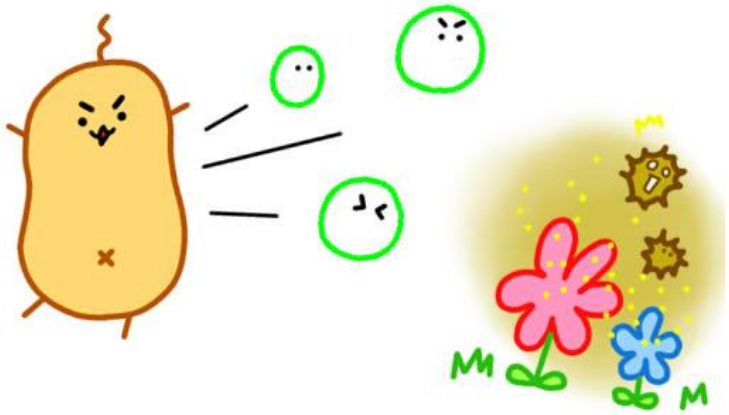
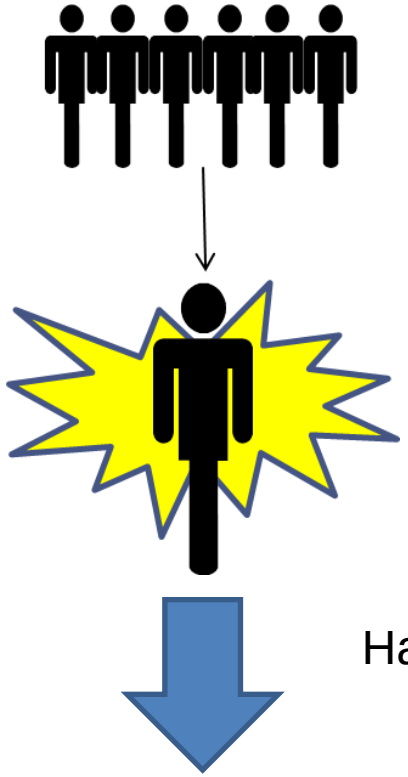
Hay fever curE.coli



KAIT-JAPAN

Background

In JAPAN



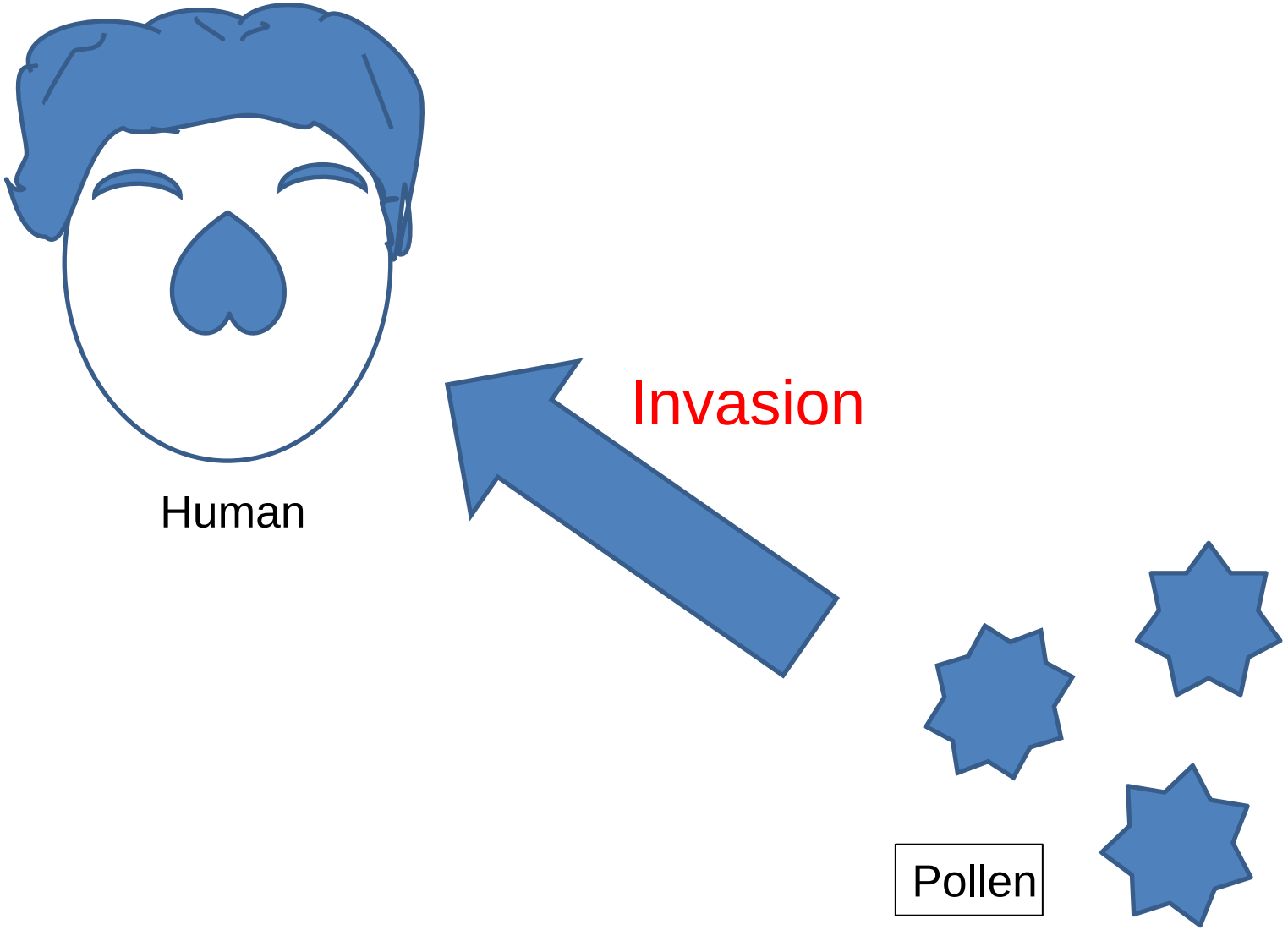
One of six person are developed hay fever.

Hay fever measures…

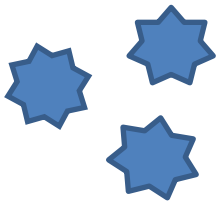
But …

There is a difference in the case of an individual drug of hay fever, some people side effects such as thirst and sleepiness may appear.

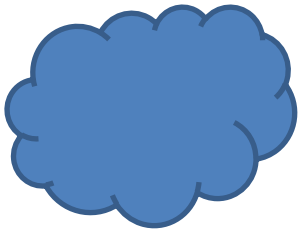
What is Hay fever?



