Synthetic Biology

based on standard parts

Team:Peking/Judging Form

Team:	Peking
iGEM Year:	2019
Track:	Foundational Advance
Project Name:	Dr. Control: A dCas9-based DNA replication control system
Project Abstract:	Many challenges impeding genetically engineered bacteria from benefiting us can be attributed to the growth rate (e.g. infections in microbial therapies) and can be solved if we can better control over it. However, previous methods for growth rate control has many disadvantages: limited application scenarios, cell function disorders, etc. Here, we developed a novel system for precise growth rate control, by using dCas9 to target the DNA replication origin. Such system is highly tunable with multiple inputs, large dynamic range and non-detectable leakage. It functions in a gentle and reversible way without harming cell activities. Furthermore, we explored the potential of replication control in synthetic biology, including control of plasmid copy number and gene expression variation. Finally, we tried to design a safe therapeutic E. coli with high targeting specificity and controllable treatment intensity, promising to reduce the infection risk, which shows the broad application prospects of our system.

Edit

iGEM Medals

All qualified teams are eligible for medals at the 2019 Giant Jamboree. Every team can win their own medal based on their achievements; there is no limit to the number of Gold, Silver, or Bronze medals that can be given at the Giant Jamboree. Standard Tracks and Special Tracks have different medal criteria. Please ensure the correct track is listed for your team.

To be judged for a specific prize or medal criterion, **you must document your achievements for that prize/criterion on the specified page**. Please see Pages for Awards for more information.

You must convince the judges that your work meets the medal criteria. If the judges are not convinced, you will not be awarded the medal.

Details for 2019 iGEM Medal Criteria can be found on the Judging/Medals page.

Requirements for a Bronze Medal (must complete all):

1 **Registration and Jamboree Attendance:** Register for iGEM, have a great iGEM season, and attend the Giant Jamboree.

2 **Competition Deliverables:** Convince the judges that you have completed the following Competition Deliverables:

- Wiki
- Poster
- Presentation
- Judging Form

3 **Attributions:** Convince the judges that you have completed Competition Deliverable #5 Attributions.
Please note: This requirement is not about citing literature references. Attributions is about describing what work your team did and what other people did for your project.

Required link: http://2019.igem.org/Team:Peking/Attributions

This team can be evaluated for this medal.

4 **Project Inspiration and Description:** Convince the judges that you have completed Competition Deliverable #8 Project Inspiration and Description.
On your Project Description page, document how and why you chose your iGEM project, and in a few sentences describe how you will achieve your goal(s). Refer to work outside or inside of iGEM that inspired your project, how you selected your project goal(s), and why you thought your project was a useful application of synthetic biology.

Required link: http://2019.igem.org/Team:Peking/Description

This team can be evaluated for this medal.

5 **Characterization:** Convince the judges that you have added quantitative experimental characterization data to an existing Part from the Registry of Standard Biological Parts. See the Measurement Hub for more information, resources, and examples of previous teams' exemplary work.

- Clearly document the experimental characterization on that Part's Main Page on the Registry (see the Registry Document Parts page for instructions).
  Judges will only look at the Registry page to evaluate your Parts, so make sure all of your information and characterization data are posted on your Part's Main Page on the Registry!
- This existing part may be a Basic or Composite part and must be BioBrick RFC10 or iGEM Type IIS compatible.
- The part that you are characterizing must NOT be from a 2019 part number range.
- It is acceptable to add new data to an already highly characterized part.
- Sample submission is not required.

Enter the existing Part Number you have characterized below.

Part Number(s):	
BBa_I0500	This part can be evaluated for this medal.
BBa_T9002	This part can be evaluated for this medal.

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

1 **Validated Part/Validated Contribution:** Convince the judges that at least one new BioBrick Part of your own design that is related to your project works as expected.

- Clearly document the experimental characterization on that Part's Main Page on the Registry (see the Registry Document Parts page for instructions).
  Judges will only look at the Registry page to evaluate your Parts, so make sure all of your information and characterization data are posted on your Part's **Main Page** on the Registry!
- This new part may be a Basic or Composite part.
- This new part must be BioBrick RFC10 or Type IIS compatible.
- If your team is creating a new part for Gold #2, the Silver #1 part must be different from the new part documented for Gold #2.
- Sample submission is not required.

