

The slide features a light yellow background with a dark blue border. It is decorated with various scientific and mathematical icons in blue, orange, and yellow. In the top left, there is a blue Erlenmeyer flask, a yellow pill bottle, a blue circle with a diagonal line, a yellow circle with a diagonal line, and a blue right-angled triangle. In the top right, there is a yellow magnifying glass, an orange Erlenmeyer flask, a blue round-bottom flask, and several asterisks. In the bottom left, there is a blue Erlenmeyer flask, a yellow syringe, a blue beaker, and several asterisks. In the bottom right, there is a blue right-angled triangle, a yellow pill, a blue molecular structure, and a yellow radiation symbol. The text is centered in the middle of the slide.

What is **iGEM** all about ?

Introduction to the competition and our project



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THE TOPIC

Describe the topic of the section





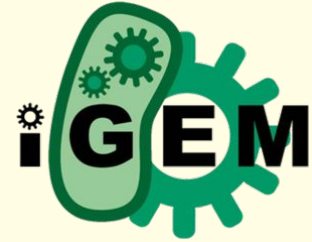
Introduction to iGEM

"Have fun with synthetic biology!"

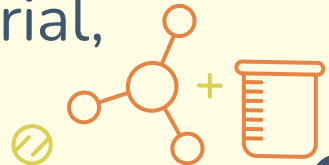




iGEM



- Stands for “International Genetic Engineering Machine Competition”
- Aims to encourage students to learn, design and build systems based on synthetic biology
- From 2003 to now, participants worldwide have contributed their work to agricultural, industrial, medical, environmental aspects



* An iGEM project consists of... *

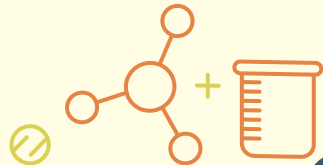
- **Wet lab:** conduct experiments and construct the biological system
- **Dry lab:** includes **modeling** and **device**
 - (1) Modeling: enables prediction of results under different input parameters
 - (2) Device: hardware/software product that assists in the operation of the biological system
- **Human practice:** connection of our project to the “outside world”
 - (1) Expert consultation: clarify project goal from a professional perspective
 - (2) Marketing and survey: understand customer needs, potential investors *
 - (3) Collaboration and partnership: create bonds with other teams
 - (4) Education: enhance understanding of synthetic biology in community
- **Others: Safety and responsibility** (Ethics, contamination...)



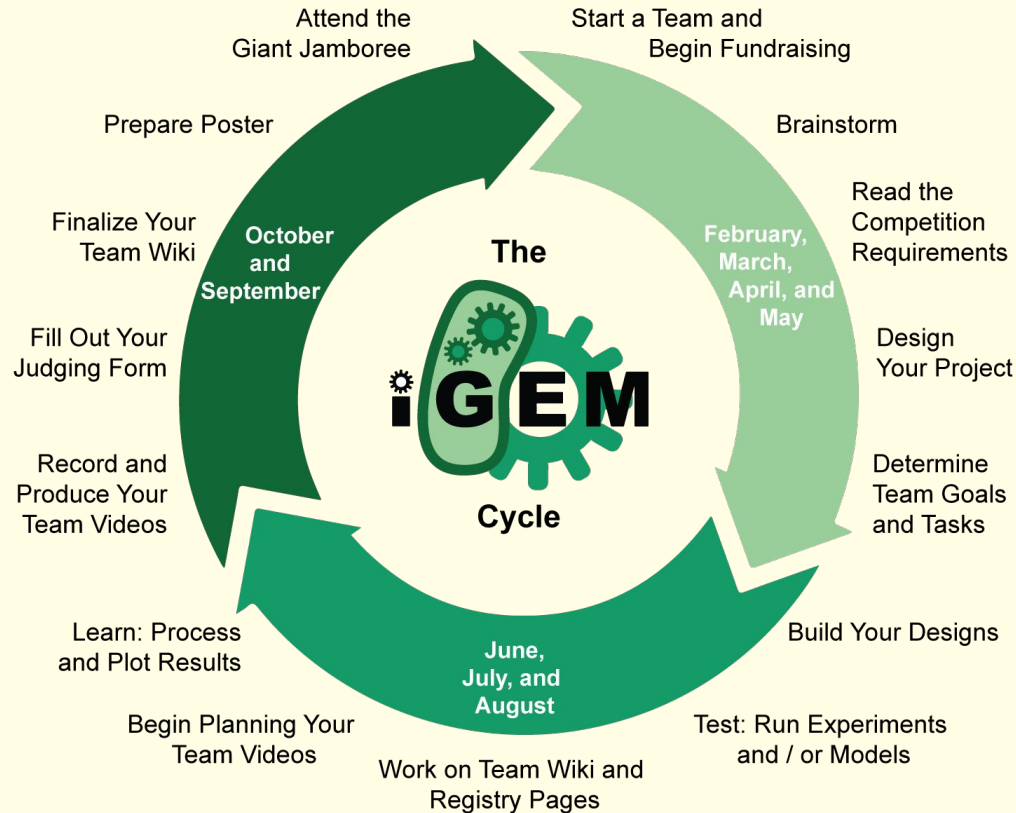
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The iGEM cycle

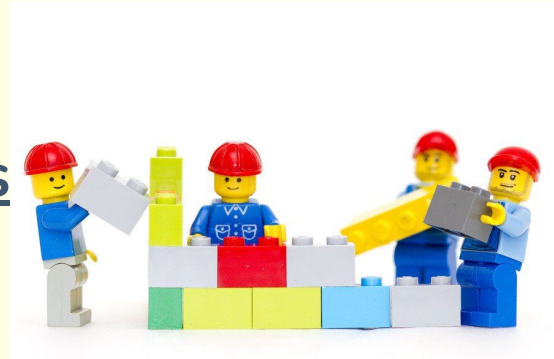


* What is Synthetic biology? *

- To apply and assemble different genetic parts to construct new devices and systems (think of lego bricks!)
- Genetic parts: plasmid backbones, primers, promoters, protein coding sequences, RBS, terminators...






















- • Where to find?

Registry of Standard Biological Parts





BioBricks

 promoter	 primer binding site
 cds	 restriction site
 ribosome entry site	 blunt restriction site
 terminator	 5' sticky restriction site
 operator	 3' sticky restriction site
 insulator	 5' overhang
 ribonuclease site	 3' overhang
 rna stability element	 assembly scar
 protease site	 signature
 protein stability element	 user defined
 origin of replication	

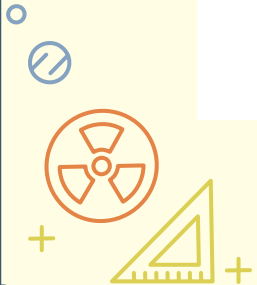
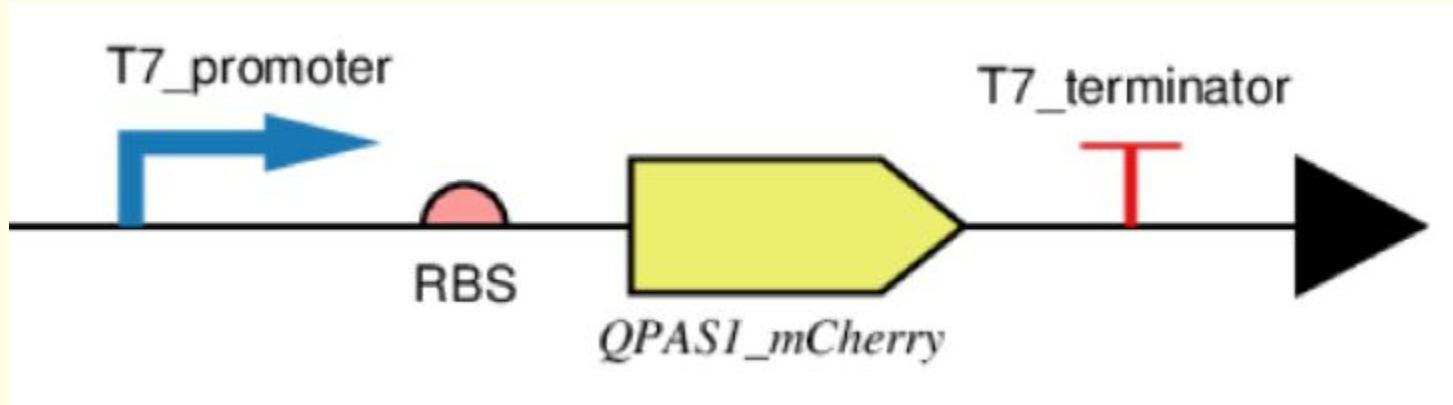


- **Promoter:**
DNA sequence that initiates transcription of downstream DNA
- **CDS:** DNA that encodes protein
- **Ribosome binding site (RBS):**
recruits ribosome during the initiation of translation
- **Terminator:**
palindromic sequence that forms a G-C rich stem loop, stops transcription
- **Primer:**
Single-strand sequence for initiation of DNA synthesis
- **Degradation tag:**
enhances degradation of upstream protein



* Main biobrick components *

- 4 main components: promoter- RBS- coding DNA sequence- terminator
- Example design:





Promoter

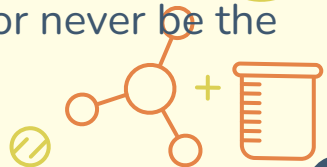
- Promoter activity is determined by **PoPS** (Polymerases per Second), the number of RNA polymerase molecules that pass by (or clear) the final base pair of the promoter and continue along the DNA as an elongation complex
- How to measure promoter activity? **Relative Promoter Units** (RPU), use pSB3K3 as the plasmid and BBa_E0240 (GFP) for measurement
<http://parts.igem.org/Measurement>
- ● **Regulated promoter:** has binding site for protein that attracts/obstructs RNAP binding*
○ to promoter (called operator)
- **Constitutive promoter:** levels of free RNA polymerase are unchanging or never be the limiting factor in transcription initiation



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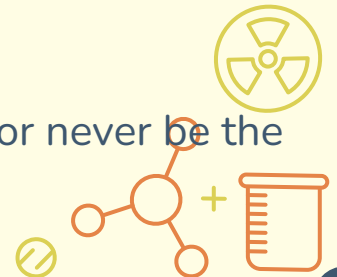
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Promoter

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Ribosome binding site (RBS)

- 150 in Registry, most used **BBa_E0034** (strong RBS)
- Bacterial ribosome binds to particular sequences on an mRNA, primarily the Ribosome Binding Site (RBS) and the start codon (AUG). RBS and AUG must be positioned approximately 6-7 nucleotides apart so they can both make contact with the appropriate parts of the ribosome complex

RBS catalog:

http://parts.igem.org/Ribosome_Binding_Sites/Catalog#Constitutive_prokaryotic_RBS



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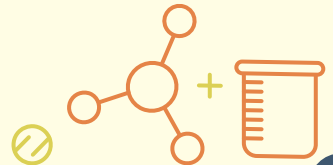
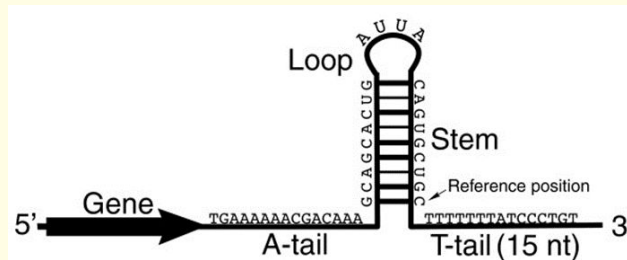




Terminator

- Usually occurs at the end of a gene or operon mRNA and **causes transcription to stop**
- Fall into two categories: (1) rho-independent terminators and (2) rho-dependent terminators. All the E. coli terminators in the Registry are **rho-independent terminators**
- Measure terminator efficiency: defined as **1 - (PoPS_out / PoPS_in)**,
PoPS_in: RNA polymerases entering the terminator (成功), PoPS_out: RNA polymerases exiting the terminator (失敗)

• Terminator catalog: <http://parts.igem.org/Terminators/Catalog>





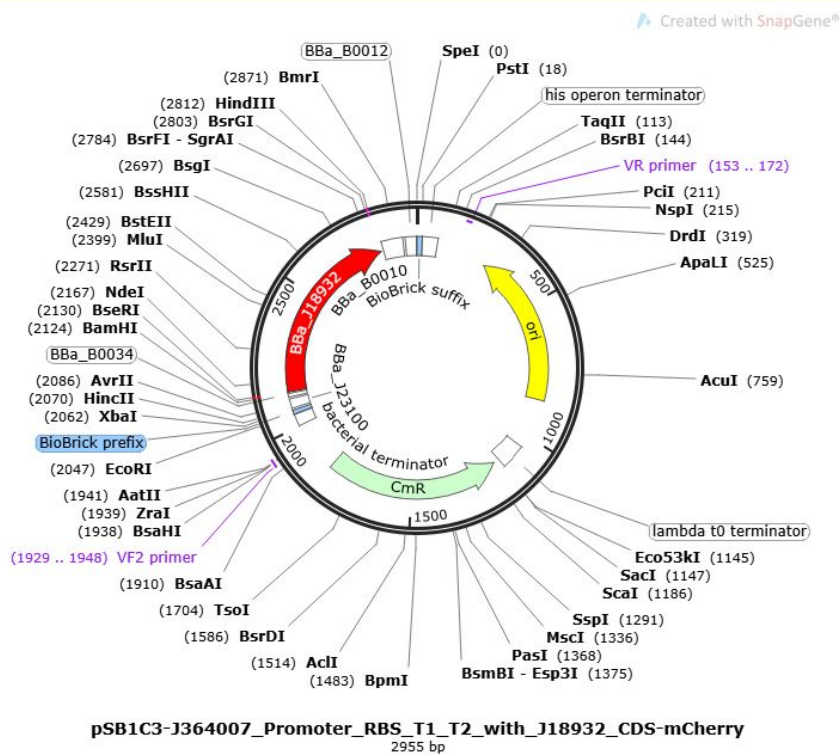
Primer

- Melting temperature T_m : temperature at which 50% of that same DNA molecule species form a stable double helix and the other **50% have been separated to single strand molecules**, calculation: $4^{\circ}\text{C} * (\# \text{ G/C nucleotides}) + 2^{\circ}\text{C} * (\# \text{ A/T nucleotides})$
- When comparing two primers with equal length, the one with the **higher GC content will have a higher melting temperature** (more base pairs)
- For PCR and sequencing applications, primers should have a T_m of **55-65°C**, which generally corresponds to **20-25 nucleotides** in length with about **40-60% GC content***
- Called “oligonucleotides”, not in Registry collection b/c commercial primer synthesis is cheap and fast
- Tools for designing primers and calculate T_m : <http://parts.igem.org/Help:Primers/Tools>

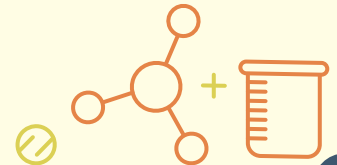




BioBrick assembly



- Create your plasmid, or modify parts of it
- Methods:
 1. Restriction enzymes and ligation
 2. Gibson assembly
 3. 3A assembly
 4. Omega PCR using megaprimers (substitution, deletion or insertion)
- Tools: Snapgene, Snapgene viewer





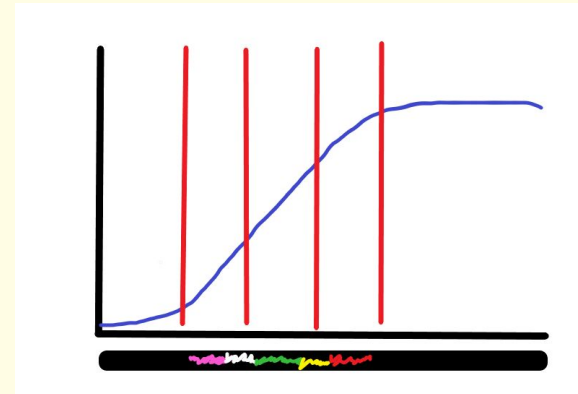
About our project





The aim of our project

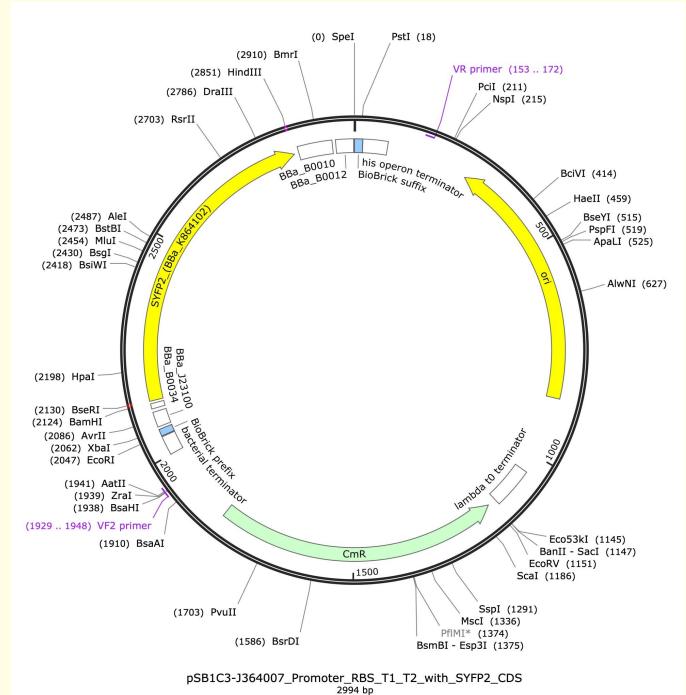
- Our project provide the engineered *E. coli* that express different color on each growth phase, transition, and cell division. This *E. coli* help the researcher to detect approximate status of E.coli without any instrument and trouble. The approximate growth curve on solid medium can also be graphed easily by our project.
- We also design the device and software to provide more accurate result.