Invasion

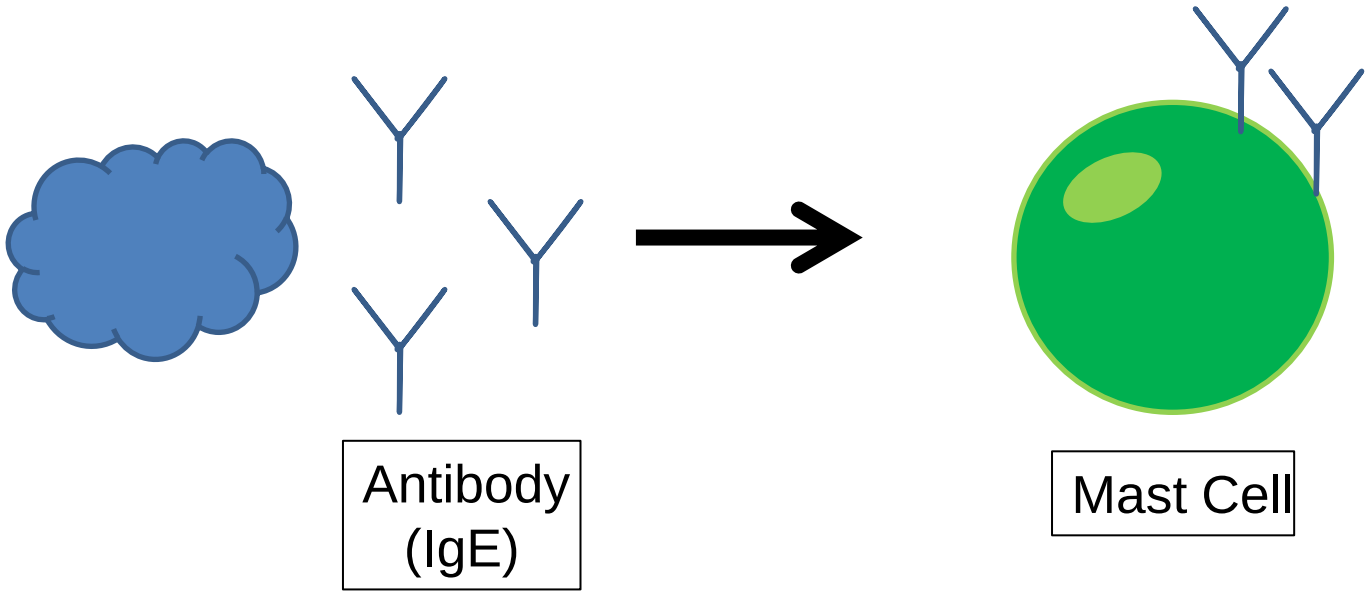


Pollen

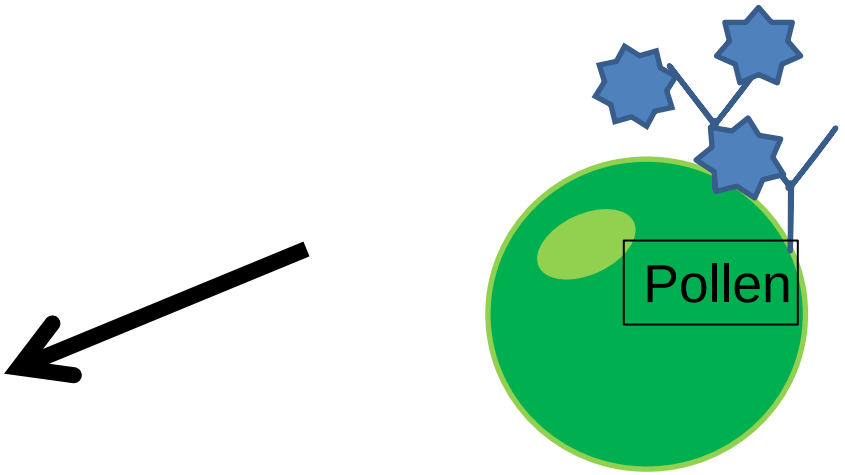


Lymphocyte

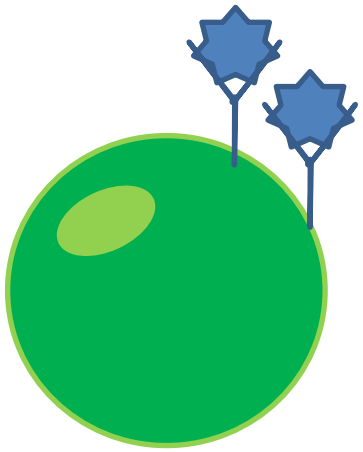
Invasion



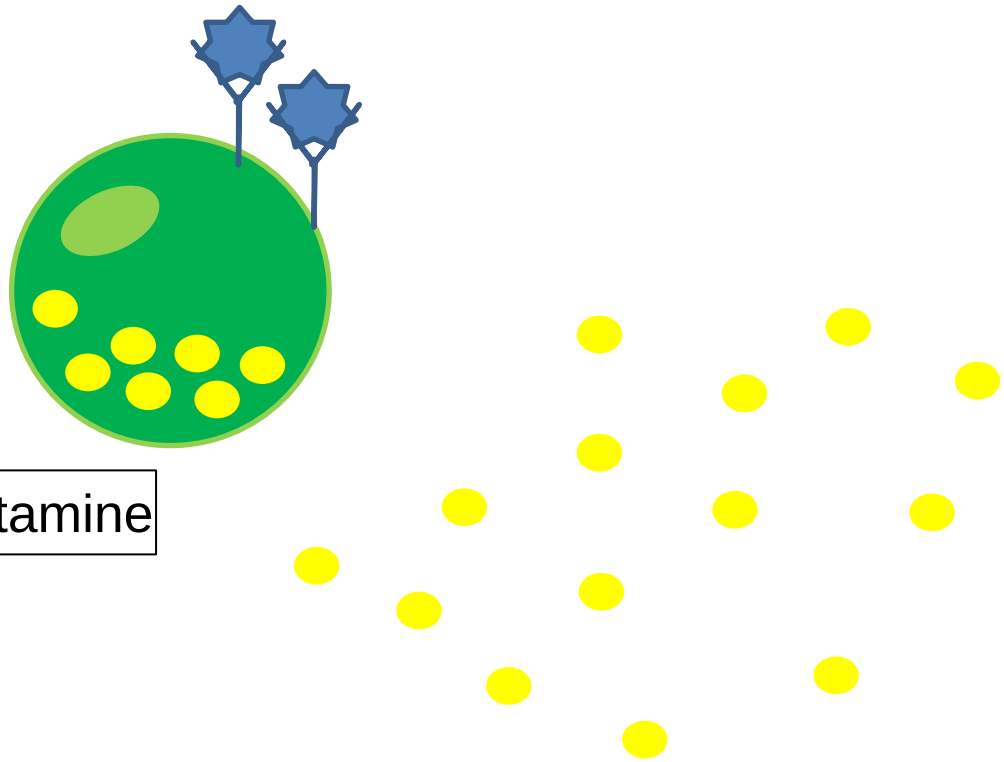
Invasion



Invasion



Invasion



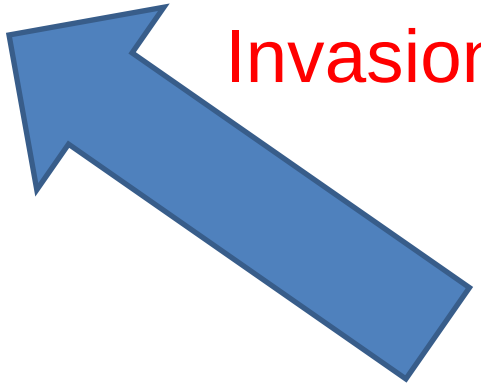
Histamine

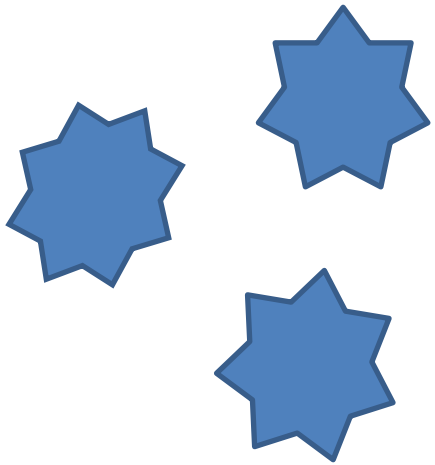
What is Hay fe

Human

Crisis

Invasion





To control hay fever…

It should control production of IgE and the histamine.

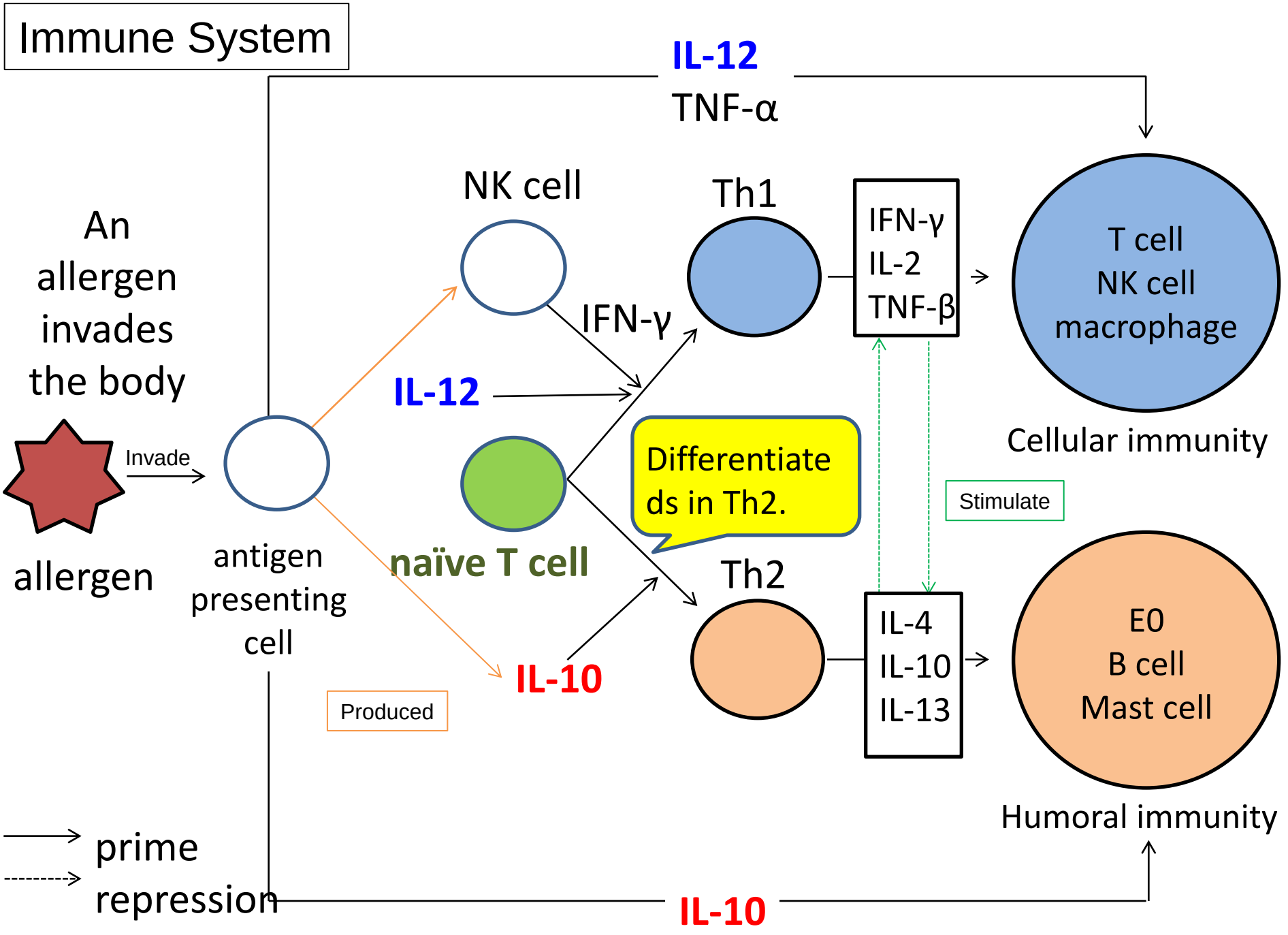
↓

It should control production of B cell and Mast cell.

