Biosensor of Tetracycline

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Part One
Background Preparation
Currently, the overuse of antibiotics has not been effectively regulated in China.

Overuse and misuse of antimicrobial agents in human medicine,

Large-scale use of antimicrobials in agriculture also contributes to the crisis.

For treatment, prevention of diseases and the need of promoting animal growth, some of the farmers use antibiotics extensively which cause the rise of antibiotic-resistant pathogens.
1.2 Policy Situation

Problems of Policy in China:

In China, the food safety management system is still bull management.

Most of China’s laws and regulations about veterinary drug residues are lack of unified planning and hard to conducive search.

The recent situation shows that parts of the standards’ version are out of date with slower update time, less coverage and irrational maximum residue limits which differs from that of CAC and FDA to a great extent.
1.3 Motivation and Innovation

After investigation, we choose tetracycline as the research object because of its extensive existence in animal husbandry.

The overuse and misuse of antibiotics makes these drugs residues in edible animal, which will cause a damage in human liver, kidney and digestive tract.

Currently antibiotics detection methods are complex, expensive.

Our group hopes to use synthetic biology to provide a simple, reliable and efficient method for the detection of antibiotics.
SCUT-NJU-SYSU three-university forum
In early April 2015, the students form Nanjing University came to Guangzhou. We took the opportunity to organize the first meeting.
Part Two

Biological Solution
Our group constructed the tetracycline inducible expression system. And through plasmid mediated, the system was transfected into Escherichia coli TOP10 and GS115 Pichia pastoris. It can generate a rtTA transcription activation factor in cells. RtTA combining with tetracycline antibiotics can activate the fluorescent protein expression system, which can instruct the tetracycline class of antibiotics by fluorescence detection.
2.2 Our advantages

(1) We construct a simple biological detector using microorganism by the method of synthetic biology and can detect the tetracycline.
(2) The detector can tell us if there is tetracycline by obvious signal, playing the role of biological test paper.
(3) It is easy to operate and has important significance for food safety.
(4) Engineering bacterium can be fermentable on large scale. This will make it cheaper than other methods.
2.3 Biosensor of Tetracycline

The design and construction of Tet-on regulation plasmid

The design and construction of Tet-on expression plasmid

Preparation of recombinant yeast and the Transformation and screening of recombinant yeast
2.3 Tet-on Expression System
2.3.1 The design and construction of Tet-on regulation plasmid

We design the primer by software and get the rtTA gene from Tet-on plasmid. Add EcoR1 and Not1 on the ends of it. We reconstruct the system because the original system is not apply to yeast.
To ensure the success of the experiment, we reomded the Plasmid. We link the rtTA or rtTA-flag with Restriction Enzyme cutting site to the Plasmid pGAPZB. Construct the Plasmids pGAPZB-rtTA and pGAPZB-rtTA-flag.

2.3.1 The design and construction of Tet-on regulation plasmid
2.3.2 The design and construction of Tet-on expression plasmid

Lead the pPICTC-EGFP Plasmid into the competent Yeast with pGAPZB-rtTA or pGAPZB-rtTA-flag to complete the construction of recombinant yeast.
2.3.3 Preparation of Recombinant Yeast

Preparation of recombinant yeast and the Transformation of recombinant yeast

Transformation steps:
1) Prepare recombinant yeast by chemical approach, 80μL/tube, use it immediately or conserve it at -80°C.
2) Add 0.1~0.2mg linearization recombinant plasmid into the recombinant yeast cells, then take them in the 0.2cm electric shock cup in the ice-bath for 5min.
3) Electric shock it by pulse cell transfection system at 1.5Kv, 200Ω, 25mF, 5ms.
4) Add 1ml 1mol/L sorbitol taken from ice-bath into the mixed liquor after electric shock. Take it to 1.5ml centrifuge tube and wait for 1.5h at 30°C.
5) Take 200μl bacterium solution, smear it on the Resistance or auxotroph plate to screen.
2.4 Results

- The design and construction of Tet-on regulation plasmid
- The design and construction of Tet-on expression plasmid
- Preparation of recombinant yeast and the Transformation and screening of recombinant yeast
2.4.1 Tet-on regulation plasmid

The design and construction of Tet-on regulation plasmid
2.4.1 Tet-on regulation plasmid

The picture on the left shows the PCR result, and the right shows the identification result of double enzymes restriction. They indicate that pGAPZB-rtTA and pGAPZB-rtTA-flag are constructed correctly. The fragment size is right.