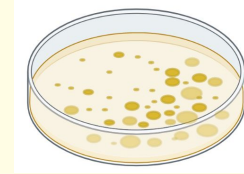
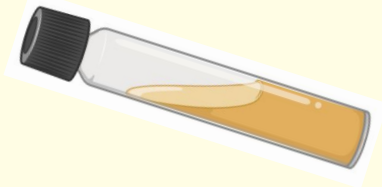
How we done it

- The repeatable, standard growth curve are established on either liquid medium and solid medium
- Each gene are cloned by PCR, and substitute the original construct by omega-PCR
- Promoter are tested with FP
- Fluorescent protein are tested to determine maturation time and degradation time
- The fluorescent intensity with desired promoter are measured and correspond to the standard growth curve we estimated
- The fluorescent intensity-time data are imported to software, providing the accurate system to determined growth curve





liquid and solid culture

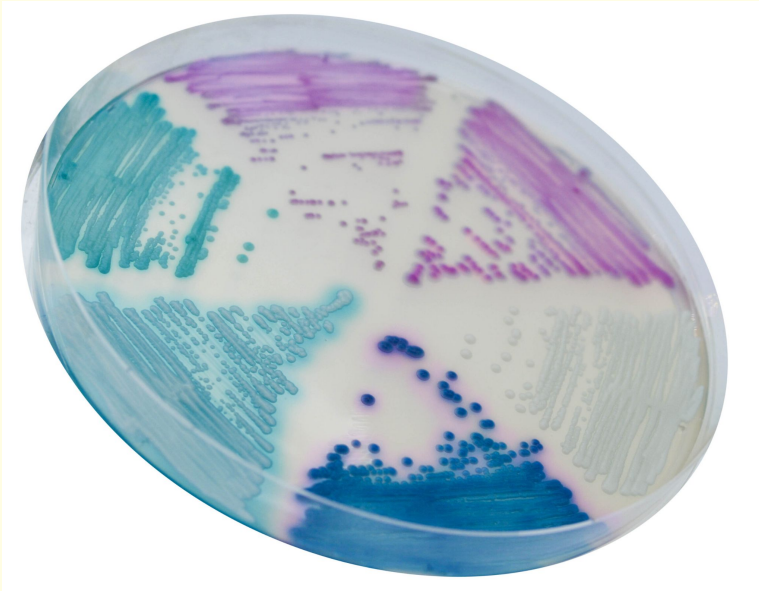


	liquid	solid
component	LB(Lysogeny broth), (Antibiotics)	LB+Agar, (Antibiotics)
colony	mixed, freely suspended	single, Biofilm
environment	low resistance, low pressure	high density, high pressure, EPS(extracellular polymeric substance)
usage	propagation, fermentation test	isolation, identification, motility test
<i>E. coli</i>	Flagellum ↑	Fimbria ↑





Additional: various Mediums



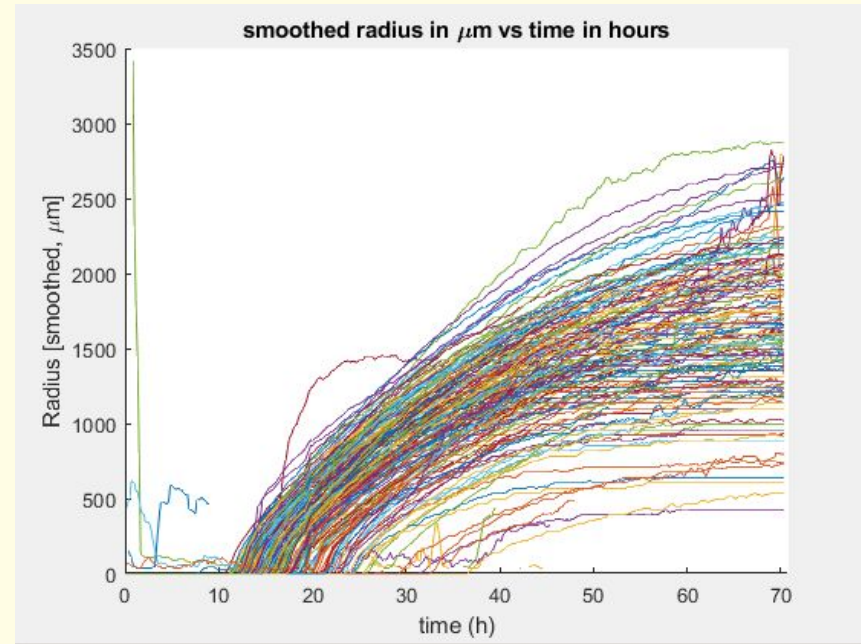


Growth curve established

Liquid culture : OD value

Solid colony : colony size and FP intensity

- Depend on the quality of picture

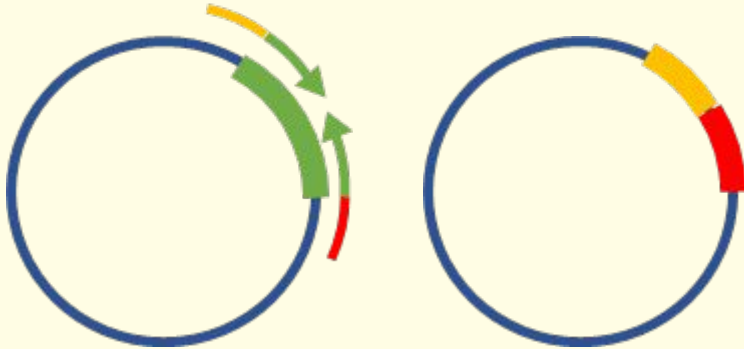




PCR cloning and omega PCR

1st PCR provide the megaprimer that contain the gene we interest

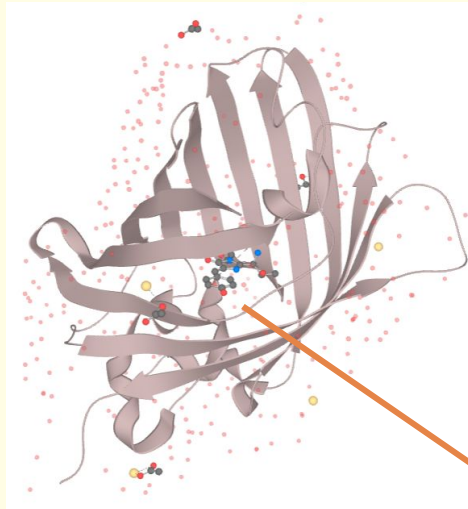
2st PCR could insert or substitute the gene



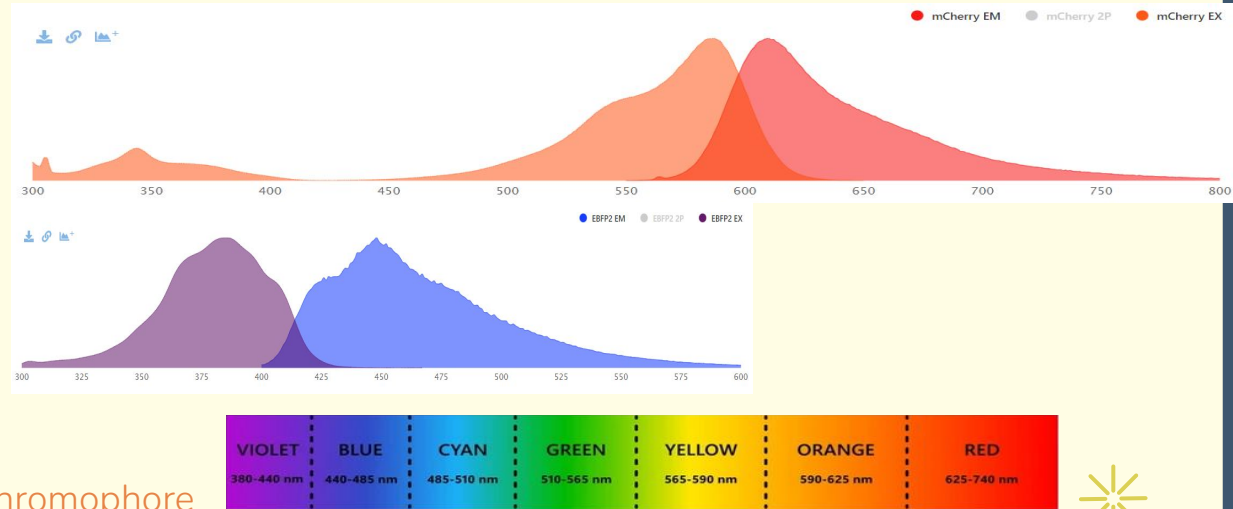


About FP and CP

- **Absorption & Emission** - the chromophore of fluorescent proteins and chromoproteins must be excited by lights with specific wavelengths and then emit light with various wavelengths.