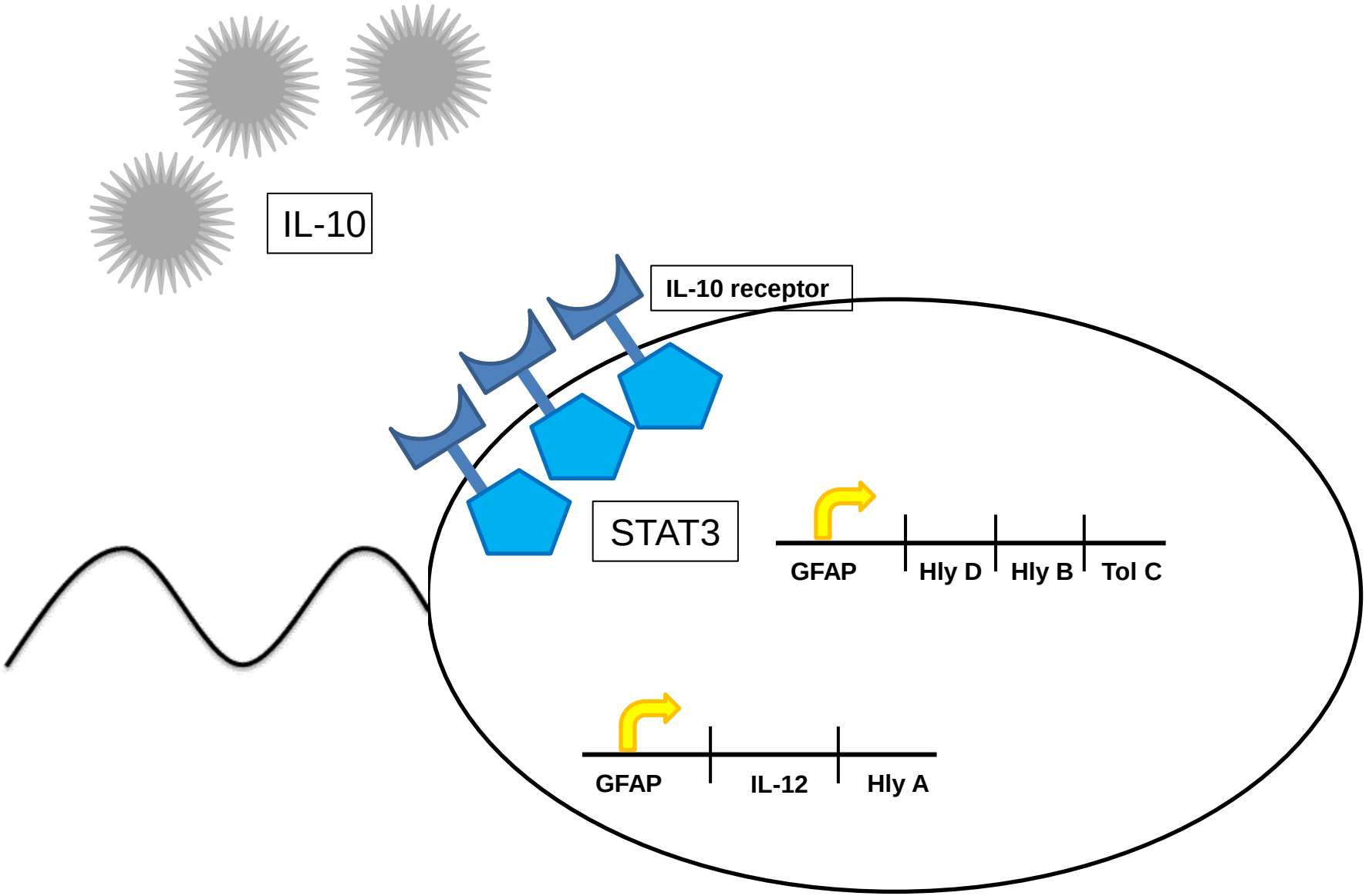
↓

It should do suppress the helper T-cell

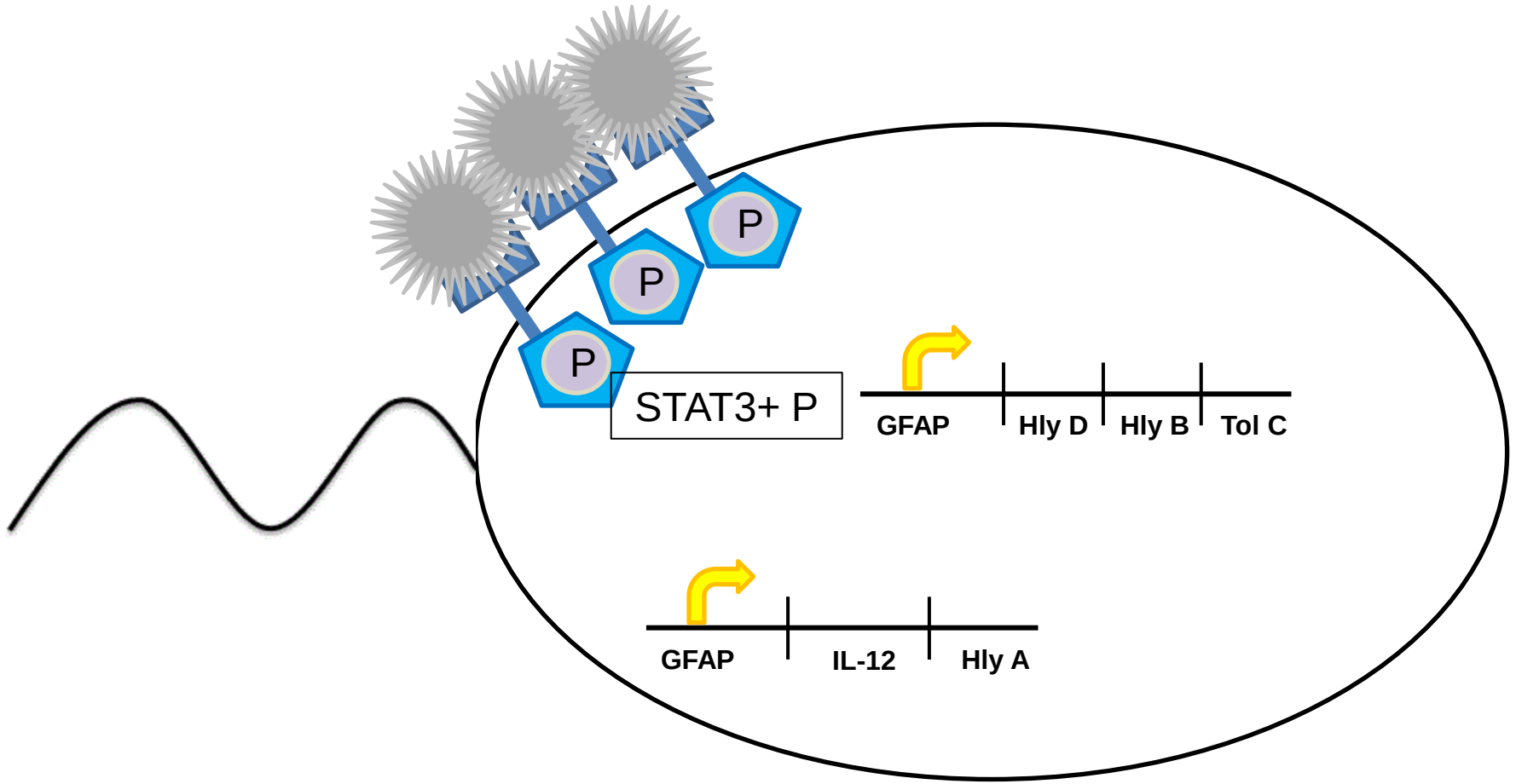
So we focused on the immune system.


Immune System
IL-12
TNF-α
NK cell
Th1
IFN-γ
IL-2
TNF-β
T cell
NK cell
macrophage
Cellular immunity
An allergen invades the body
IFN-γ
IL-12
Differentiate ds in Th2.
Stimulate
Invade
allergen
antigen presenting cell
naïve T cell
Th2
IL-4
IL-10
IL-13
E0
B cell
Mast cell
Humoral immunity
IL-10
Produced
prime
repression
IL-10

