the Fantastic Lysisbox

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0. INTRODUCTION

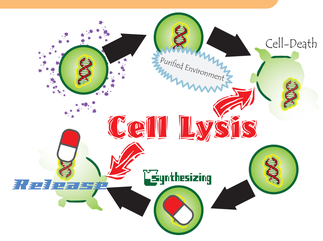
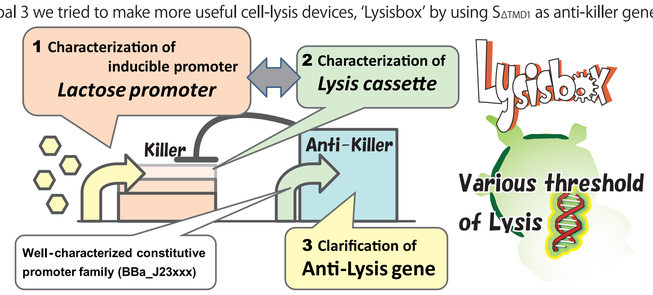


Fig.0-1 We need cell-lysis devices!!
Cell-lysis device has a broad range of applications, such as bioremediation and medicine manufacturing and so on. In the BioBrick partsregistry, there have been already several cell-lysis devices, but those experimental data are unsatisfied. Many previous teams had much difficulty to use cell-lysis devices and accomplish their projects. To overcome this situation, we iGEM Kyoto attempted to make well-characterized useful cell-lysis devices.

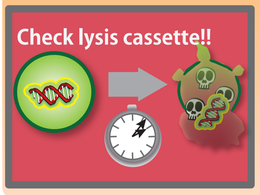
Fig.0-2 Our Goal — `Lysisbox`
To make well-characterized useful cell-lysis devices 'Lysisnox' , we established three goals below.

Goal 1. Characterization of Lactose promoter R0011
Goal 2. Characterization of Killer gene, lysis cassette
Goal 3. Characterization of Anti-Killer gene

In our experiment, we used lactose promoter R0011 as a component of cell-lysis devices, we characterized R0011. Goal 1 is essential for the characterization of cell-lysis devices. Lysis cassette is the most popular cell-lysis device. So, we first characterized this lysis cassette. This is Goal 2. In addition, in Goal 3 we tried to make more useful cell-lysis devices, 'Lysisbox' by using $S_{\Delta TMD1}$ as anti-killer gene.



GOAL 2:CHARACTERIZATION OF LYSIS CASSETTE



To characterize lysis cassette, we made the construct below. We measured OD550, the number of E.coli transformed with the construct in medium with various IPTG concentration.

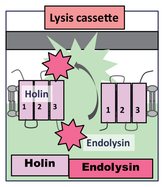


Fig.2-1 What is lysis cassette?
Lysis cassette consists of S and R gene. S gene encodes holin and R gene encodes endolysin. Holin made holes within innermembrane (IM) and this hole enables endolysin to access to the periplasm, and quickly leads to cell lysis.

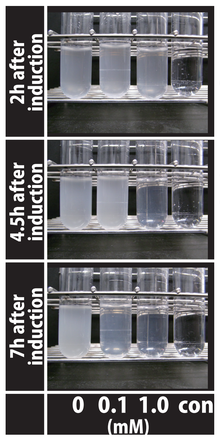


Fig.2-2 The result of lysis induction
Top: 2h (after induction), added 0mM, 0.1mM, 1.0mM IPTG,control (only medium).
Center: 4.5h, 0mM, 0.1mM, 1.0mM IPTG, control.
Bottom: 7h, 0mM, 0.1mM, 1.0mM IPTG, control.
The tube with 1.0mM IPTG got clear as time goes by. This suggests that the E.coli in the M9 medium with 1.0mM IPTG is killed by induction of lysis cassette.