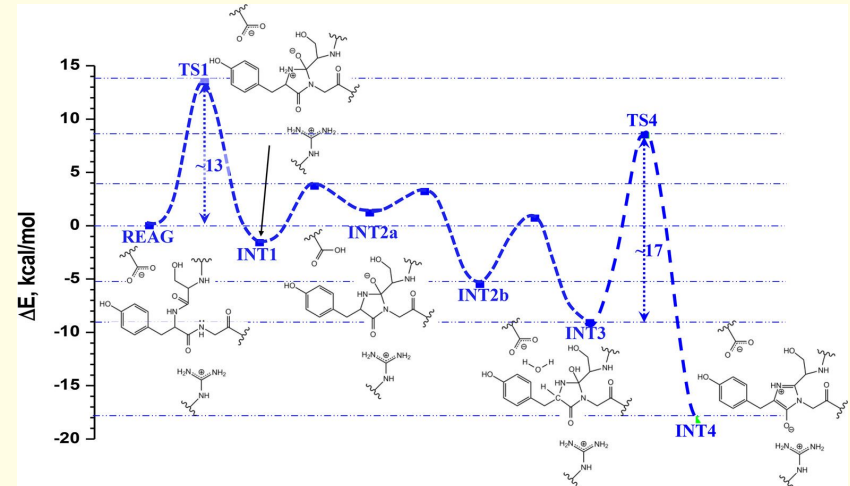
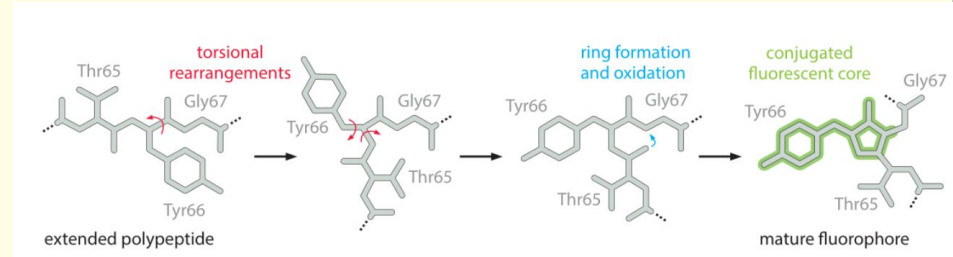
chromophore





About FP and CP

- **Maturation** - normally, protein has rather short maturation time, however, FP has long maturation time
 - Folding
 - Torsional adjustments
 - Ring formation
 - Oxidation

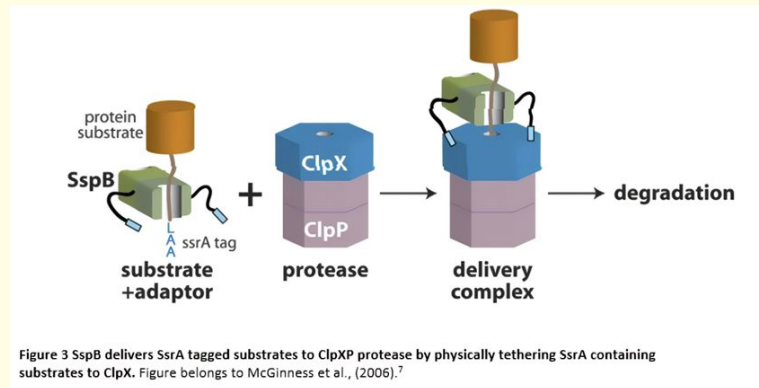




About FP and CP

- **Half life (degradation)**

- SsrA peptide-tag system is conserved across prokaryotic species
- when ribosome get stuck on a broken mRNA, ssRNA or tmRNA will added degradation tag and stop codon, so ribosome can release and be degradation
- Recognize by protease “ClpXP”





Additional: FRET

Förster resonance energy transfer(FRET) - using energy transfer between 2 fluorescent proteins to detect protein-protein interactions or conformation change.

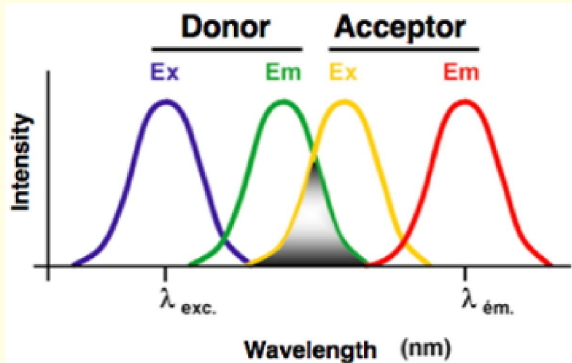
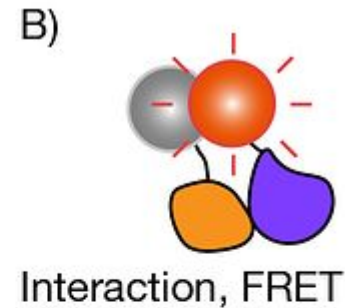
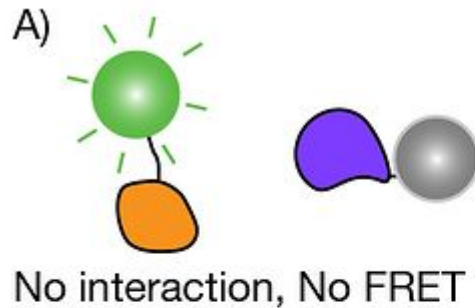
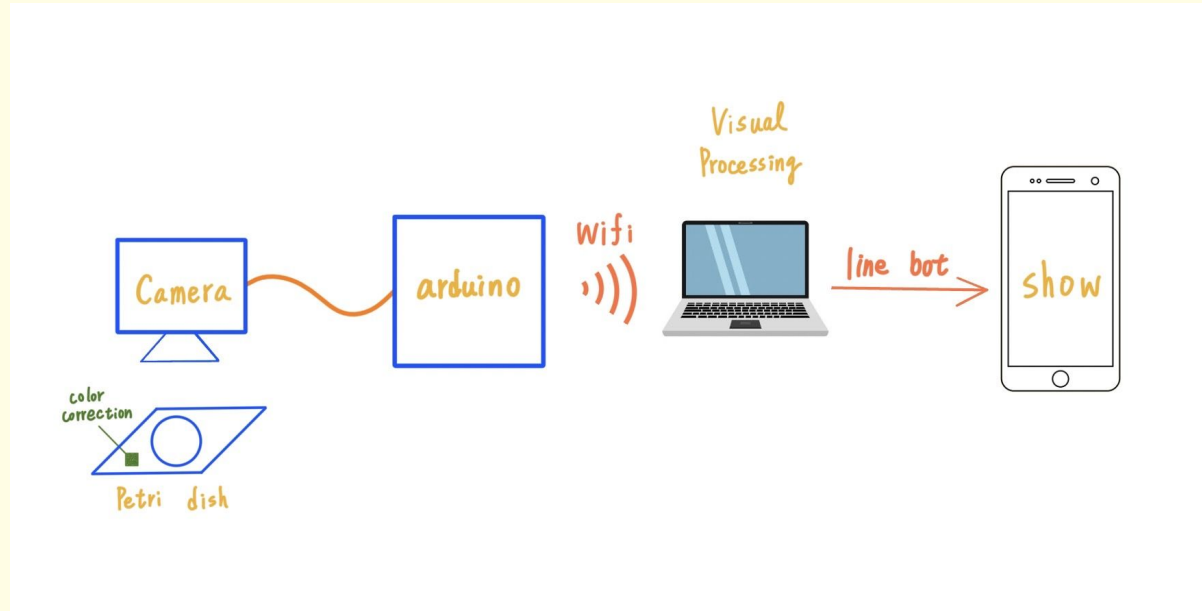


Figure 1: Spectral overlap required for FRET to occur. Modified from Wikipedia, original author Maurel Damien. Accessed 11/4/2014.





Software and device





Advantage of our project

- The remote detection of *E. coli* status
- The approximate measurement without any instrument
- Easy growth curve measurement on solid medium
- Easy and real time cell division measurement of *E. coli*



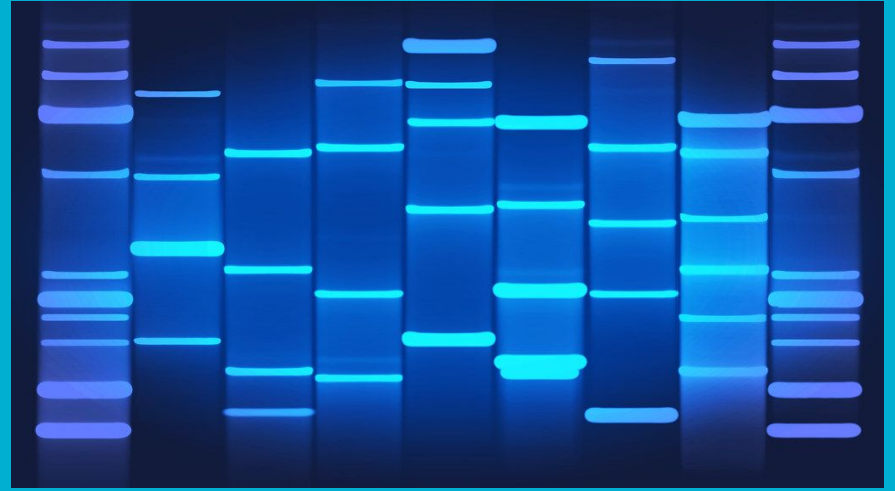


END



Electrophoresis

Introduction by
NYCU Taipei Igem 2022 Team



A technique used to **visualize** (directly observe) the fragments of DNA

After

1. PCR reaction (making many copies of a target DNA region)
2. DNA cloning (trying to "paste" a gene into a circular DNA plasmid)

>>>> Gel electrophoresis

How can we check and see whether our PCR worked, or whether our plasmid has the right gene in it.