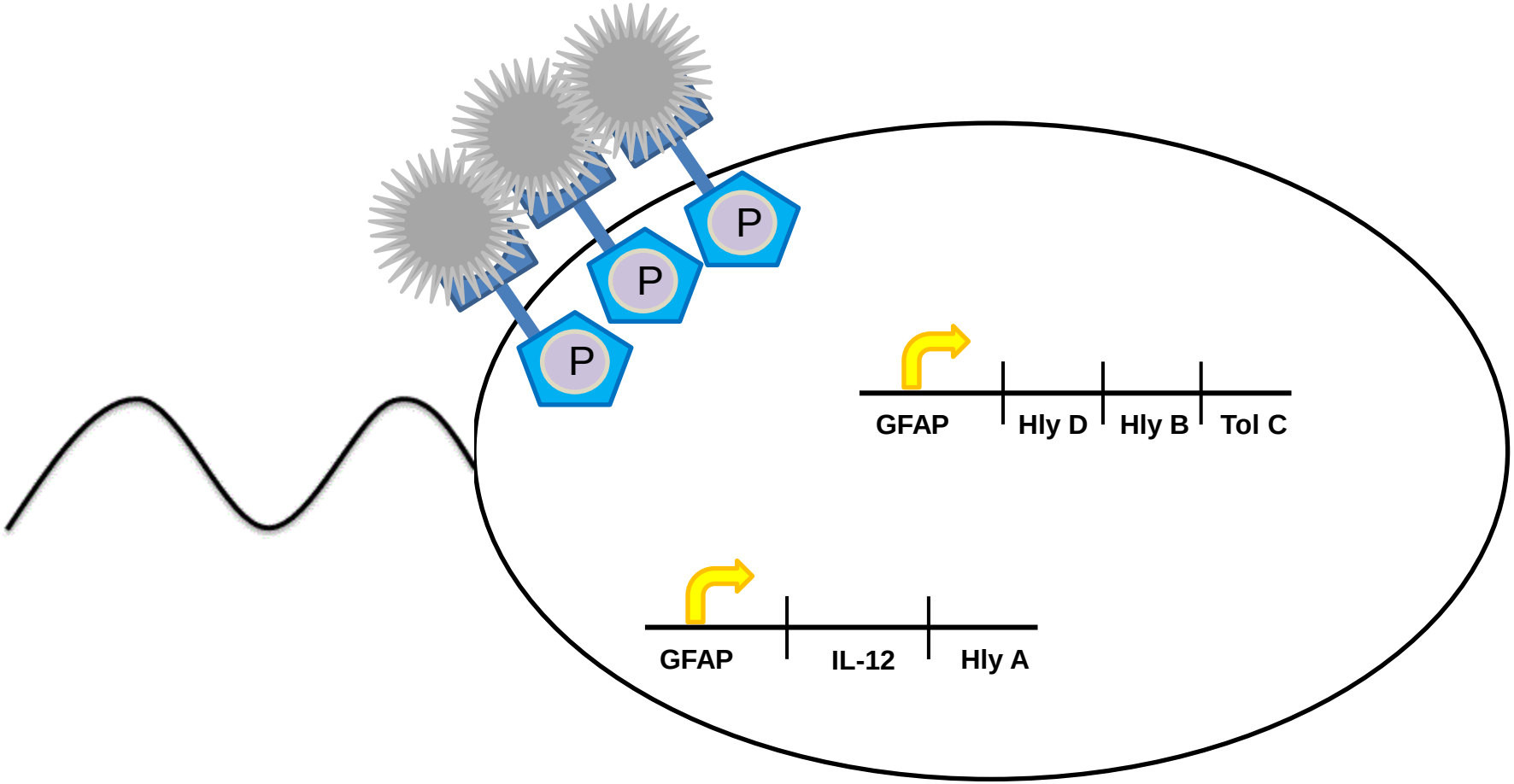
Project



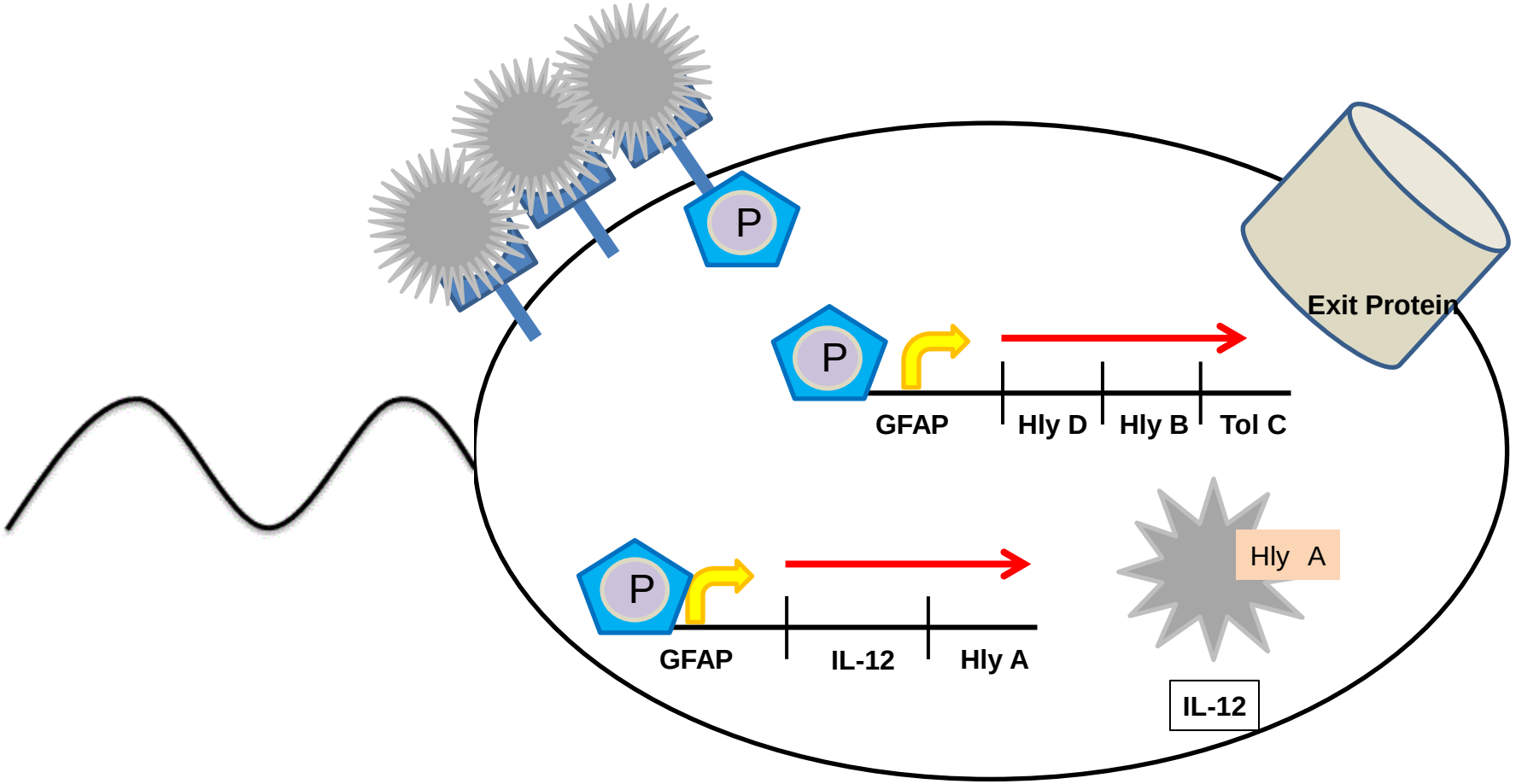
Project



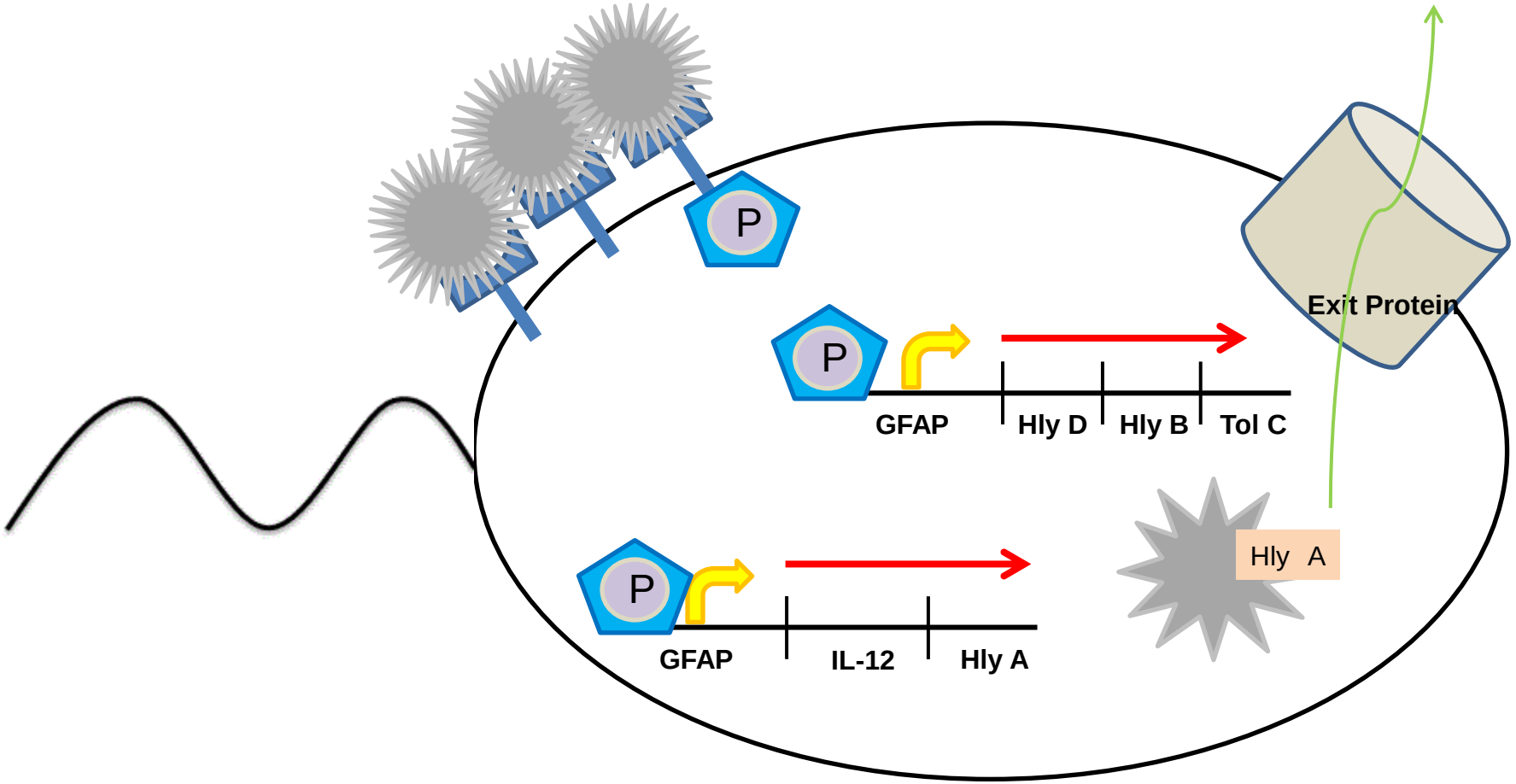
Project



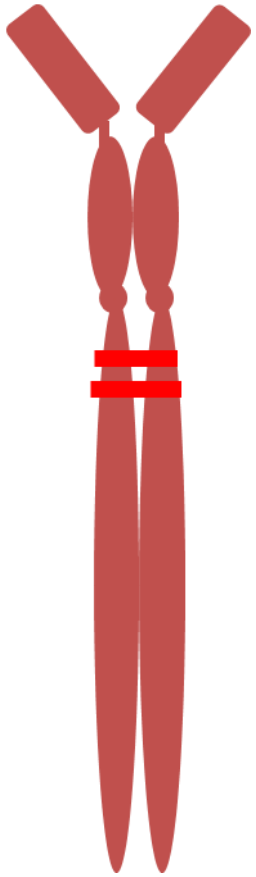
Project


P
Exit Protein
P
GFAP
Hly D
Hly B
Tol C
P
GFAP
IL-12
Hly A
Hly A
IL-12

Project



1. Recepted IL10



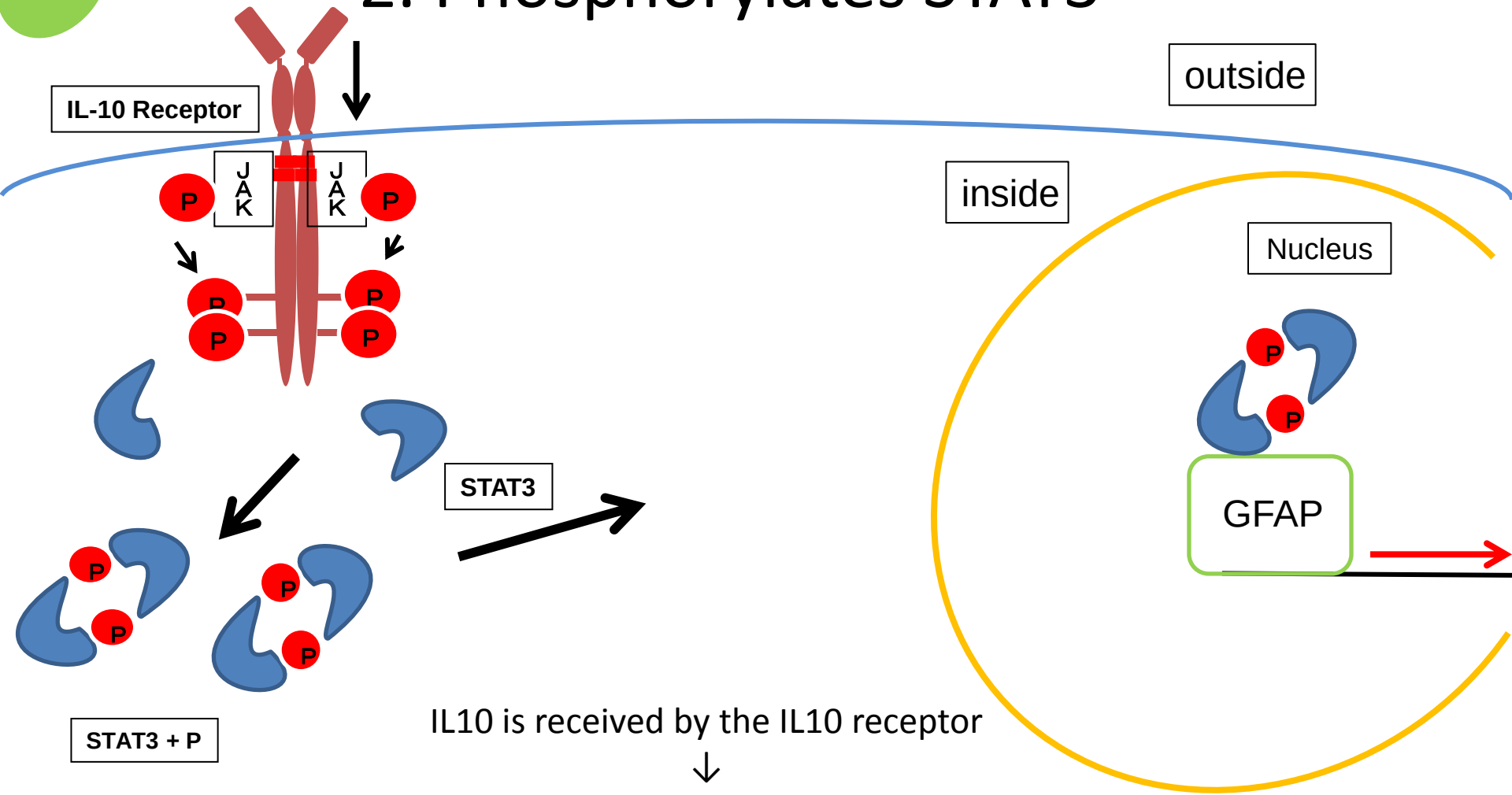
IL10 repotor

- Two subunits consisting of α chain and β chain

α chain: ligand binding subunit, and hight affinity.
β chain: for signalization

IL-10

2. Phosphorylates STAT3