Part Number(s):

BBa_K3081053	This part can be evaluated for this medal.
BBa_K3081054	This part can be evaluated for this medal.
BBa_K3081002	This part can be evaluated for this medal.

2 **Collaboration:** Convince the judges you have significantly worked with one (or more) currently registered 2019 iGEM team(s) in a meaningful way. For example, mentor a team (or be mentored by a team), characterize a part, troubleshoot a project, host a meetup, model/simulate a system, or validate a software/hardware solution to a synthetic biology problem.

Document your collaboration in detail on your wiki. Judges will look at your collaborator's wiki to see what they say about your interaction. Simply filling out a survey for a team is not enough to demonstrate a significant interaction.

Required link: http://2019.igem.org/Team:Peking/Collaborations

This team can be evaluated for this medal.

3 **Human Practices:** Convince the judges you have thought carefully and creatively about whether your work is responsible and good for the world. Document how you have investigated these issues, how you engaged with communities relevant to your goals, why you chose this approach, what you have learned, and the potential impact of your project's success. See the Human Practices Hub for more information and examples of previous teams' exemplary work. Please note that surveys will not fulfill this criteria unless you follow scientifically valid methods.

Required link: http://2019.igem.org/Team:Peking/Human_Practices

This team can be evaluated for this medal.

Additional Requirements for a Gold Medal: (Must complete two OR more)

1 **Integrated Human Practices:** Expand on your silver medal activity by demonstrating how you have integrated the investigated issues into the purpose, design, and/or execution of your project. Document your process and describe how your human practices work informed and shaped your project at different stages. See the Human Practices Hub for more information and examples of previous teams' exemplary work.

Required link: http://2019.igem.org/Team:Peking/Human_Practices

This team can be evaluated for this medal.

2 **Improve a Previous Part or Project:** Convince the judges that you have created a new Part that has a functional improvement of an existing Part. You must perform experiments with both parts to demonstrate this improvement.

- Clearly document the quantitative experimental characterization data on the Part's Main Page on the Registry for both the existing and new parts (see the Registry Document Parts page for instructions).
- The new part must be BioBrick RFC10 or Type IIS compatible.
- The sequences of the new and existing parts must be different. Making an existing part compatible to RFC10 or Type IIS is not sufficient to fulfill this criterion.
- The existing part must NOT be from your 2019 part number range.
- The existing part must be different from the part you used in Bronze #5.
- The new part you create must be different from the new part documented in Silver #1.
- Sample submission is not required.

Enter the part number for the existing part you are improving in the box below:

Part Number:	
BBa_K1150000	This part can be evaluated for this medal.

Enter the part number of your new part in the box below:

Part Number:	
BBa_K3081055	This part can be evaluated for this medal.

3 **Model Your Project:** Convince the judges that your project's design and/or implementation is based on insight you have gained from modeling. This could be either a new model you develop or the implementation of a model from a previous team. You must thoroughly document your model's contribution to your project on your team's wiki, including assumptions, relevant data, model results, and a clear explanation of your model that anyone can understand.
The model should impact your project design in a meaningful way. Modeling may include, but is not limited to, deterministic, exploratory, molecular dynamic, and stochastic models. Teams may also explore the physical modeling of a single component within a system or utilize mathematical modeling for predicting function of a more complex device.

Required link: http://2019.igem.org/Team:Peking/Model

This team can be evaluated for this medal.

4 **Demonstration of Your Work:** Convince the judges that your engineered system works.
Your engineered system has to work under realistic conditions. Your system must comply with all rules and policies approved by the iGEM Safety Committee. Your system can derive from or make functional a previous iGEM project by your team or by another team. For multi-component projects, the judges may consider the function of individual components.

Required link: http://2019.igem.org/Team:Peking/Demonstrate

This team can be evaluated for this medal.

iGEM Special Prizes

All teams are eligible for special prizes at the Jamborees. Your team will be evaluated by the judges if (1) you have documented your special prize activity on your wiki on the specified page, AND (2) you have explained on this form why you think your team is eligible for this prize. More information on special prizes

- **Integrated Human Practices**

Required link: http://2019.igem.org/Team:Peking/Human_Practices

This team can be evaluated for this prize.

