Introduction

◆ Overview

We tried to develop a cheap, fast and effective tuberculosis antigen detecting system using a modified antibody in yeast. Our project is based on a FGFR-STAT1 pathway in human. We have a plan to use a fusion antibody and GFP to display existence of antigens. In other words, this yeast emits fluorescent light when it is attached to antigens.

We can diagnose not only tuberculosis but also other diseases that have antigens, such as malaria, HIV or cancer. By changing an Ig-like domain of a fusion antibody receptor in '*DiscoverY*', we can use '*DiscoverY*' as a universal yeast disease diagnosis chassis to a rapid and effective diagnosis.

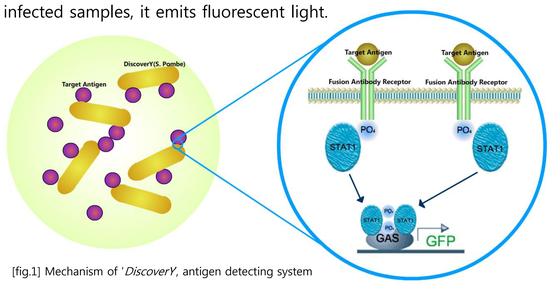
◆ Motivation

In developing countries, many people are suffering from various diseases like tuberculosis, HIV, malaria, etc. They are dying even without knowing themselves having the diseases. We tried to make a portable, effective, and high-potential diagnosis chassis for those unfortunate people in the world.

Concept

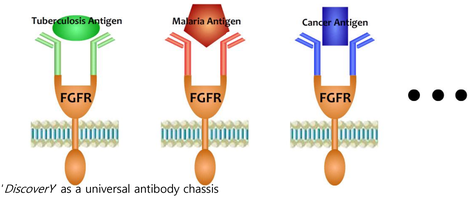
◆ Mechanism

The mechanism of '*DiscoverY*' is composed of three parts; an antigen recognition by a fusion antibody receptor, a STAT1 pathway, and a GFP expression promoted by a GAS promoter. When '*DiscoverY*' is spread on infected samples, it emits fluorescent light.



[fig.1] Mechanism of '*DiscoverY*', antigen detecting system

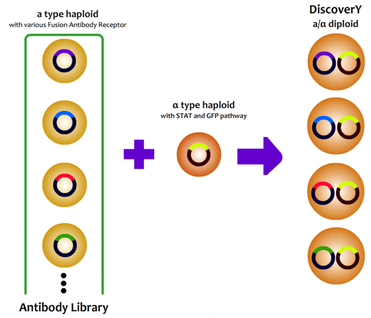
The ultimate model of '*DiscoverY*' is a '***Universal Antibody Chassis***'. By altering the Ig-like part of the fusion antibody receptor, the chassis can work as a diagnosis kit for various diseases.



[fig.2] '*DiscoverY* as a universal antibody chassis

Idea & Logics

To develop a universal diagnosis chassis, we came up with an idea using a life cycle of a fission yeast. Under normal conditions, a fission yeast stays as a haploid. When the condition changes, it mates with another type of haploid(a/α) to become a diploid.



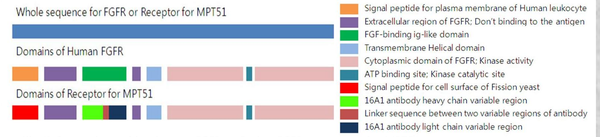
[fig.3] A universal diagnosis chassis using the life cycle of fission yeast

From this characteristic, we can construct an antibody library. An 'a-type' haploid having a target antibody and an 'α-type' haploid having STAT1 and GFP mate together to build '*DiscoverY*' for a target disease.

The strong point of a haploid library is that it is easy to construct the library and more stable in yeast. Since two inserted plasmids are in the 'α-type', and the 'a-type' haploid has only the homologously recombined gene, the antibody library including the 'a-type' haploid can be preserved in the best condition.

Modeling

◆ Fusion-Antibody Receptor



[fig.4] Comparison with a human FGFR vs Fusion FGFR, a receptor for MPT51

◆ Foundations & Basis

Papers
1. [Reconstitution of Fibroblast Growth Factor Receptor Interactions in the Yeast Two Hybrid System] by Ronit A Ioni-Grinstein, Andrew Seddon, and Avner Yayon
• Yeast two hybrid system to show that an FGFR interaction is possible in yeast → • **FGFR can be expressed completely in yeast** without any functional disability • located in outer cellular membrane completely
2. [Jak2-Stat5 Interactions Analyzed in Yeast (Received for publication, January 30, 1998, and in revised form, March 2, 1998) by Fariba Barahmand-Pour, Andreas Meinke, Bernd Groner, and Thomas Decker
• JAK-STAT pathway is operated completely in yeast • JAK in yeast phosphorylates STAT5 without any trouble → • **FGFR-STAT1 pathway will be operated completely in yeast** (∵FGFR,JAK ∈ same protein family & STAT1,STAT5 ∈ same protein family)
3. [Fibroblast Growth Factor Receptor-Induced Phosphorylation of STAT1 at the Golgi Apparatus Without Translocation to the Nucleus] by LUCI'A CITORES, LING BAL2 VIGDIS SØRENSEN, AND SJUR OLSNES2
• FGFR phosphorylates STAT1 at the golgi apparatus in human cell → • **FGFR can phosphorylates STAT1** • STAT1 will be dimerized to activate GAS promoter
4. [Novel Recognition Motif on Fibroblast Growth Factor Receptor Mediates Direct Association and Activation of SNT Adapter proteins] (Received for publication, April 13, 1998) by Hong Xu, Kyung W. Lee, and Mitchell Goldfarb
• Identified fusion FGFR was phosphorylated itself automatically when it is combined with antigen called SNT, → • **Our fusion-FGFR would be phosphorylated properly** when combined with the targeted antigen.