IL10 is received by the IL10 receptor

↓

The intracellular domain(JAK:Janus Kinase) of the receptor is phosphorylated.

↓

STAT3 is tyrosine phosphorylated.

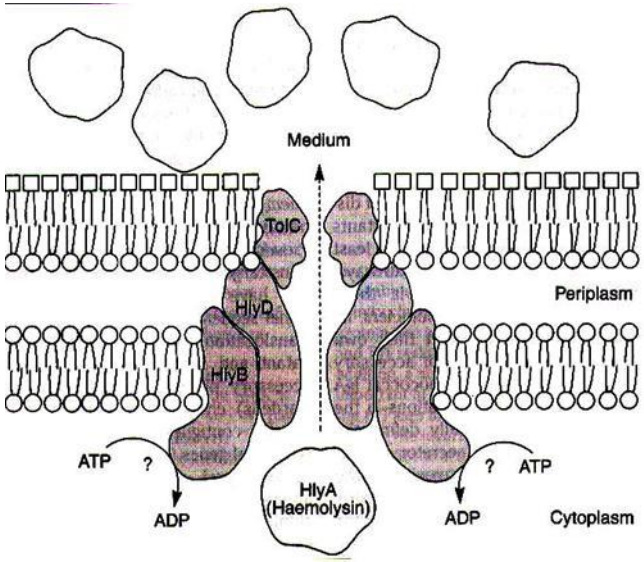
↓

The process proceeds to the nucleus to form a dimer.

3. Promoter and Downstream gene

Promoter is GFAP(Glial fibrillary acidic protein)gene.
There is the part where phosphoylated STAT3 binds to in GFAP

When phosphorylated STAT3 is combine GFAP IL12+HlyA and HlyB+HLyD+TolC of downstream gene starts transcription.