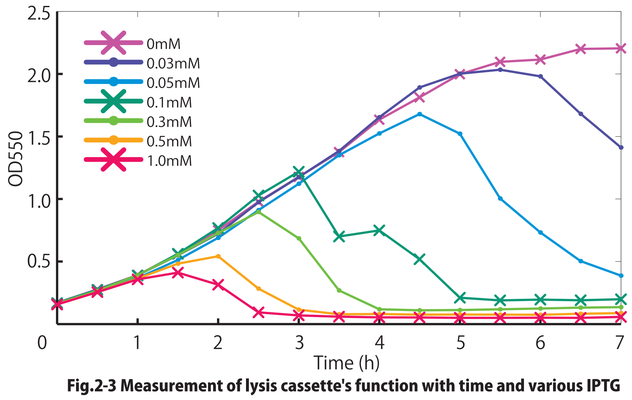


Fig.2-3 Measurement of lysis cassette's function with time and various IPTG concentration
This figure suggests that lysis cassette works adequately if induced by more 0.05 mM IPTG. In addition, the higher IPTG concentration is, the earlier the time OD550 starts to decrease.

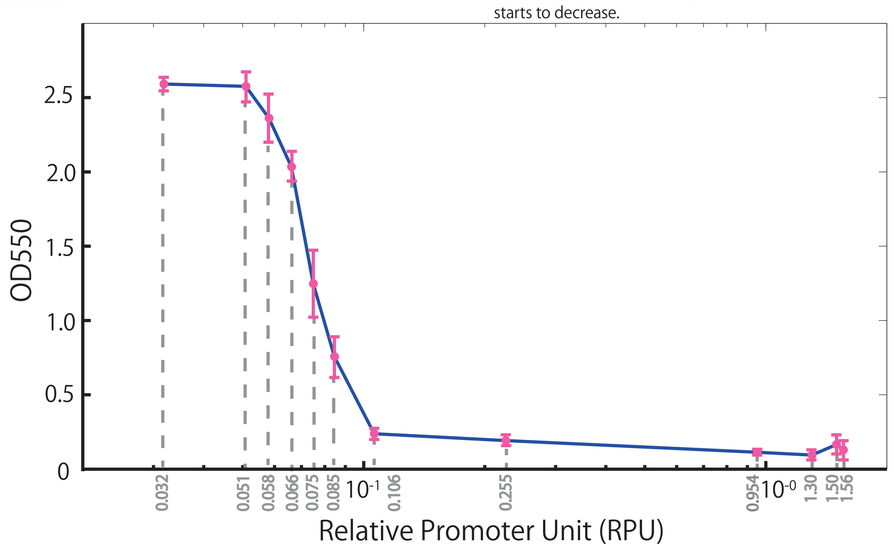
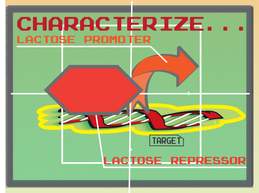


Fig.2-4 Measurement of lysis cassette's function with various RPU

We measured OD550 (≒cell number) with various IPTG concentration at 18h after induction. We confirmed that OD550 hardly changed at 18h after induction. When RPU is from 0.032 to 0.051, the rate of lysing is not sharp. When RPU is from 0.058 to 0.106 , that rate becomes sharp dramatically. When RPU is over 0.106 , OD550 is under 0.3 and does not change largely. In addition, we measured CFU to make sure how many cells are alive (data not shown on this poster).

Complete characterization of lysis cassette

GOAL 1:CHARACTERIZATION OF LACTOSE PROMOTER R0011



To characterize lysis cassette from lambda phage, we regulated lysis cassette by lactose promoter R0011, which is induced by IPTG. So, we wanted to know how the expression level of R0011 varies depending on IPTG concentration. Accordingly, we measured the activity of R0011 with Relative Promoter Unit (RPU). Many teams have used this lactose promoter, so we insist that this quantitative experimental data are very useful and important for them.