Please write about 150 words on why you think your team is eligible for this prize:

Safety is an important issue in bacteria therapy, which is mentioned in many lectures. In this summer, we consulted many experts to know the safety concerns in clinical research. They provide us with information that uncontrolled bacteria growth will cause serious infections and cytokine storm. To solve these problems, we focused on the key factor, bacteria density, and we came up with the idea to design a DNA replication control system, which could affect bacteria growth directly.
One of the experts, Dr. Dong Yuxuan agrees with our theory. He told us bacteria density control is very helpful to keep a balance between bacterial toxicity and therapeutic effects. After sharing the preliminary data with him, He was very curious about our bacteria’ s physical condition, which may have an important impact on the therapeutic efficacy of engineered

of bacteria. Another interviewee, Prof. Zhang Lixin attaches great importance to precise control of cell density, and appreciates our project which can produce slow-growing strain easily. All the feedbacks motivated us to optimize the project continually. Human practices helped us to find the application scenarios for our project and proposed the optimization of the project design. From the start to the end, we leared a lot from human practices, and we hope our project would be valuable for humanbeings.

- **Education and Public Engagement**

Required link: http://2019.igem.org/Team:Peking/Public_Engagement

This team can be evaluated for this prize.

Please write about 150 words on why you think your team is eligible for this prize:

Genome editing using CRISPR/Cas9 has the potential to make great breakthrough in many areas, including industry, agriculture and medicine. However, a few ethical issues need to be resolved, such as “can we edit germline cells”, etc. We designed a questionnaire to understand public attitude and opinions about this technology. Then, we interviewed scientists, ethicists, doctors and several patients to identify the present and future possibilities in genome editing, as well as relevant ethical dilemma. It turns out that some people agree to use gene editing in human. But all the experts we interviewed are cautious about gene editing in human being. A wide-spread general agreement about the topic still needs to be achieved. As we can learn from the results above, there is a growing gap between the frontier science and the public. So, making scientific knowledge accessible to all members of society is really important. We build up a WeChat platform and release many scientific articles to our subscribers. These articles include basic biology, synthetic biology, mathematical modeling, experimental technology and many other things and most of them received positive feedbacks.

- **Model**

Required link: http://2019.igem.org/Team:Peking/Model

This team can be evaluated for this prize.

Please write about 150 words on why you think your team is eligible for this prize:

To make the DNA replication control system precise and versatile, we focused on bacterial behaviors related to DNA replication, such as cell growth, etc. We built a single-cell stochastic model to describe that DNA replication would be delayed due to the expression of dCas9-sgRNA. Another model predicted the system could improve the metabolic efficiency, like to produce more high value bio-products by interfering DNA replication. The prediction was proved by our experiments. We also used models to simulate the behaviors of plasmid replication control system and quorum sensing-based DNA replication control system. These phenomena were validated by subsequent experiments. Last but not least, with the modelling, we have explored our system enabled to control the gene expression noise due to the variation of gene copy number. All the theoretical models helped us along the way of fully exploit potentials of "Dr. Control".

- **Measurement**

Required link: http://2019.igem.org/Team:Peking/Measurement

This team can be evaluated for this prize.

Please write about 150 words on why you think your team is eligible for this prize:

The overall state of bacteria has proved to be one of the most essential factors that affect operation of functions of genetically engineered bacteria. Herein, we developed a CRISPR-based replication interference system to bridge the synthetic parts and bacterial overall states. A rounded characterization system, including multiple measurement methods, is well developed as a full-scale quantitative description of E. coli general states, while three parameters are chosen as measure: cell growth, cell morphology and irrelative protein productivity. Besides common biochemical methods, unique 4-channel and multi-view microfluidic system is developed for observation of the cell division and cell morphology. Another designed microfluidic chip enables us to directly record cell adhesion with different flow velocity. Nucleo-cytoplasmic ratio is measured using a mature nucleoid staining method followed by observation under laser scanning confocal microscope. Each approach we utilized is highly repeatable and portable and we helped Tsinghua iGEM with observation of cell division using our microfluidic system. Most of observed phenomenon from one method can be proved by another, to ensure the reliability of our results. High robustness and precision of our measurement system helped us build a quantitative insight into the inner link among different parameters of E. coli, which provides a potential characterization and evaluation framework to extend to any artificial bacteria system.