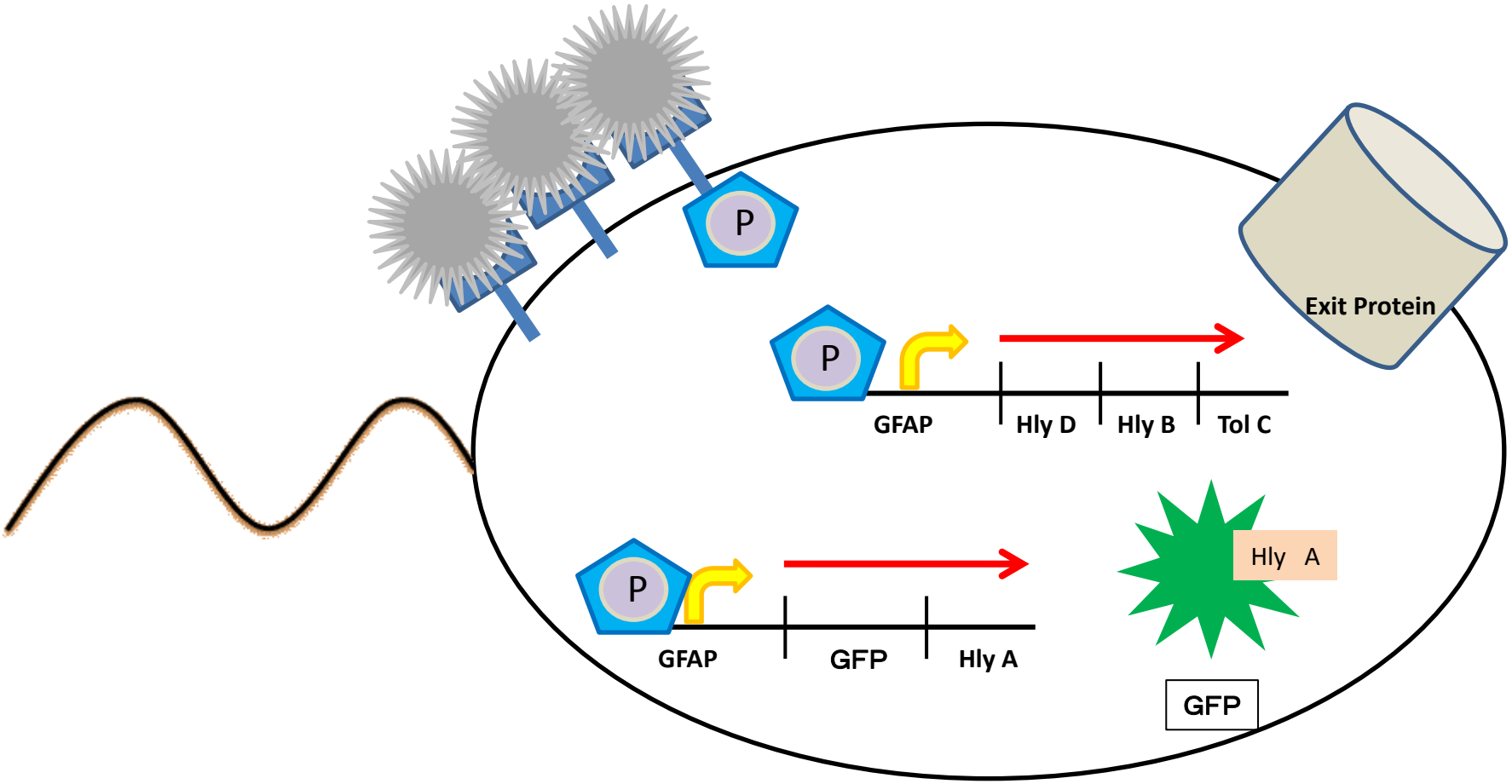
HlyA is signal peptide.
HlyB+HlyD+TolC is membrane protein.
There is HlyB+HlyD to inner membrene,and there is TolC to out membrene.

↓

We produce IL12 with this structure.

3. Promoter and Downstream gene

IL12 activity test is difficult in the short term, it was thought that instead of the GFP. It would be repleaced by IL12 if successful.

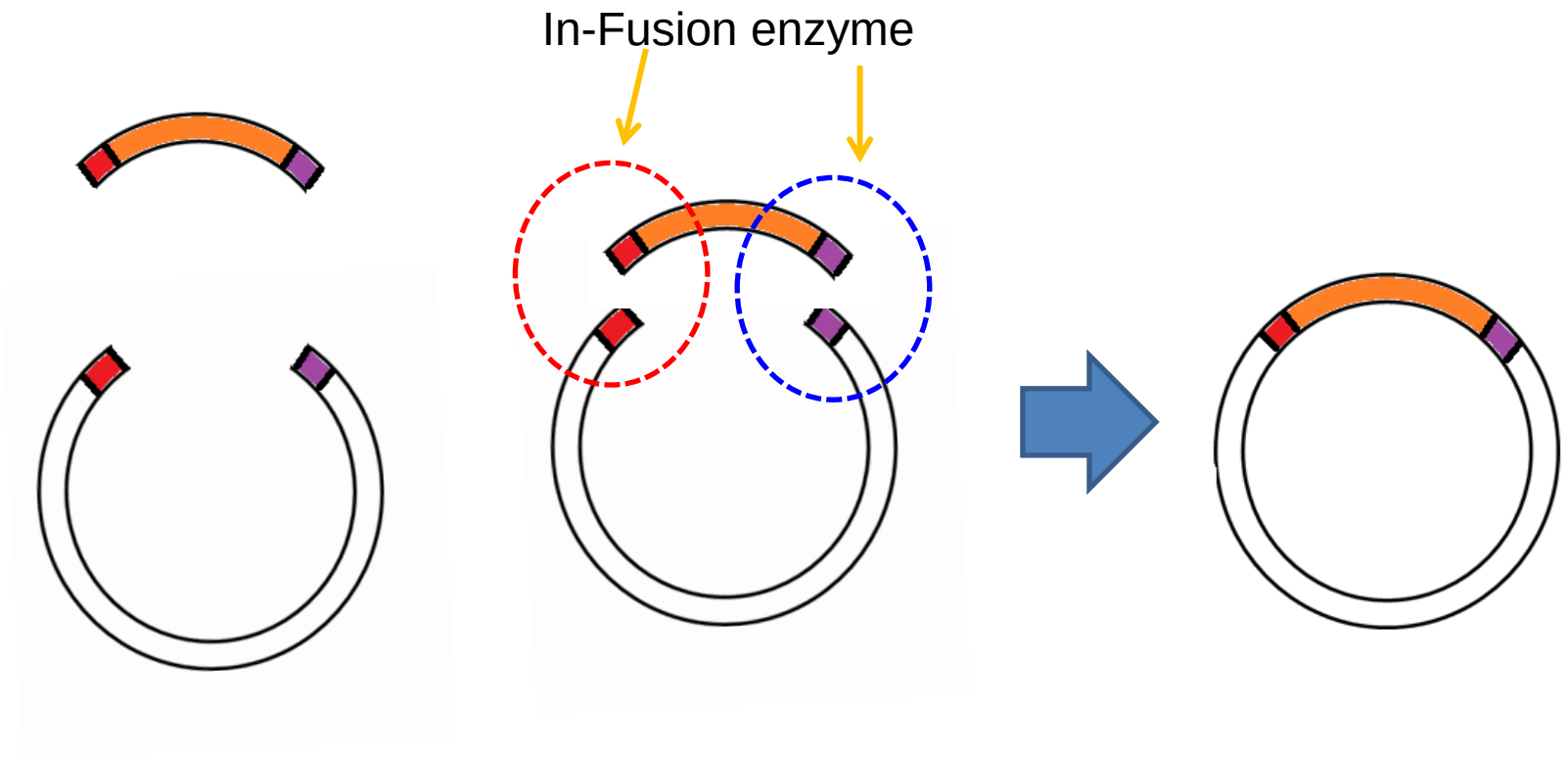


Method

This time, we used the In-Fusion kit (purchased from Clontech) instead of restriction enzyme digestion and ligation.

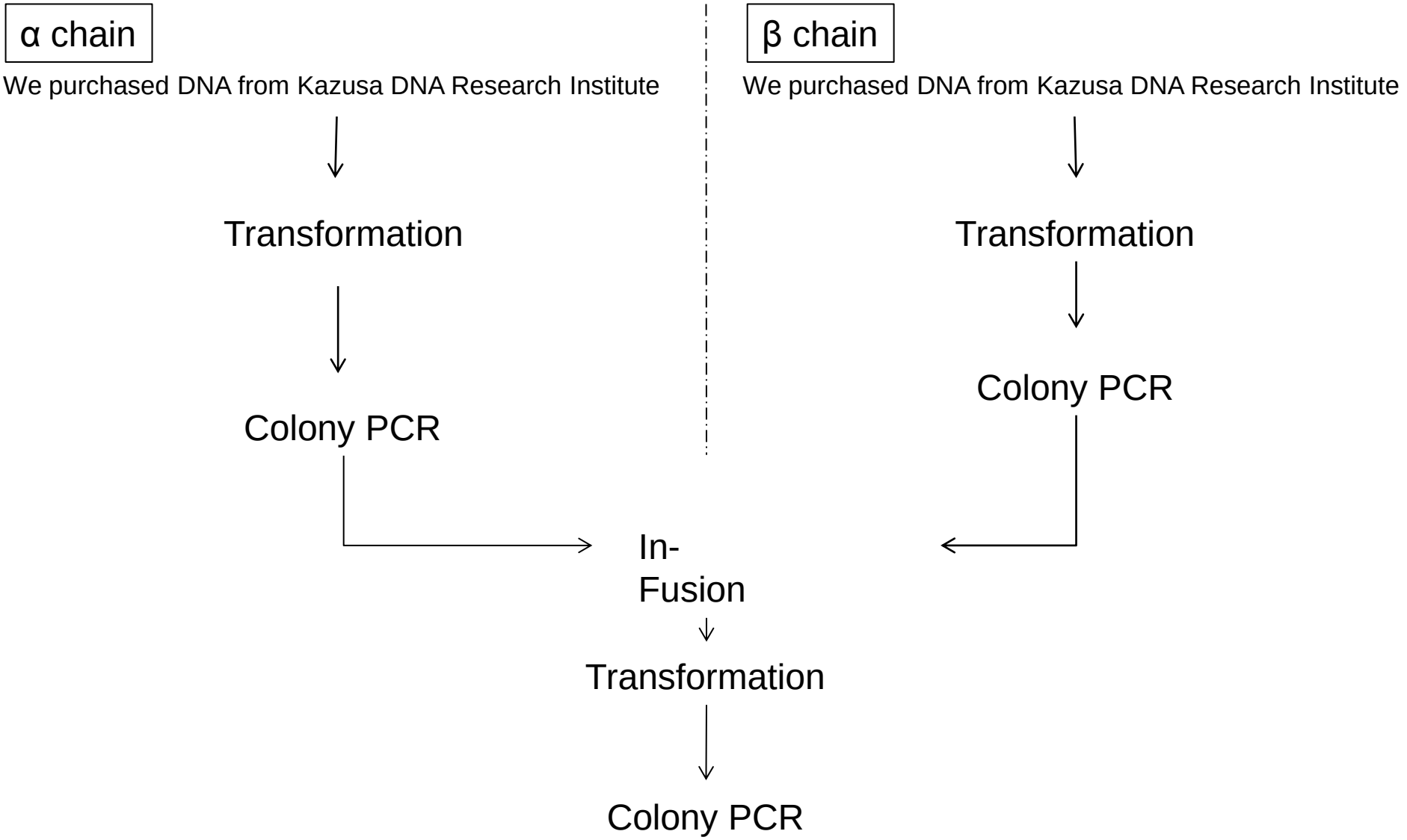
What is In-Fusion…

In-Fusion method is on the DNA recombinant method. The enzyme (In-Fusion enzyme) recognize complement 15 base pair of both end of the DNA. The objective gene is inserted into the Vector by the enzyme..