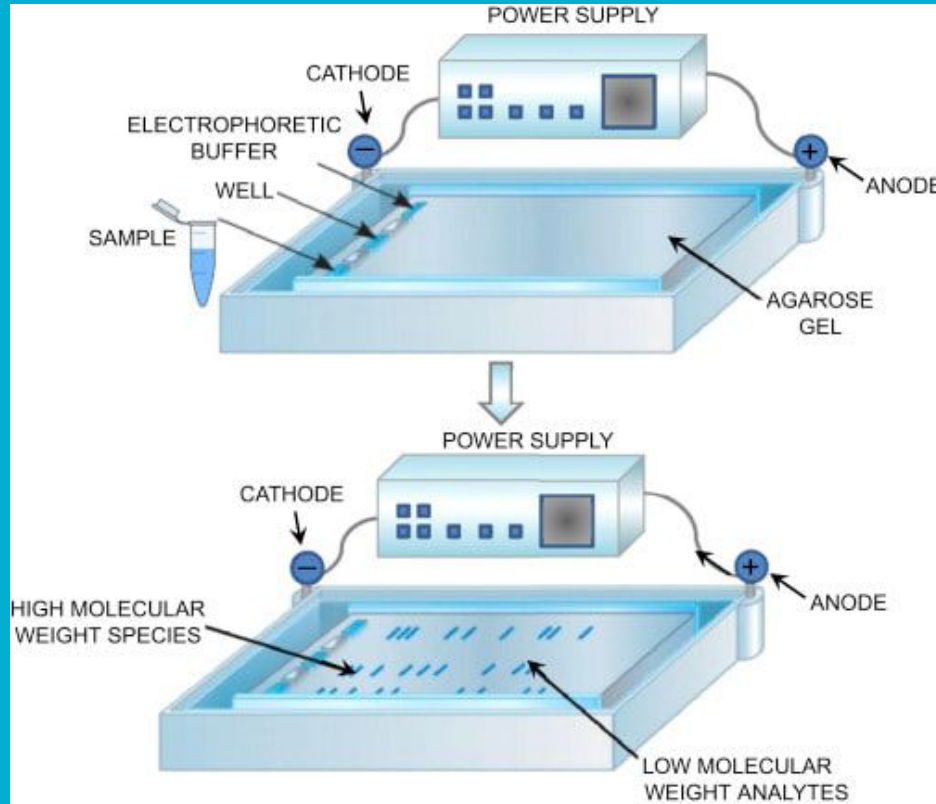


Intro

Gel electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their **size and charge**. Electrophoresis involves **running a current through a gel** containing the molecules of interest. Based on their size and charge, the molecules will travel through the gel in different directions or at different speeds, allowing them to be separated from one another.

All DNA molecules have **the same amount of charge per mass**. Because of this, gel electrophoresis of DNA fragments separates them based on size only. Using electrophoresis, we can see how many different DNA fragments are present in a sample and how large they are relative to one another. We can also determine the absolute size of a piece of DNA.

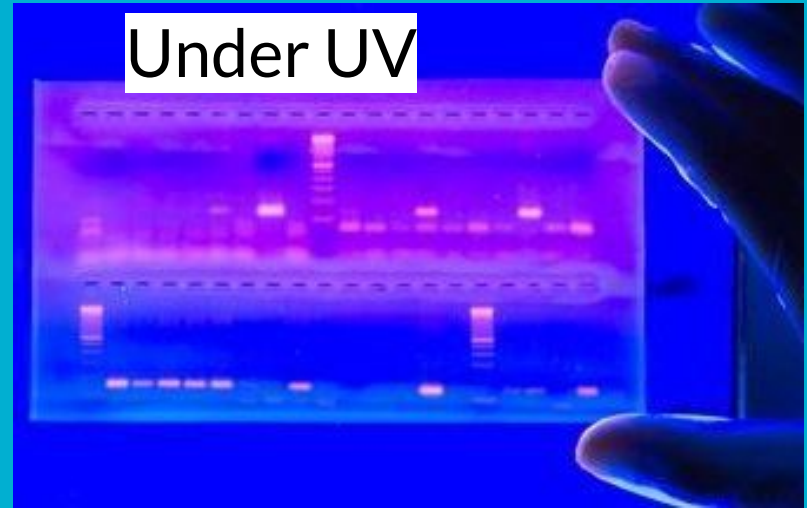
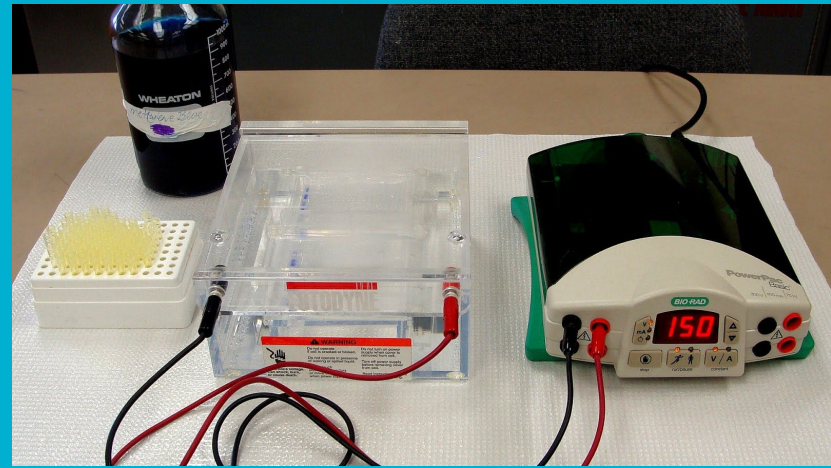
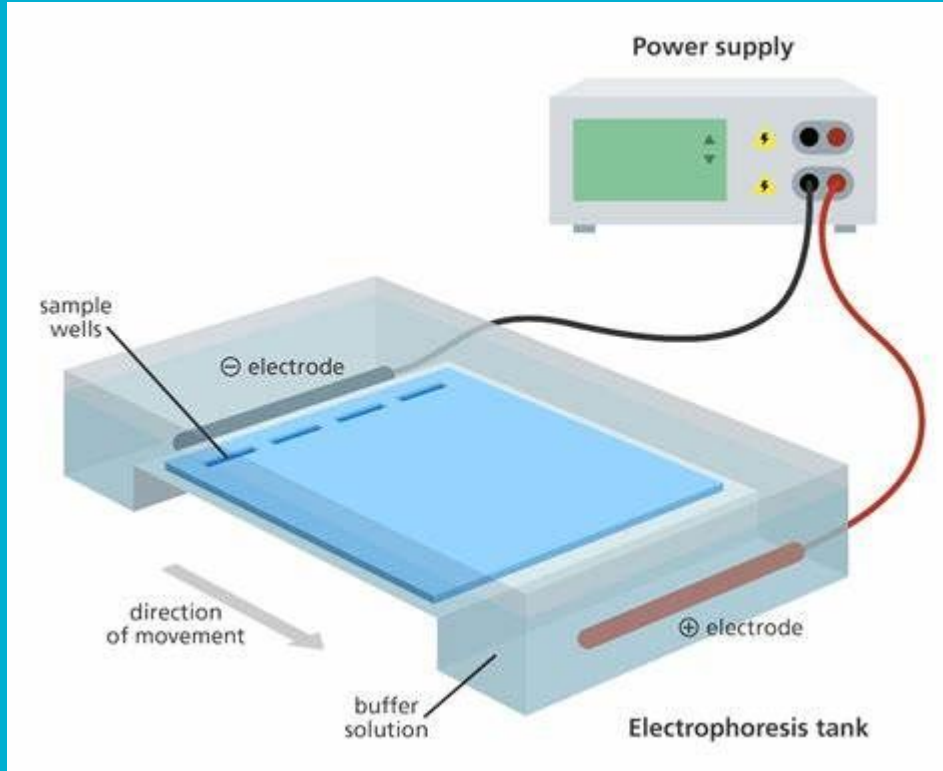
Principle



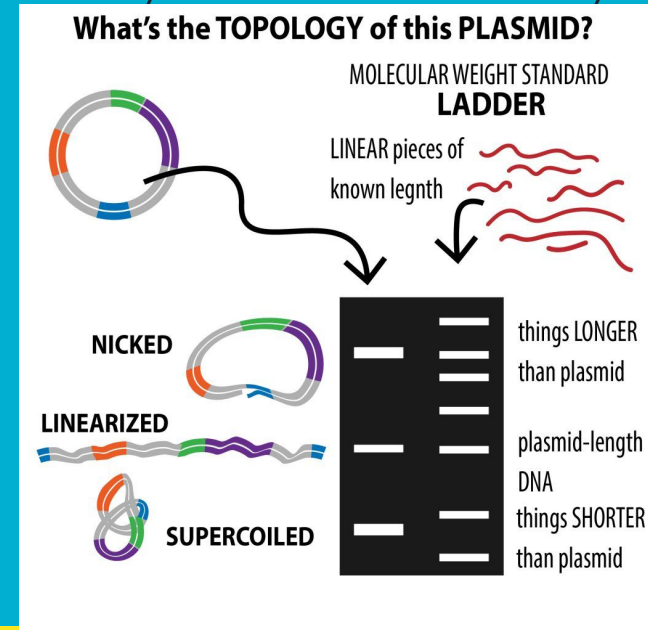
charge-to-mass ratio

this ratio significantly affects the mobility of a **macromolecule** through a **solution** when driven by an electric field (two molecules of identical **mass** but different **charge** will move at different rates in an electric field). Since at neutral pH, the majority of the net **charge** on **DNA** is neg . As **DNA** increases in size, the total **charge** increases at the same rate. The resulting **charge-to-mass** ratio therefore remains constant, and **DNA** fragments of different sizes all move at about the same rate in an electric field. (the only difference is length)