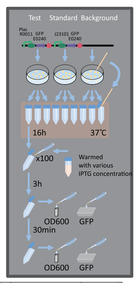
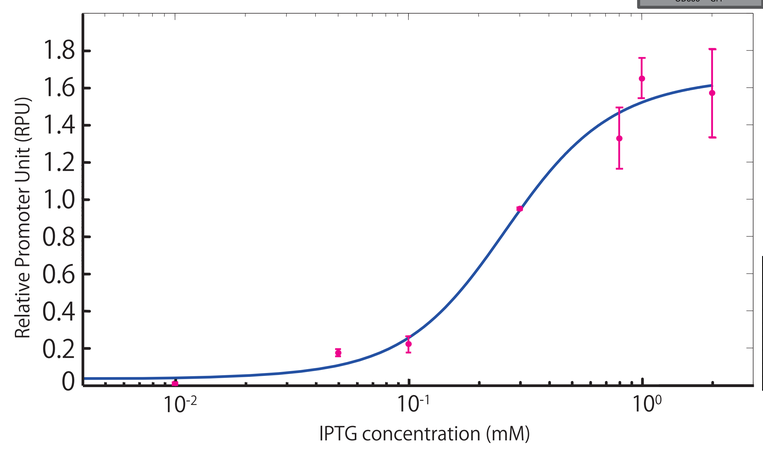


Fig.1-1 Measurement procedure of RPU
We used low copy vector: pSB4K5 and E.coli strain: KRX (LacIq).
We picked out three colonies from each plate, and cultivated them in M9 medium with 50 μg/mL of kanamycine and various IPTG concentration at 37℃ for about 16h. We diluted the precultures to 1:100 with the same IPTG concentration pre-warmed fresh M9 medium and incubated them with shake at 37℃. After they were incubated for 3h and 3.5h, we measured OD600 (≒cell number) followed by GFP fluorescence of the cultures.



IPTG (mM)	RPU A	B	C	Average	s.d.	CV
0	*	0.0122	0.0147	0.0134	0.00821	0.611
0.01	0.0191	0.00963	0.00244	0.0104	0.0084	0.806
0.03	0.177	0.155	0.198	0.174	0.0173	0.0997
0.1	0.265	0.191	0.269	0.221	0.0416	0.188
0.3	0.938	0.955	0.955	0.949	0.00981	0.0103
0.8	1.44	*	1.21	1.37	0.161	0.122
1	1.8	1.58	1.85	1.61	0.108	0.064
2	1.49	1.83	1.39	1.57	0.233	0.148

Fig.1-2, Table1 The relationship between IPTG concentration and RPU

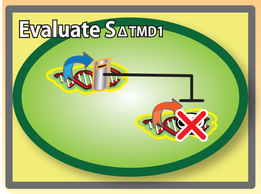
This Table1 shows that the highest RPU is 160 times higher than the lowest one. Coefficient of Variation (CV) of IPTG 0mM and 0.01mM are high. From this result, we modeled this lactose promoter's activity. The Fig.1-2 above shows this model. Error bars are experimental data. Blue line is expectation by mathematical model. Because R0011 has two operator regions, we assumed the equilibrium reaction below.

$$(\text{Promoter Activity}) = \frac{aK_1K_2 + \frac{\beta K_2 X_T}{1+\left(\frac{[S_1]}{K_1}\right)^n}}{K_1K_2 + \frac{K_2X_T}{1+\left(\frac{[S_1]}{K_1}\right)^n} + \frac{X_T^2}{1+\left(\frac{[S_1]}{K_1}\right)^n}}$$

This model was fitted to the result of experiments and parameters were decided by using MATLAB 7.10.0 (MathWorks).

Complete characterization of R0011

GOAL 3:CHARACTERIZATION OF ANTI-KILLER GENE



Lysis cassette, which is the only available cell-death device on the BioBrick partsregistry, has room to be improved: given a certain concentration of an inducer and the inducible promoter, we can not modulate what inducer concentration leads cell lysis.
We made the key for solving this defect: anti-killer gene, $S_{\Delta TMD1}$. By using it, we propose new cell-lysis device, 'Lysisbox'.