Method

1. IL10 receptor



Method

2. STAT3

STAT3 plasmid was purchased from Kazusa DNA Research Institute

↓

Transformation

↓

Colony PCR

↓

In-Fusion

↓

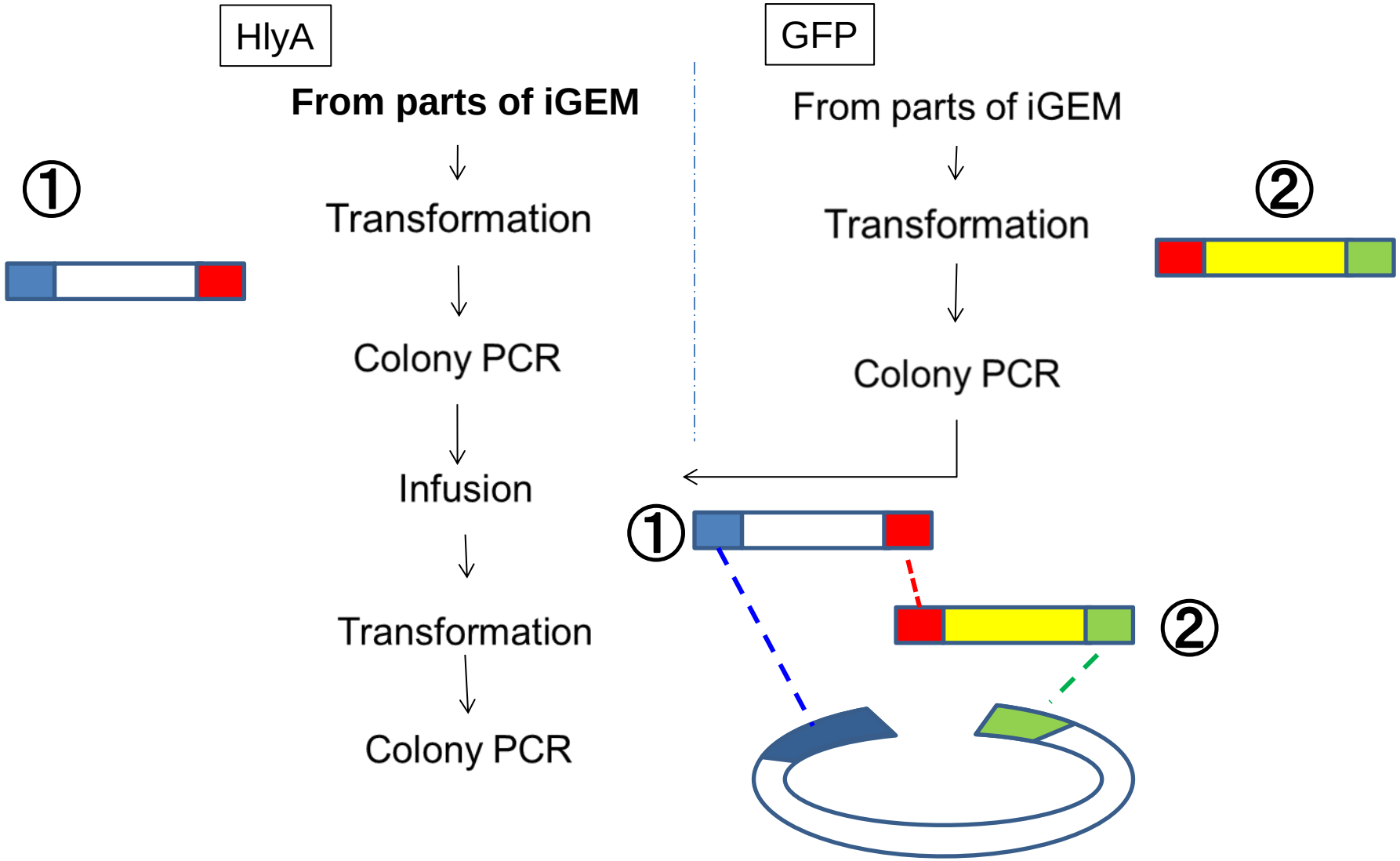
Transformation

↓

Colony PCR

Method

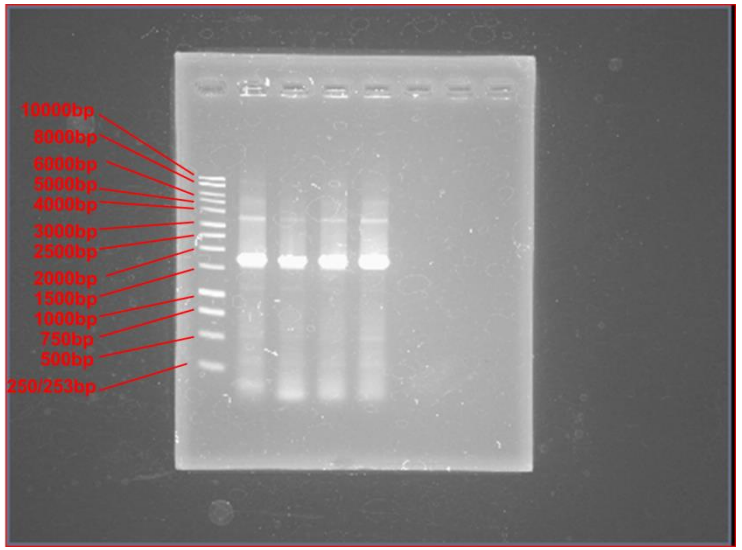
3. Downstream gene



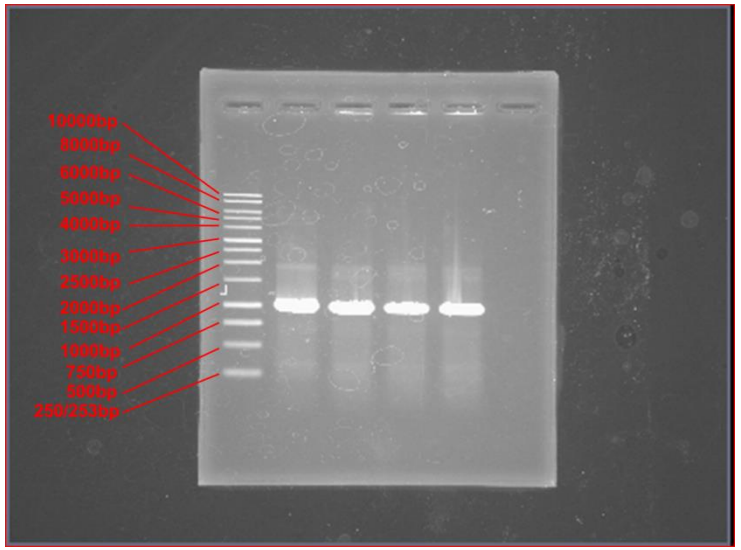
Result

1.IL10 receptor

It was possible to clone DNA.



IL10 receptor α chain(1734bp)



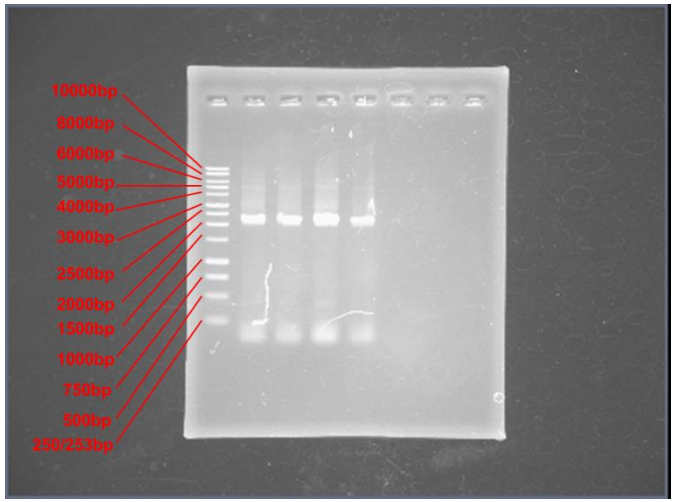
IL10 receptor β chain(975bp)

We succeeded cloning of IL-10 α and β chain DNA sequence with attached the tag sequence for In-Fusion method.
However, We couldn't construct the vector by the In-Fusion method.

Result

2. STAT3

It was possible to clone DNA.