We proved its correctness by sequencing.
2.4.2 Tet-on expression plasmid
2.4.2 Tet-on expression plasmid

We proved its correctness by sequencing.

The result of fusion PCR show that the fusion segments are right.
2.4.3 Preparation of recombinant yeast

We lead the linearization plasmid into the inner of yeast cell. The linearization plasmid will occur homologous recombination with the genome of yeast to lead our target gene into the genome of yeast. We can screen the transformant by cultivate the cell on resistance or auxotroph plate.

Next, we will test the sensitivity and explore the possibility of business cooperation.
### 2.4.4 Registry Part

Here is the complete list of new parts submitted to the iGEM registry. Each BioBrick is sent in pSB1C3.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Designer</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K1778000</td>
<td>DNA</td>
<td>CYC1TATA sequence is derived from the TATA box specific sequence of yeast Saccharomyces cerevisiae which related to synthetic pigment CYC1 gene promoter.</td>
<td>Haonan Qi</td>
<td>153</td>
</tr>
<tr>
<td>BBa_K1778001</td>
<td>DNA</td>
<td>TRE is closely related to promoter regulation, can correctly specific binding to regulatory protein rtTA, control regulation mechanism. This part act as signal receptor in the project.</td>
<td>Haonan Qi</td>
<td>312</td>
</tr>
<tr>
<td>BBa_K1778002</td>
<td>Composite</td>
<td>TRE-CYC1TATA is a recombinant promoter, which is constructed in order to make the Tet-on system function in Pichia pastoris, which is connected in series by TRE promoter and CYC1TATA sequence.</td>
<td>Haonan Qi</td>
<td>465</td>
</tr>
<tr>
<td>BBa_K1778003</td>
<td>Composite</td>
<td>rtTA-flag is a regulatory protein that can regulate the binding of tetracycline analogues.</td>
<td>Haonan Qi</td>
<td>1032</td>
</tr>
<tr>
<td>BBa_K1778004</td>
<td>Protein_Domain</td>
<td>rtTA is a regulatory protein which can combined with tetracycline analogues. Like the protein in Biological signaling pathways.</td>
<td>Haonan Qi</td>
<td>1008</td>
</tr>
<tr>
<td>BBa_K1778005</td>
<td>Protein_Domain</td>
<td>eGFP: enhanced Green Fluorescent Protein. It’s the mutant of GFP. It is widely used as report gene</td>
<td>Haonan Qi</td>
<td>720</td>
</tr>
<tr>
<td>BBa_K1778006</td>
<td>DNA</td>
<td>Kana-His is a selective marker gene in experiment. The escherichia coli with Kana-His gene has the resistance of kanamycin. The pichia pastoris with Kana-His gene can grow in the culture medium without histidine.</td>
<td>Haonan Qi</td>
<td>5287</td>
</tr>
</tbody>
</table>
2.4.5 Contribution

Our team improved the function and characterization of previously existing BioBrick Parts (created by one of our university teams SCUT in 2014 of iGEM), and entered this information in the part's page on the Registry (These parts do not come from our team's 2015 range of part numbers)

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>BBa_K1462430</td>
<td>Composite</td>
<td>pTEF2+GFP+tADH1</td>
<td>Haonan Qi</td>
<td>1486</td>
</tr>
<tr>
<td>BBa_K1462440</td>
<td>Composite</td>
<td>pTDH3+GFP+tADH1</td>
<td>Haonan Qi</td>
<td>1583</td>
</tr>
<tr>
<td>BBa_K1462450</td>
<td>Composite</td>
<td>pGAL1+GFP+tADH1</td>
<td>Haonan Qi</td>
<td>1632</td>
</tr>
</tbody>
</table>
Part Three
Propaganda
3.1 Wechat
3.3 Team Identity

Team Flag

Team Uniform

Bookmark

Visual Identity
Part Four

Collaboration
4 Mentoring Program

This program included six parts which can also be called six mentoring courses, including:

- iGEM program introduction
- synthetic biology knowledge sharing
- experiment skill mentoring
- bio-brick standardization
- modeling
- human practice design guidance
- web building
05 Part Five
Entrepreneurship
5.1 Scient Visit

