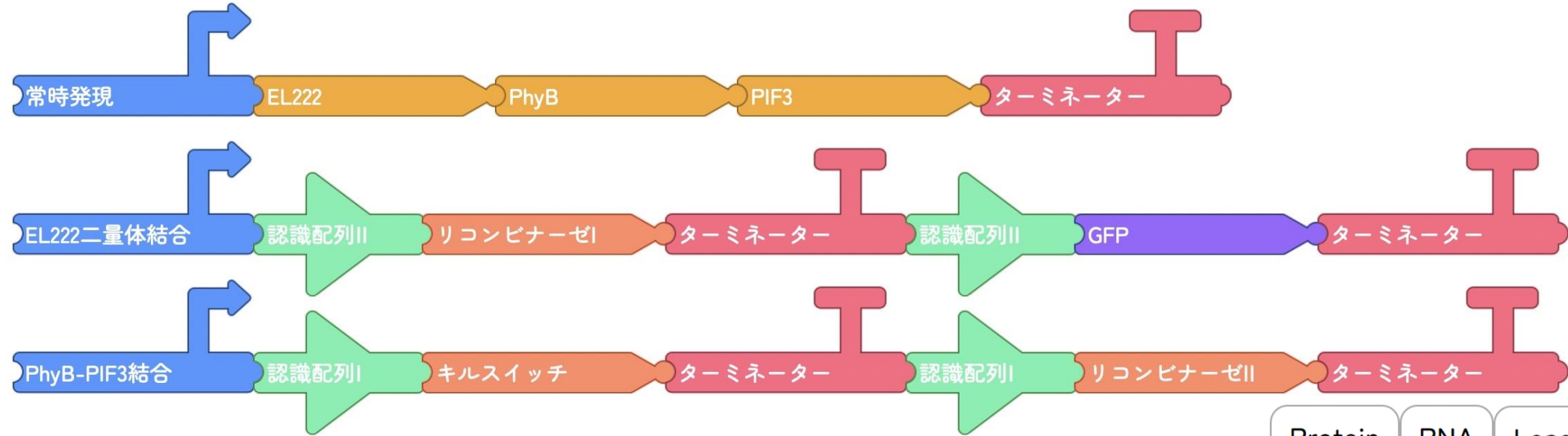
- Eliminate gene expression by cutting out sequences flanked by loxPs.
- Attach the Cre gene to a tissue-specific promoter and cut out the terminator between the loxPs, resulting in Cre-dependent high expression of the gene, etc.

## 2-3. Cre/loxP System

Order-recognition system.

Design genes with a light stimulus-dependent promoter upstream, flanked by loxP sequences on either side of the kill switch gene, so that genes further downstream are expressed only when they are cut out.

By using multiple Cre and loxP pairs, the kill switch gene can only be correctly triggered by light stimulation in a specific order, resulting in normal expression (otherwise the kill switch is activated).



Protein

RNA

Load

Import UTokyo 2022 Project

# Cre/loxP System (Genochemistry)

### 3. Material production to be locked

Investigate how the project can be implemented in society, mainly through human practices.

→ Antibody medicine and biosecurity and its use in economic security and material technology.

# Conclusion

So far, a mechanism has been developed whereby **red** / **blue** / **red** + blue light causes gene expression.

iGEM UTokyo aims to create a system that produces different substances in different orders, such as **red** → **blue** and **blue** → **red**.

→ Various patterns of expression are possible with fewer types of light!