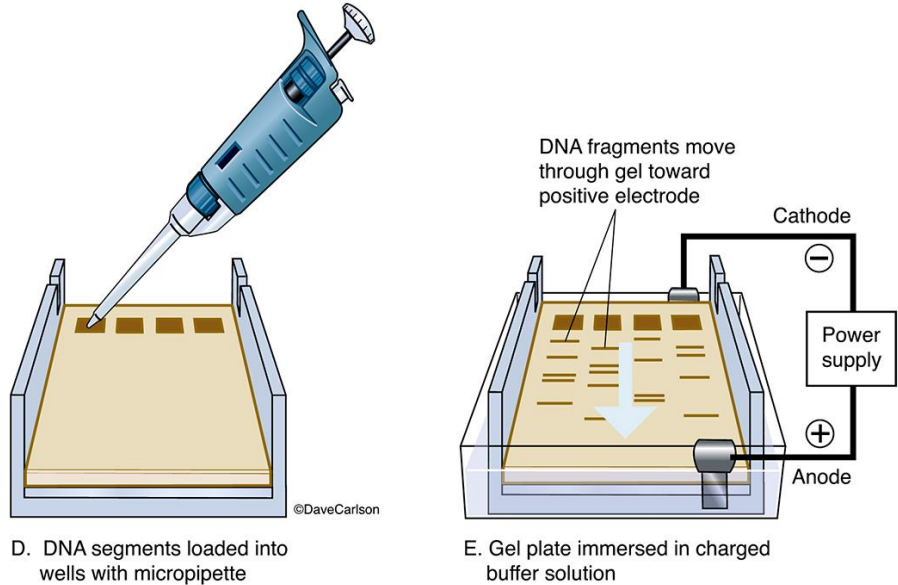
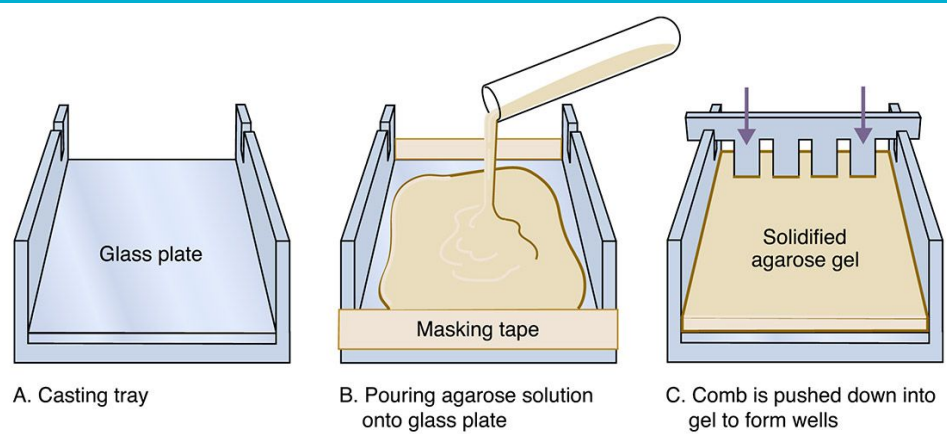
It looks like this :



A collection of DNA fragments separate by length because they are all the same type of molecule. In general, the only meaningful difference between the various fragments should be their length. However, there are some exceptions to this rule. For instance, some DNA molecules are circular (like bacterial plasmids), while others are linear. Circular DNA molecules may run differently than linear ones through a gel. Plasmids, for example, can exist in a form called "supercoiled," in which they actually move *faster* through a gel than they should for their size, because they have twisted into a skinny shape that can move through the gel more easily.



Procedure



- **DNA ladder**= a standard reference that contains DNA fragments of known lengths.

Commercial DNA ladders come in different size ranges

The *bp* next to each number in the ladder indicates how many *base pairs* long the DNA fragment is.

- A **lane** is a corridor through which DNA passes as it leaves a well.)
- A **band**= a well-defined “line” of DNA on a gel .

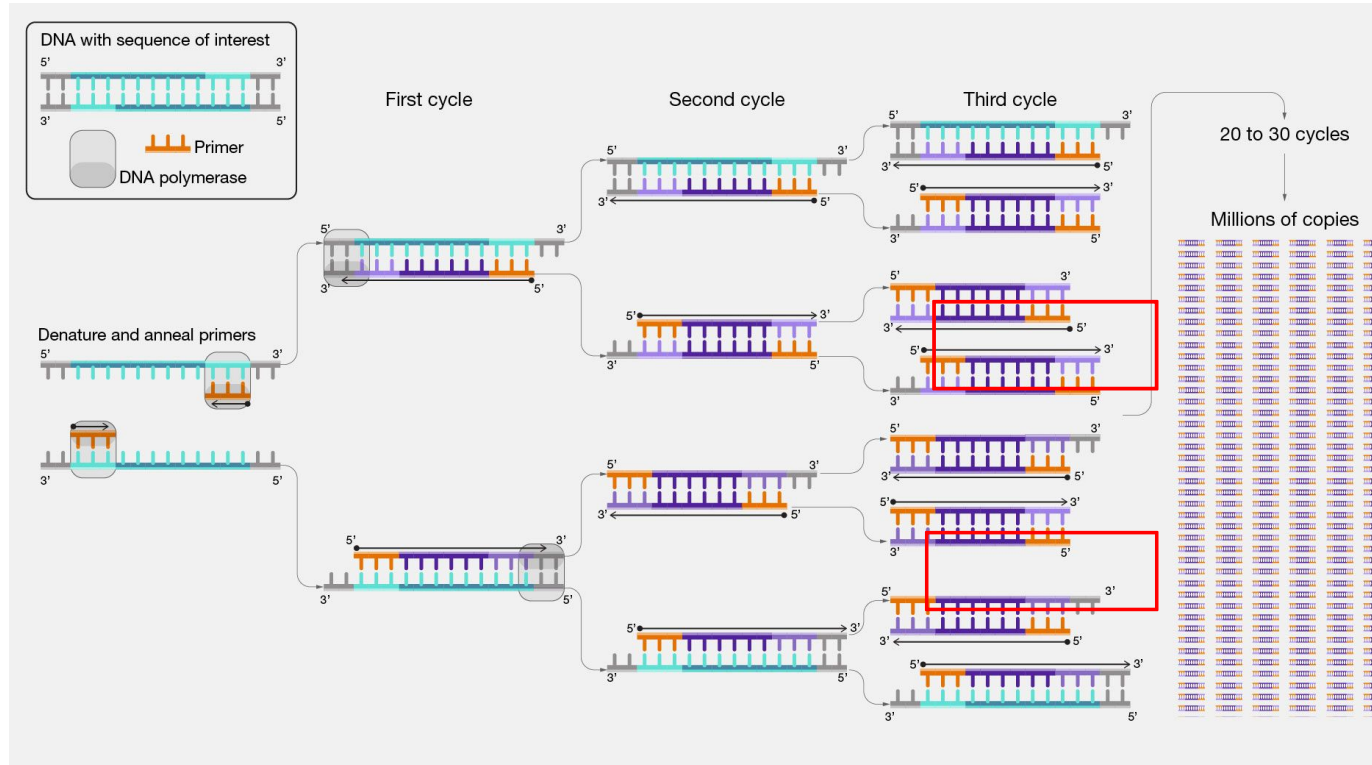
Each band contains a large number of DNA fragments .A single DNA fragment (or even a small group of DNA fragments) would not be visible by itself on a gel.By comparing to the DNA ladder, we can determine their approximate sizes. For instance, the bright band on the gel above is roughly 700 base pairs (bp) in size.



Introduction to the PCR technique

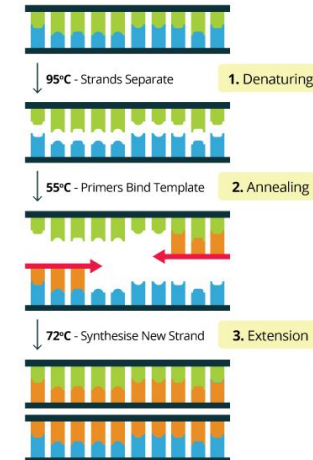
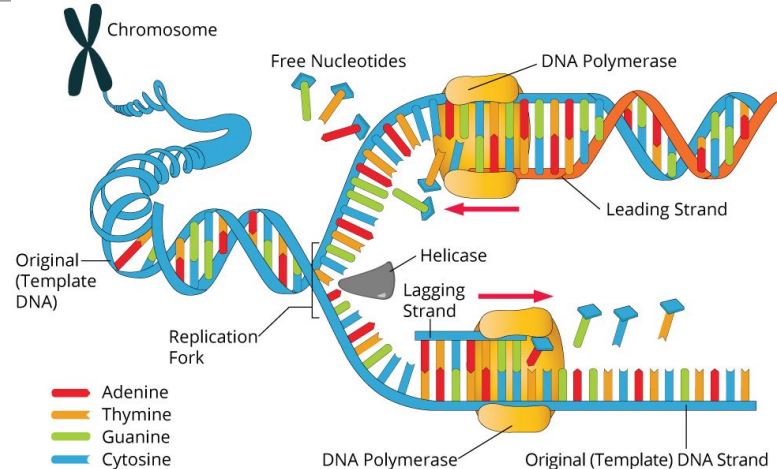
2022.08.01

Outline of PCR



PCR vs DNA replication

Steps	DNA replication	PCR
Double strand unwinding	helicase and topoisomerase	raising temperature
Primer annealing	generated by primase	synthesized primer, anneal by temperature cool down
Complementary strand synthesis	DNA polymerase + dNTP	



The history of PCR: DNA replication in vitro

- Manual PCR first invented in 1983 by Kary Mullis
- Automated PCR after discovery of the “thermostable” Taq polymerase from *T. aquaticus*



Thermus aquaticus in Yellowstone national park



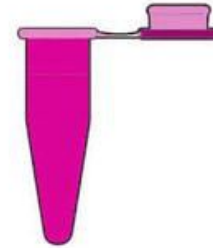
Prof. Chien Chia Yun

What do we need? The basics...