[table.1] Papers that support our mechanism work properly, and the details of each paper guarantees.

DiscoverY

Universal Yeast Disease Diagnosis Chassis

KAIST Korea

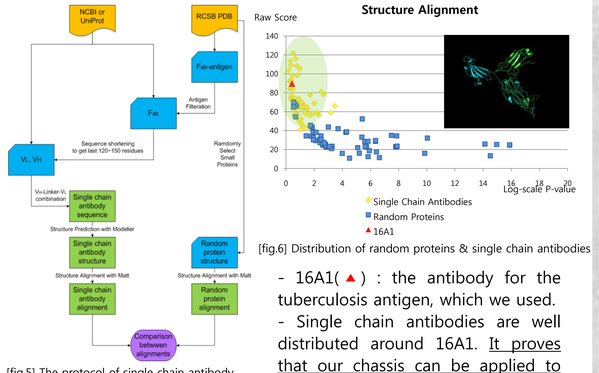
Korea Advanced Institute of Science and Technology

NamBin Yim, EunSoo Kim, HanSol Kim
HwanJun Yun, JeongHyun Lee, SuHwan Kim
SeungBum Yang, DongChan Yang, JaeBong Jung

◆ Possibility

To verify the possibility that our fusion antibody receptor model can be applied to a wide variety of antibodies, we compared the structure of Ig domain of single chain antibodies and a FGF binding domain of FGFR.

We combined a sequence of a variable region of antibodies with a linker sequence to make a single chain antibody sequence. After predicting the structure of the single chain antibody with a structure prediction program, we aligned these structures of Ig domain of the antibodies with the structure of the FGF binding domain of FGFR.



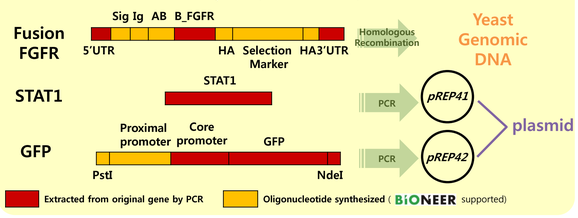
[fig.5] The protocol of single chain antibody structural alignment

[fig.6] Distribution of random proteins & single chain antibodies

- 16A1(▲) : the antibody for the tuberculosis antigen, which we used.
- Single chain antibodies are well distributed around 16A1. It proves that our chassis can be applied to various antibodies.

Experiment

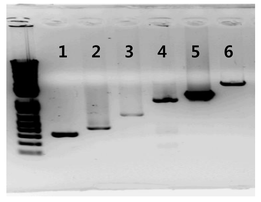
- Yeast gene with 'Fusion FGFR' gene by homologous recombination
- 2 plasmids in the same yeast , one for GFP, the other for STAT1



◆ PCR

We used a PCR method in two processes of our experiment.

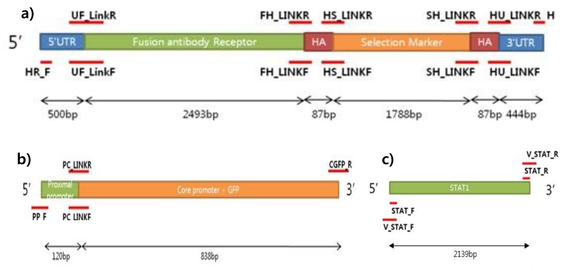
1. To extract genes that we want from the whole genome.
2. To connect parts (Sig, Ig-like, AB, B_FGFR) of the Fusion FGFR.



[fig.7] The result of a PCR experiment

Num	Gene
1	Ig_like
2	Signal+Ig_like
3	Antibody
4	**Fusion FGFR** (Signal+Ig_like+Antibody)
5	Back bone of original FGFR
6	STAT1

[table.2] The result of a PCR experiment

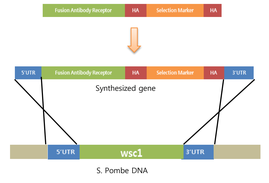


[fig.8] Final genes with primers and linkers to insert into a yeast. a) Fusion FGFR with selection marker for homologous recombination. b) GFP with the core promoter to insert into 'pREP41' plasmid. c) STAT1 to insert into 'pREP42' plasmid.

◆ Homologous Recombination

We will insert our fusion-FGFR DNA sequences that we designed to the yeast, *S. pombe* by using 'Homologous recombination'.

We are going to replace 'wsc1' gene in *S. pombe* with our fusion antibody receptor gene.



[fig.9] Homologous recombination of 'fusion-antibody receptor' gene in the place of wsc1 gene in *S. pombe*

Conclusion

◆ Bio-Bricks

- We finished the oligonucleotide synthesis of 'Signal', 'Ig_like', 'Antibody' and 'proximal promoter' gene parts.
- Also, we finished extracting a 'B_FGFR' and a 'STAT1' parts from an original FGFR and a vector containing STAT1.
- These are the basic bricks to make a 'fusion-antibody receptor' gene.
- Furthermore, we combined 'Signal', 'Ig_like', and 'Antibody' parts together.
- Now, we are trying to ligate a 'fusion-FGFR' and 'B_FGFR' gene parts together.

Description	Length (bp)
First Ig-like region of a basic human FGFR	339
First Ig-like region of a Human basic FGFR with a signal peptide for *S. pombe* surface	246
Single chain antibody for a *Mycobacterium tuberculosis* MPT51 antigen	669
Synthesized Fusion Antibody Receptor (Front part of the modified FGFR)	1095
Back bone part of the modified FGFR	1398

[table.3] The result of the 'bio-bricks' in our experiment.