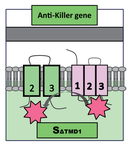


Fig.3-1 $S_{\Delta TMD1}$
$S_{\Delta TMD1}$ is a S allele with transmembrane domain 1 (TMD1) of S deleted and dominant-negatively inhibits lysis cassette by preventing holins from forming holes (Young et.al.).

Reference
Rebecca White, Tram Anh T. Tran, Chelsey A. Dankenbring, John Deaton, and Ry Young.2010.The N-Terminal Transmembrane Domain of _ S Is Required for Holin but Not Antiholin Function._ J. Bacteriol. 192: 725–733

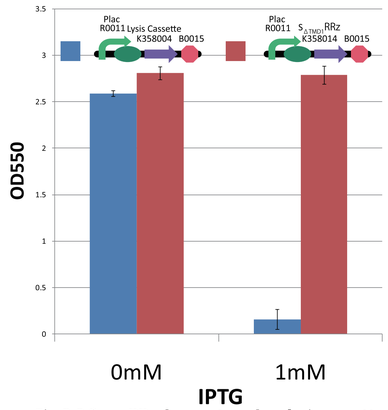


Fig.3-2 $S_{\Delta TMD1}$RRz does not work as lysis cassette
Data of 18h after induction. The red bar is OD550 of cultures of the E. coli transformed with the construct above, the blue is OD550 of cultures of the one transformed with the construct in the Goal 2. This result indicates that $S_{\Delta TMD1}$ cannot form a hole and inhibits cell lysis.

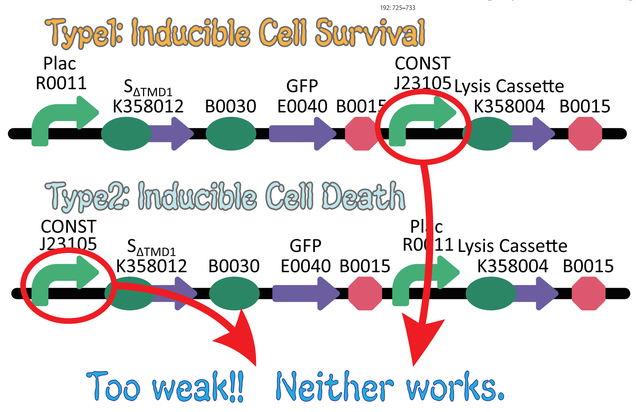


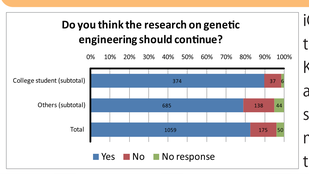
Fig.3-3 The function of Lysisbox has yet been characterized.
We checked the function of Lysisbox. Whatever IPTG concentration induced the each gene regulated by lac promoter, the E.coli transformed with Lysisbox type1 could not be dead and the one transformed with Lysisbox type2 was dead (data not shown on this poster). We think this is because the constitutive promoter J23105 we used is too weak.

Finish constructing $S_{\Delta TMD1}$ and Check its function

4. FUTURE WORK

As future works, we will characterize the 2 types of Lysisbox quantitatively after we change the constitutive promoter J23105 to a stronger one by using the data of the constitutive promoters' activity (RFU) which is listed by UC Berkley (2006) in BioBrick partsregistry.
We could not accomplish this characterization in this experiment, but we must do it because we guess why we could not. Then, given any inducible promoter, we can modulate what inducer concentration leads cell lysis.

5. HUMAN PRACTICE



iGEM Kyoto suggested a nationwide survey to all the iGEM teams in Japan this year. Five universities (including Kyoto) participated in this project. We Kyoto team led other teams in the project, and successfully got over 1,500 answers in total! From the survey, we find that opinions differ between students and non-students. To our surprise, the survey also showed that the majority of Japanese regard biotechnology favorably, while at the same time they worry very much about the safety.