1. **Our DNA template**
2. **Primers** (forward + reverse)
3. **dNTP** (dATP, dGTP, dTTP, dCTP)
4. **DNA polymerase**
5. **PCR buffer/ PCR tube/ thermocycler**



Buffer solution



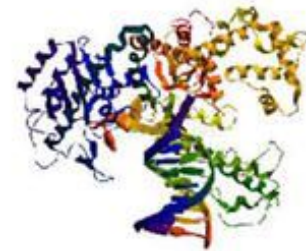
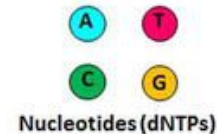
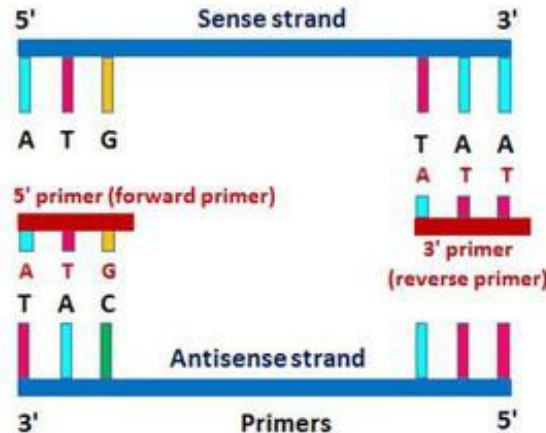
PCR Tube



Thermocycler



DNA template (DNA region to be amplified)



Taq polymerase

Different types of PCR

RT-PCR

- Reverse transcription PCR: synthesize DNA from RNA fragment
- Materials: RNA template, primers, dNTP, reverse transcriptase, DNA polymerase
- Applications: test whether a person is infected by virus (genome mostly RNA)

Step 1. Reverse transcription: Reverse transcriptase synthesizes single-strand cDNA from RNA fragment

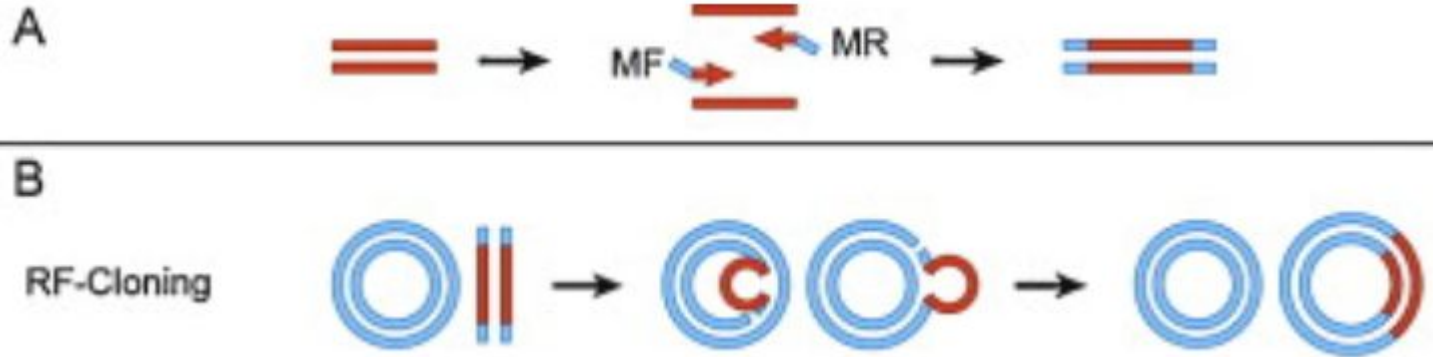
Step 2. PCR: DNA polymerase synthesizes the complementary strand of DNA to form double strand



<https://www.youtube.com/watch?v=XH6vIBLwC2M>

RF(restriction free)-PCR

Restriction-free (RF) cloning provides a simple, universal method to precisely insert a DNA fragment into any desired location within a circular plasmid, independent of restriction sites, ligation, or alterations in either the vector or the gene of interest. (from PubMed)

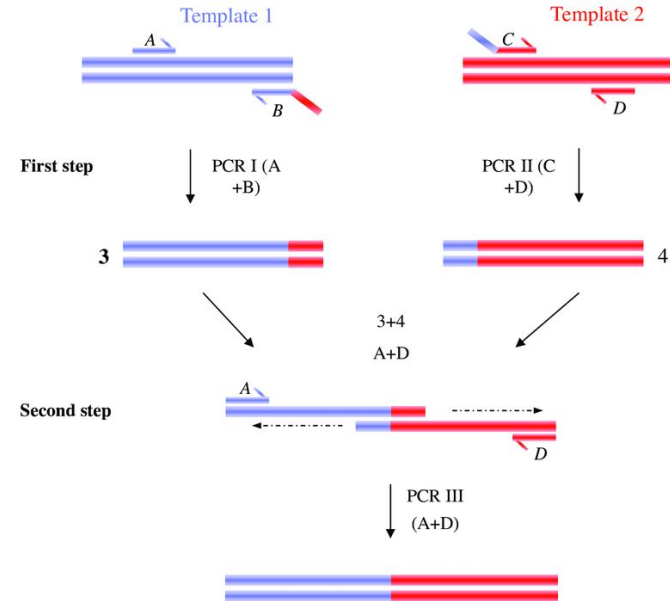


overlap extension PCR (OE-PCR)

enables the ligation of two fragments without using restriction enzyme

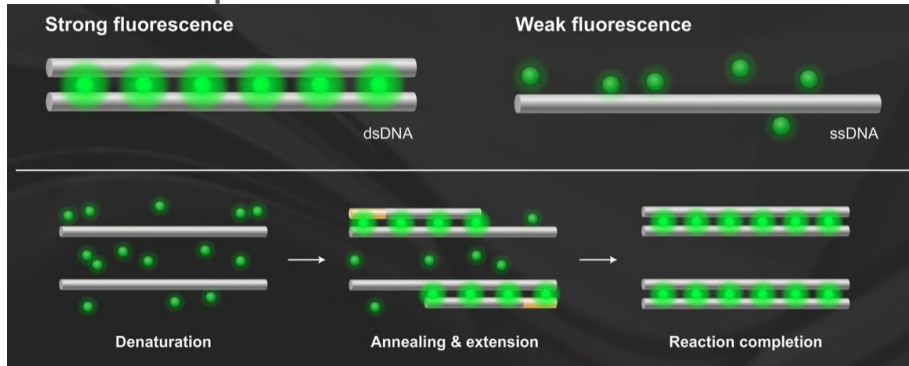
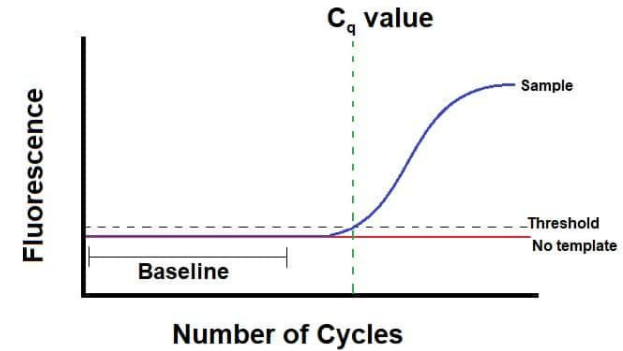
First step. Make fragments with flanking region by specially-designed primers by PCR

Second step. perform overlap PCR

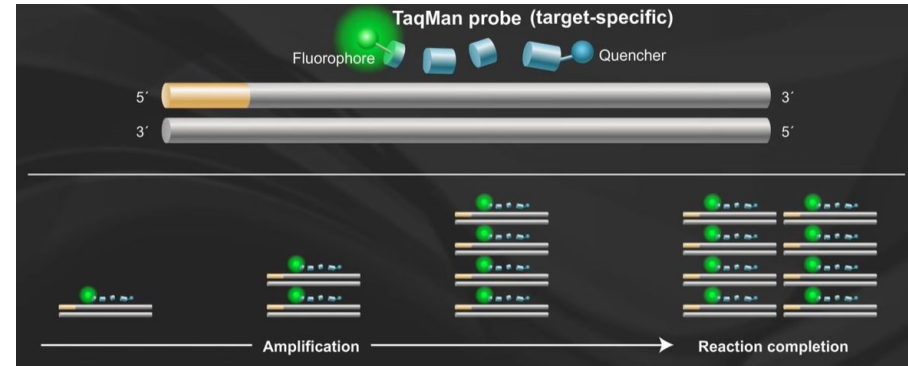


real-time PCR / qPCR

- materials: thermocycler + light source + fluorimeter
- measures DNA amplification by fluorescent signal increase at each PCR cycle (real-time)
- C_q value is the PCR cycle number at which your sample's reaction curve intersects the threshold line



SYBR assay (DNA-binding dye)