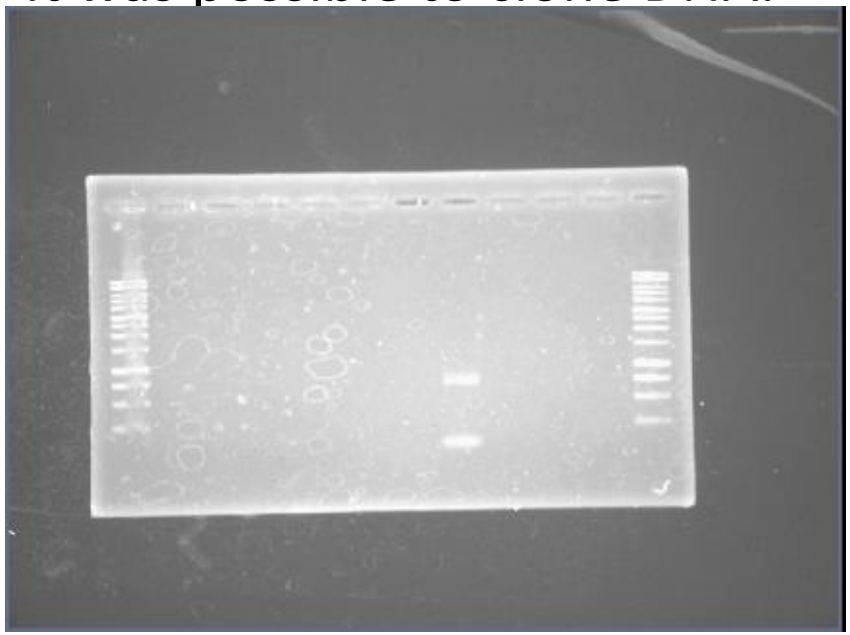
STAT3(2307bp)

We also succeeded cloning of STAT3 with attached the tag for In-Fusion method.
However, We couldn’t construct the vector by the In-Fusion method.

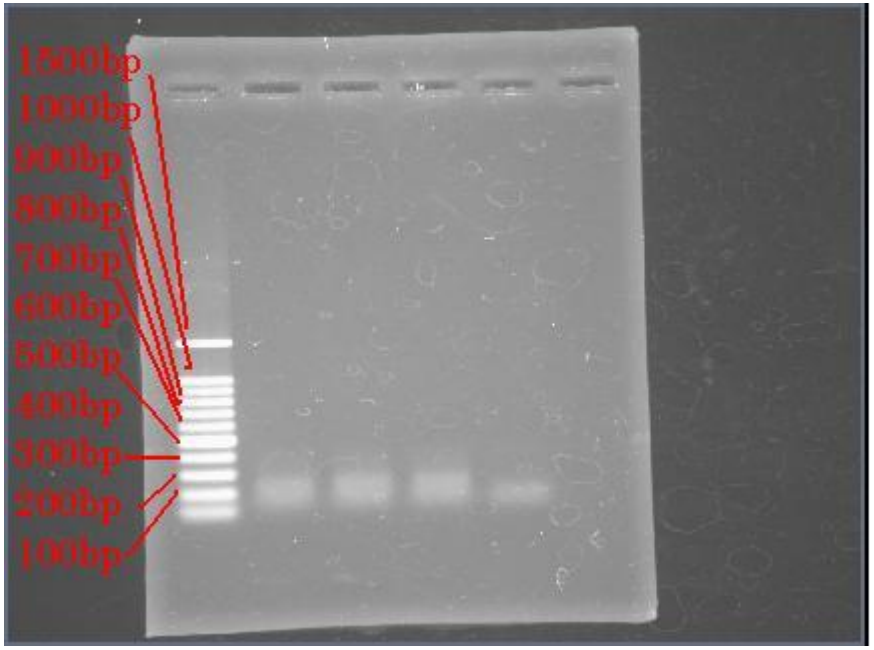
Result

3.Downstream gene

It was possible to clone DNA.



GFP (720bp)



HlyA(187bp)

We succeed ed cloning of down stream gene with attached the tag for In-Fusion method.
However, we also couldn’t construct the vector.

Future Works

- To succeed In-Fusion method and to construct the vector for iGEM entry.
- Objective gene should be inserted to expression vector.
- To evaluate the activity of produced interleukin.

1.Overview

“How do you think about gene recombination?”

- It is adverse effects to a body.
- It might change ecosystem.
- It is untrustworthy.
- It is difficult.

2.Posters

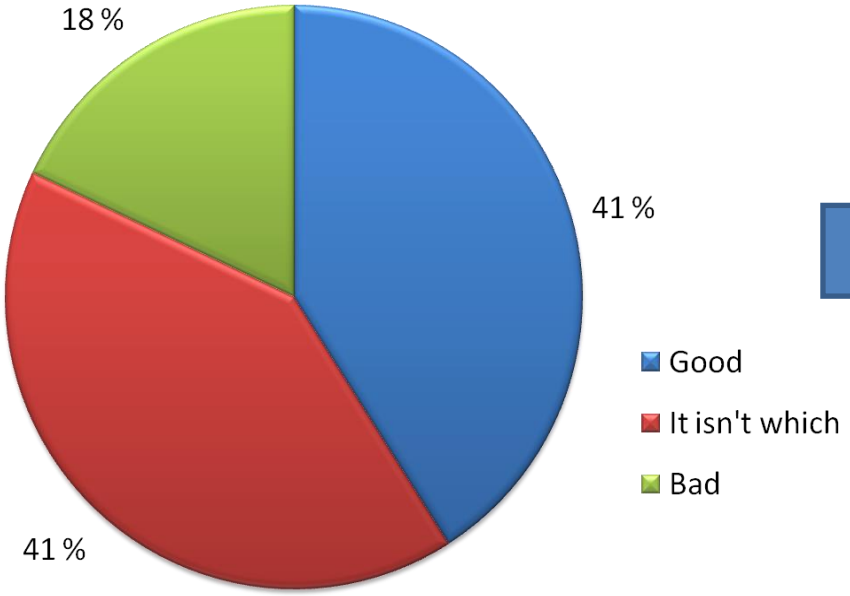
There is international decisions for nonproliferation of the gene recombination creature for the environment.

- P1, P2, P3 level in the laboratory
- Cartagena Protocol

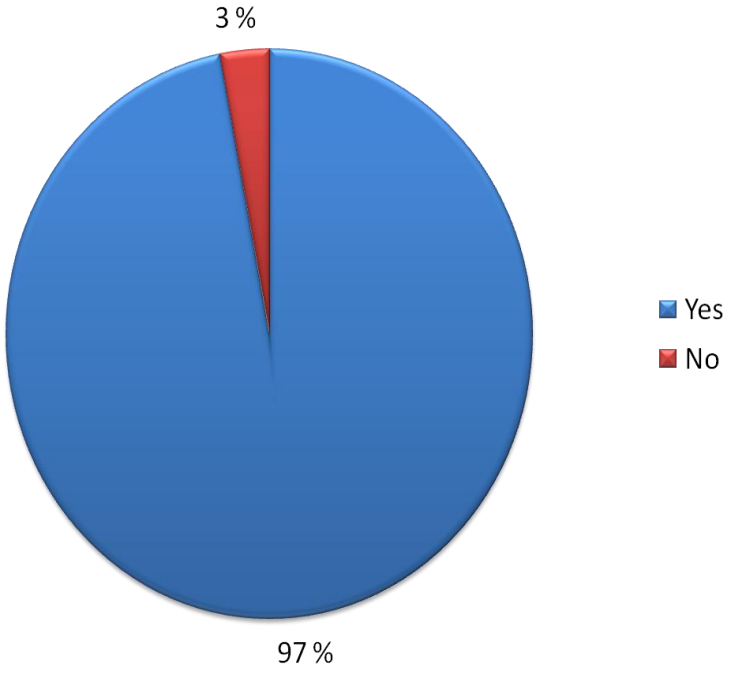
we made the poster about and explain these things in our iGEM project.

3.Questionnaire

Q1.What kind of impression did you have about genetic modification before hearing this explanation?



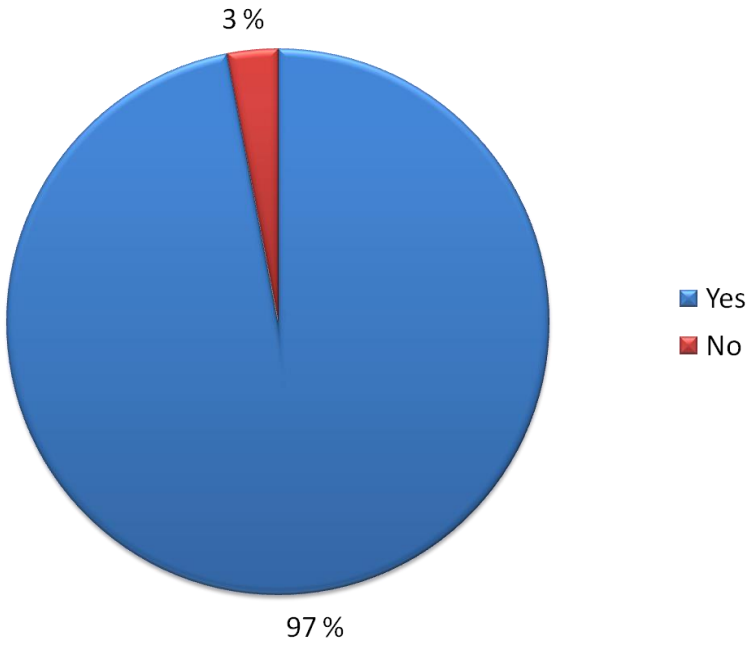
Q2.Were you interested in genetic modification?



3.Questionnaire

Q1.Are you interested in genetic modification?

Q5.Were you interested in genetic modification?



Thank you for listening！