- **Entrepreneurship**

Required link: http://2019.igem.org/Team:Peking/Entrepreneurship

This team will not be evaluated for this prize because the required page still contains the 'judges-will-not-evaluate' div
This team will not be evaluated for this prize because they have not entered an explanation below

Please write about 150 words on why you think your team is eligible for this prize:

- **Software Tool**

Required link: http://2019.igem.org/Team:Peking/Software

This team will not be evaluated for this prize because the required page still contains the 'judges-will-not-evaluate' div
This team will not be evaluated for this prize because they have not entered an explanation below

Please write about 150 words on why you think your team is eligible for this prize:

- **Hardware**

Required link: http://2019.igem.org/Team:Peking/Hardware

This team will not be evaluated for this prize because the required page still contains the 'judges-will-not-evaluate' div
This team will not be evaluated for this prize because they have not entered an explanation below

Please write about 150 words on why you think your team is eligible for this prize:

- **Plant Synthetic Biology**

  Required link: http://2019.igem.org/Team:Peking/Plant

  This team will not be evaluated for this prize because the required page still contains the 'judges-will-not-evaluate' div
  This team will not be evaluated for this prize because they have not entered an explanation below

  Please write about 150 words on why you think your team is eligible for this prize:

Part Awards

To help the judges evaluate your parts, please identify your highest quality part for each of the following prizes:

- **Best New Basic Part**

  Provide the name of your one best Basic Part.

  Each part must be fully documented in the Registry, but you can also provide an overview on your wiki Basic Part page.

  Part Number:

  BBa_K3081055 This part can be evaluated for this prize.

- **Best New Composite Part**

  Provide the name of your one best Composite Part.

  Each part must be fully documented in the Registry, but you can also provide an overview on your wiki Composite Part page.

  Part Number:

  BBa_K3081007 This part can be evaluated for this prize.

- **Best Part Collection**

  List all of the Parts in this collection. You must provide a minimum of three parts to be considered for the Best Part Collection Prize.

  Each part must be fully documented in the Registry, but you can also provide an overview on your wiki Part Collection page.

  Part Number(s):

  BBa_K3081058 This part can be evaluated for this prize.
  BBa_K3081006 This part can be evaluated for this prize.
  BBa_K3081007 This part can be evaluated for this prize.
  BBa_K3081008 This part can be evaluated for this prize.
  BBa_K3081009 This part can be evaluated for this prize.
  BBa_K3081010 This part can be evaluated for this prize.
  BBa_K3081011 This part can be evaluated for this prize.
  BBa_K3081012 This part can be evaluated for this prize.
  BBa_K3081013 This part can be evaluated for this prize.
  BBa_K3081014 This part can be evaluated for this prize.
  BBa_K3081015 This part can be evaluated for this prize.
  BBa_K3081016 This part can be evaluated for this prize.
  BBa_K3081053 This part can be evaluated for this prize.
  BBa_K3081054 This part can be evaluated for this prize.
  BBa_K3081060 This part can be evaluated for this prize.
  BBa_K3081057 This part can be evaluated for this prize.
  BBa_K3081041 This part can be evaluated for this prize.
  BBa_K3081042 This part can be evaluated for this prize.
  BBa_K3081043 This part can be evaluated for this prize.
  BBa_K3081031 This part can be evaluated for this prize.
  BBa_K3081027 This part can be evaluated for this prize.

  Please write about 150 words describing the function of this Part Collection, and give the Range of Part Numbers.

  2019 Peking iGEM constructed a large part collection focusing on dCas9-based DNA replication control. The dCas9 expression module has been optimized and expanded by using different regulatory elements, adding degradation tag, fusing a fluorescent protein reporter, etc. The updated modules enable our system to function in a low-leakage, quantifiable and reversible manner, and a series of application scenarios. We also designed a new expression module for sgRNA. The new expression system of two inputs not only benefit our project but also can be a new paradigm of CRISPRi for subsequent researchers. We have also constructed a library of sgRNA to target the replication origin of E. coli genome or plasmids (p15A and pSC101). sgRNAs are different in target sites, length and number, thus can perform a wide range of control effects. All the parts are thoroughly characterized by different methods: microscopic imaging, spectrophotometer, cytometer, microfluidic devices, qPCR, etc.