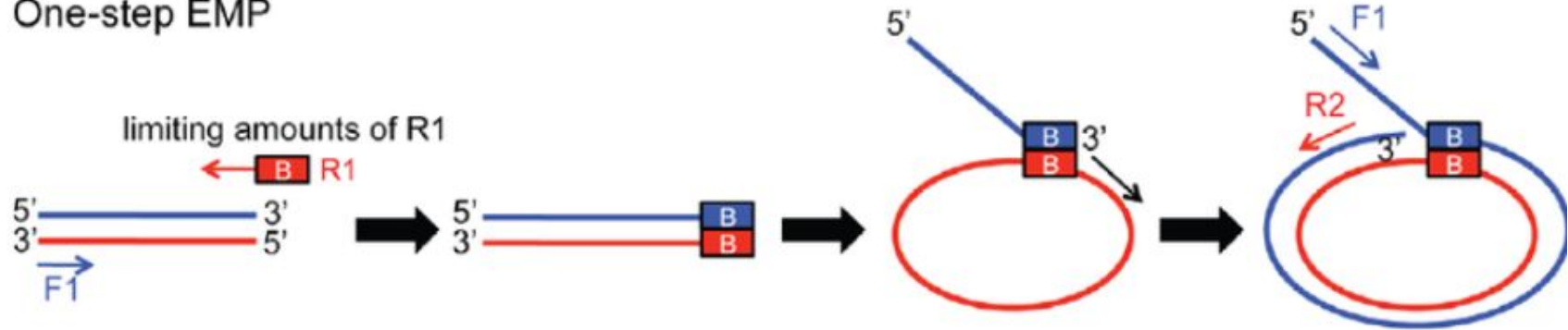


TaqMan assay (target-specific probe)

Exponential Megapriming PCR (EMP) Cloning

compare with RF-PCR

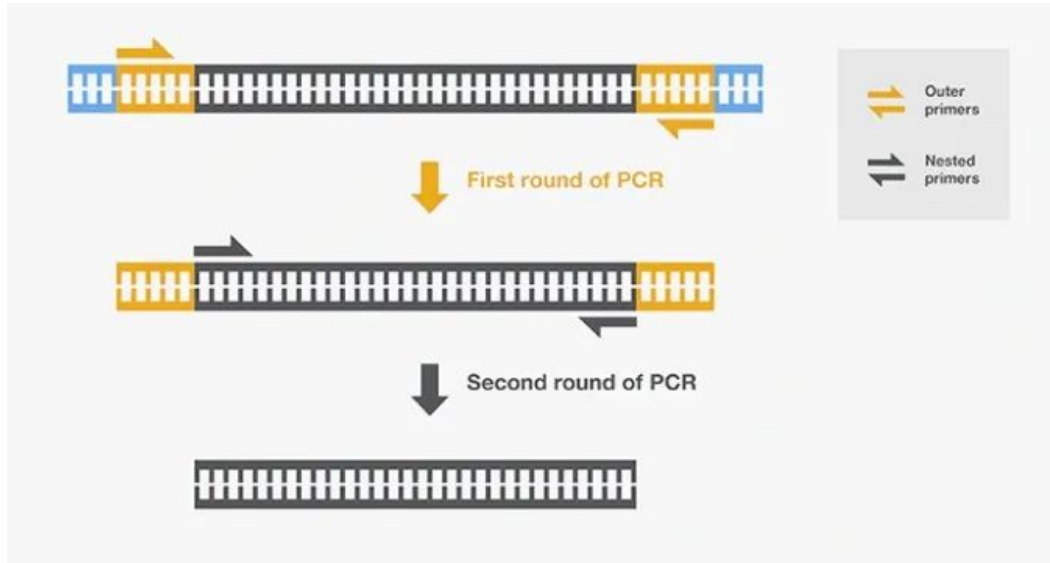
One-step EMP



nested PCR

nested PCR provide more specific of our pcr product.

it narrow down the range of 2st pcr thus enhance the specificity

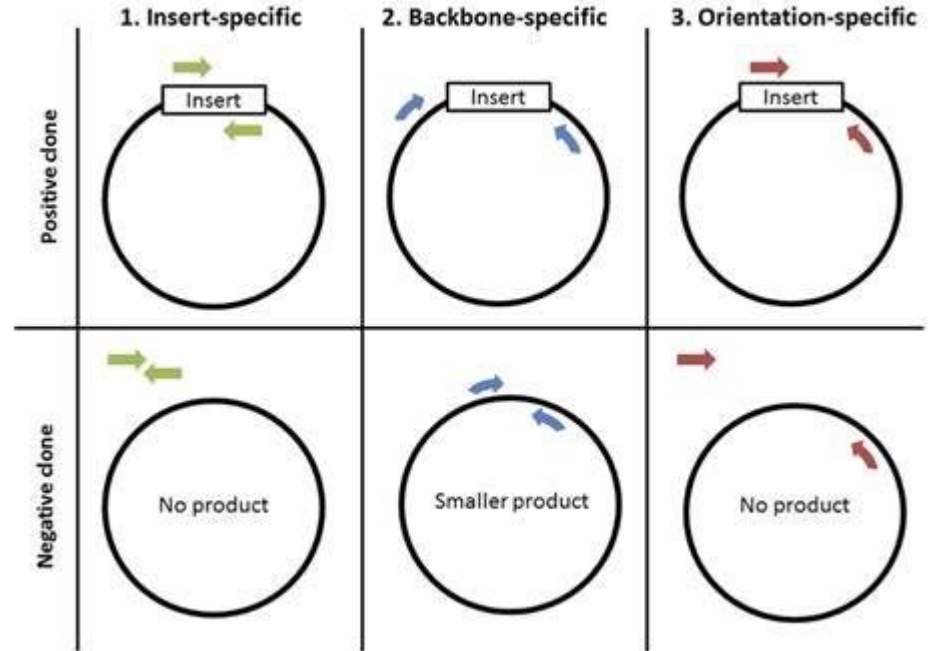


<https://www.youtube.com/watch?v=nHCjgo2Ze0o>

colony PCR

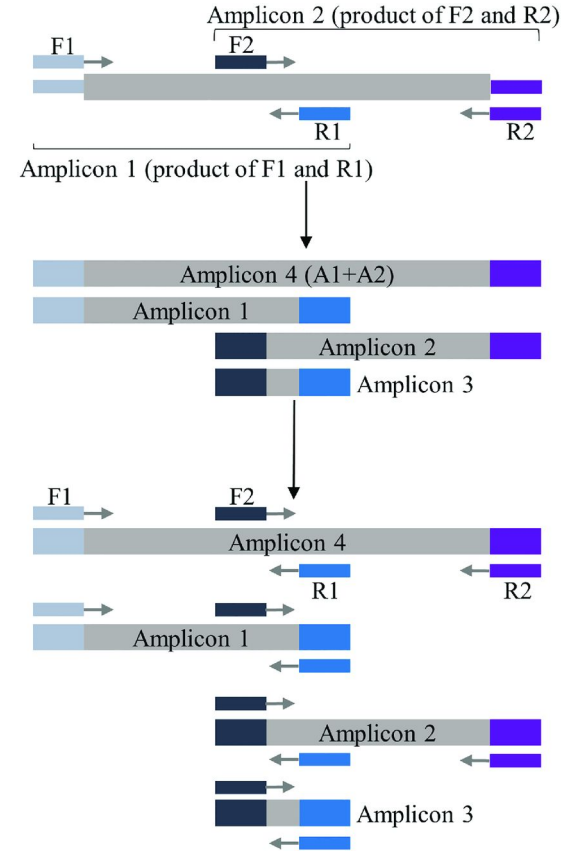
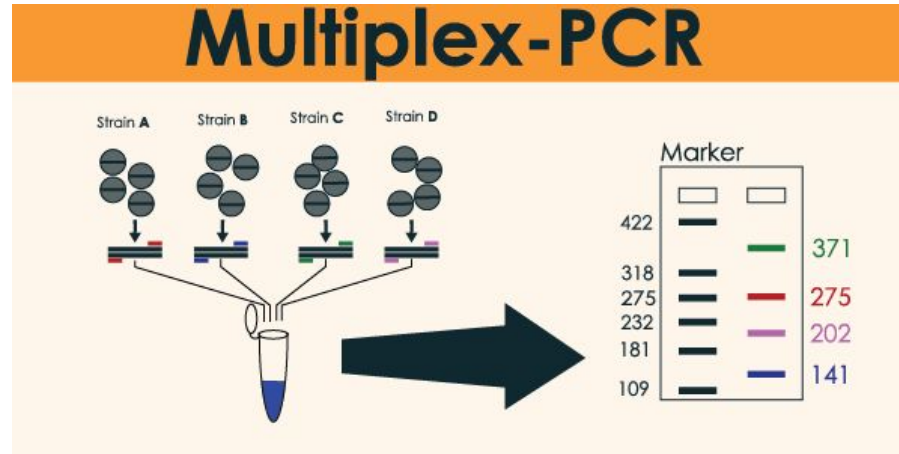
colony pcr is the way to check whether the transformation is success.

note that there is the error in photo



multiplex PCR

multiplex PCR enable simultaneous amplification of several interset target by more than one pair of primer



TD-PCR vs. Gradient PCR

[Touch down PCR - YouTube](#)

[Gradient PCR](#)

digital PCR

digital PCR