iGEM TSINGHUA 2013

MOBILE HEALTH PATHOGEN DETECTOR

Pathogen detection is a major topic related to the access to health care. We designed a novel portable pathogen detector for mobile health monitoring. The system consists of two separate modules, the sensing module which senses AHL from pathogens and the reporting module which warns the user by color change, combined by yeast mating. Fixing the yeast powder onto test paper, we expect to obtain the product applicable to medical diagnosis as well as food and environment quality monitoring.

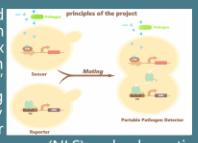
BACKGROUND

Life-threatening pathogenic infections cause thousands of deaths every week all around the world. An effective and efficient identification of the infectious pathogens would facilitate the clinical treatments of these diseases. Nevertheless, current methods for identifying pathogens have many drawbacks including long period for testing and high cost. Clinical diagnosis of infectious pathogens would benefit significantly from specific and sensitive pathogen identification methods.

DESIGN

PPD sensor

We reconstructed bacteria quorum sensing LuxR-pLux system in an "eukaryotic way" when introducing it into yeast, by adding nuclear localization sequence (NLS) and eukaryotic activating elements.



PPD reporter

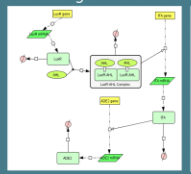
We used ADE2 gene as a reporter gene, which, once expressed, could switch the color from red to white in ADE2 knockout yeast strain. Tet-off system is a linkage between the sensor and reporter system.

PPD switch

Mating ability of yeast contributes to the combination of sensor and reporter. Combination of different sensors and reporters will also increase the diversity of PPD for the detection of more pathogens.

MODELING

Simulation of the model predicts how yeast color changes with time.



Overview of the biochemical process.

Name	Description
k_1	basal expression rate under constitutive promoter
k_2	dimerization rate of AHL and LuxR
k_3	degradation rate of LuxR
k_4	degradation rate of LuxRC
k_5	expression rate of tTA
k_6	activation coefficient of LuxRC
k_7	degradation rate of tTA
k_8	basal expression rate of tTA
k_9	expression rate of ADE2
k_{10}	activation coefficient of tTA
k_{11}	degradation rate of ADE2
k_{12}	basal expression rate of ADE2

The kinetics parameters are listed above.

Equations:

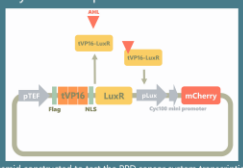
$$\frac{d[LuxR]}{dt} = k_1 - k_2[AHL]^2[LuxR]^2 - k_3[LuxR] \qquad \frac{d[tTA]}{dt} = k_5\frac{[LuxRC]}{k_6 + [LuxRC]} - k_7[tTA] + k_8$$

$$\frac{d[LuxRC]}{dt} = k_2[AHL]^2[LuxR]^2 - k_4[LuxRC] \qquad \frac{d[ADE2]}{dt} = k_9\frac{[tTA]}{k_{10} + [tTA]} - k_{11}[ADE2] + k_{12}$$

RESULTS

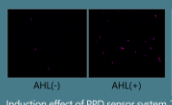
PPD sensor

To test the function of PPD sensor, we used mCherry to report the transcription ability of pLux+cyc100 mini promoter after AHL induction.

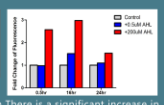


The plasmid constructed to test the PPD sensor system transcriptional potency.

After 24hr induction, compared with control group, the AHL inducing group had more mCherry positive cells. The efficiency of PPD sensor system had been quantified by flow cytometry. They were induced by different concentrations of AHL (0 μM, 0.5 μM, 200 μM). The fluorescence intensity was tested after three different time periods (0.5hr, 16hr and 24hr).



Induction effect of PPD sensor system by 0.5 uM AHL, 24hr.



There is a significant increase in mCherry fluorescence intensity with the increase of AHL concentration. In 200 μM group, mCherry fluorescence intensity fold change increase from 0.5hr to 16hr, and peaked before 24hr.

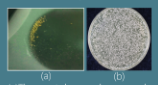
PPD reporter

In order to test if the Tet-off switching system and the reporter worked as expected, we constructed pTF6 plasmid. We tested the function of pTF6 in ADE2 knockout yeast strain. We observed an obvious color change, using cm184 (pTF6 with ADE2 gene deletion) transformation group as control.

Product testing

The final goal of our project is to make a portable test paper, which requires the test paper to be not only carriable without any special treatment, but also effective during a certain period of time.We transformed the reconstructed yeast into dry powder successfully.

The pTF6 transformed yeast obviously changed color, which indicated the Tet-off system worked as expected.



(a) (b)

(a)The yeast dry powder we made via the protocol investigated by ourselves.
(b)The 1:1000 diluted yeast dry powder grew intensively and covered the surface of medium.

After up to 3 weeks, we reactivated yeast by adding water. As indicated in the plate, after 12hr incubation, the yeast was reactivated and grew very well.

FUTURE WORK

1. Extend the application of the established system to detect other pathogens involved in human diseases, food safety issues and environmental pollutions.
2. Create more possible portable pathogen detectors by combining different sensors and reporters.
3. Improve our products for commercial use.
4. Create a standard user-friendly product instruction for disease diagnosis.

OUTREACH

Human practice

Publicizing iGEM and synthetic biology.

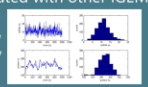
Motivation of project brainstorming.

Movie Series.

The human practice part of Tsinghua iGEM team includes routine events of the Association of Synthetic Biology in Tsinghua, scientific movie series and surveys targeting to a wide range of people from different backgrounds.

Collaboration

We established a long-term collaboration with OUC-CHINA team and helped them build the mathematical model, the Gillespiessa Algorithm, for their project. We also collaborated with other iGEM teams, including Nanjing, Tianjing, BIT and BIT-CHINA, via various forms.



Gillespie Stochastic Simulation.

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