On July 31, 2015, our group members visited the headquarters of Scient (China) Infant Nutrition Co., Ltd in Guangzhou, China. In the process of visiting, we introduced IGEM and Antibiotics detection to Miss Chen and showed her our experimental results. Miss Chen said, “Scient is lack of the testing method of tetracycline, and we are very interested in your project. If there is a chance, we can collaborate in the future.”
5.2 Meet Ups — Introduction of the meeting about entrepreneurship which SCUT-Champion-Park participated

Innovation & Entrepreneurship Fair for College Students in Shenzhen

At first, We set the goal to sold our product in the market In order to successfully achieve the goal, we find some venture capital firms to talk about cooperation, also participate in Innovation & Entrepreneurship Fair for College Students in Shenzhen.
06 Part Six Attributions
Our operating model is designed to deliver faster decisions. Each of us has clear duties and tasks. The 15 student members are divided into three big groups, and they are experimental group, social practice group, visual design group.
6 Attributions
6 Attributions
6 Attributions
07

Part Seven

Judging form
7 Judging form

Bronze

Requirements for a Bronze Medal (must complete all):

1. Register for iGEM and attend the Giant Jamboree.
2. Successfully complete and submit this iGEM 2015 Judging form.
3. Create the iGEM wiki, and document the team’s parts successfully.
4. Plan to present a Poster and Talk at the iGEM Jamboree.
5. Create a page on your team wiki with clear attribution of each aspect of your project.

http://2015.igem.org/Team:Scut-Champion-Park/Attributions

6. Document at least one new standard BioBrick Part and submit this part to the iGEM Registry. You may also document a new application of a BioBrick part from a previous iGEM year, adding that documentation to the part’s main page.

http://2015.igem.org/Team:Scut-Champion-Park/Parts

Part Number(s): BBa_K1778000, BBa_K1778001, BBa_K1778002, BBa_K1778003, BBa_K1778004, BBa_K1778005, BBa_K1778006
7 Judging form

Silver

Additional Requirements for a Silver Medal (must complete all):

1. Experimentally validate that at least one new BioBrick Part or Device of your own design and construction works as expected. Document the characterization of this part in the Main Page section of the Registry entry for that Part/Device. This working part must be different from the part you documented in Bronze medal criterion #6.
   http://2015.igem.org/Team:Scut-Champion-Park/Parts
   Part Number(s): BBa_K1778000, BBa_K1778001, BBa_K1778002, BBa_K1778003, BBa_K1778004, BBa_K1778005, BBa_K1778006

2. Submit this new part to the iGEM Parts Registry. This part must be different from the part you documented in Bronze medal criterion #6. (Submissions must adhere to the iGEM Registry guidelines.)
   http://2015.igem.org/Team:Scut-Champion-Park/Parts
   Part Number(s): BBa_K1778000, BBa_K1778001, BBa_K1778002, BBa_K1778003, BBa_K1778004, BBa_K1778005, BBa_K1778006

3. iGEM projects involve important questions beyond the bench, for example relating to (but not limited to) ethics, sustainability, social justice, safety, security, and intellectual property rights.
7 Judging form

Gold

Additional Requirements for a Gold Medal: (Two OR more):

1. Choose one of these two options: (1) Expand on your silver medal Human Practices activity by demonstrating how you have integrated the investigated issues into the design and/or execution of your project. OR (2) Demonstrate an innovative Human Practices activity that relates to your project (this typically involves educational, public engagement, and/or public perception activities. See the Human Practices Hub for information and examples of innovative activities from previous teams).
   http://2015.igem.org/Team:Scut-Champion-Park/Practices

2. Help any registered iGEM team from a high-school, different track, another university, or institution in a significant way by, for example, mentoring a new team, modeling/simulating their system.
   http://2015.igem.org/Team:Scut-Champion-Park/Collaborations

3. Improve the function OR characterization of a previously existing BioBrick Part or Device (created by another team, or by your own team in a previous year of iGEM), and enter this information in the part's page on the Registry. Please see the Registry Contribution help page for help on documenting a contribution to an existing part. This part must not come from your team's 2015 range of part numbers.
   http://2015.igem.org/Team:Scut-Champion-Park/Description
   Part Number(s): B8a_K1462450, B8a_K1462430, B8a_K1462440

4. Demonstrate a functional prototype of your project. Your prototype can derive from a previous project (that was not demonstrated to work) by your team or by another team. Show this system working under real-world conditions that you simulate in the lab. (Remember, biological materials may not be taken outside the lab.)
Acknowledgement
THANK YOU

SCUT-Champion_Park