Project Title:

Bacterial photography and edge detection: engineering light sensitive circuits in *E. coli*

Report Title:

Bacterial photography and edge detection: engineering light sensitive circuits in *E. coli*

Summary

A very short summary or abstract of your project and the main outcomes (200 word max)

Our project aimed to lead a group of undergraduate students through the recapitulation of the results of Tabor et al. (*Cell 2009*) in producing a synthetic genetic circuit that programmed *E. coli* bacteria to detect edges in images. Our students reproduced a computational model of the gene circuit's dynamics, solving the reaction-diffusion equation to track the evolution of different chemical species on a petri dish over time. We then used Benchling to design primers for Gibson Assembly of the appropriate constructs from the paper, and executed the required PCR reactions. The project culminated in a series of five workshops taking (a new set of) students from various academic backgrounds through the necessary steps to implement the circuit, including design, PCR, assembly, bacterial transformation, and mathematical modelling.

Report and outcomes

The report should be a short written description of what you accomplished and how, including an evaluation of where things didn't go to plan! Please include links to or copies of any outputs (papers, posters, data, code, hardware designs, photos, blogs, videos) - these do not need to be duplicated in the report. If you are attaching supplementary files, please refer to them in the text or add a list with a brief description at the end e.g. OpenPlantReport.pdf: Formatted version of full project report. FooSequences.fasta: Sequence file for DNA parts ACB123 and DEF345. Refer to the reporting guidelines for more information.

https://github.com/bill-jia/cusbsedgedetectionmodelling

http://cusbs.soc.srcf.net/bio-project/

Modelling

Tabor et al. presented a computational model of the gene circuit dynamics in their paper. The model tracked the time-evolution of edge detection in response to an input, by simulating the dynamics of AHL diffusion, production, and decay under the reaction-diffusion equation. Our team wrote a program in Python to reproduce these results from the differential equations presented in the paper. The software implements central-difference numerical approximation routines for the reaction-diffusion equation, and allows for simulation at different time and space resolutions, with any arbitrary light input pattern. It produces images that map the progression of concentrations of AHL and beta-galactosidase, as well as plots of transfer functions corresponding to the outputs of P_{OmpC} and $P_{lux-\lambda}$ (the light-sensing and logic promoters respectively).

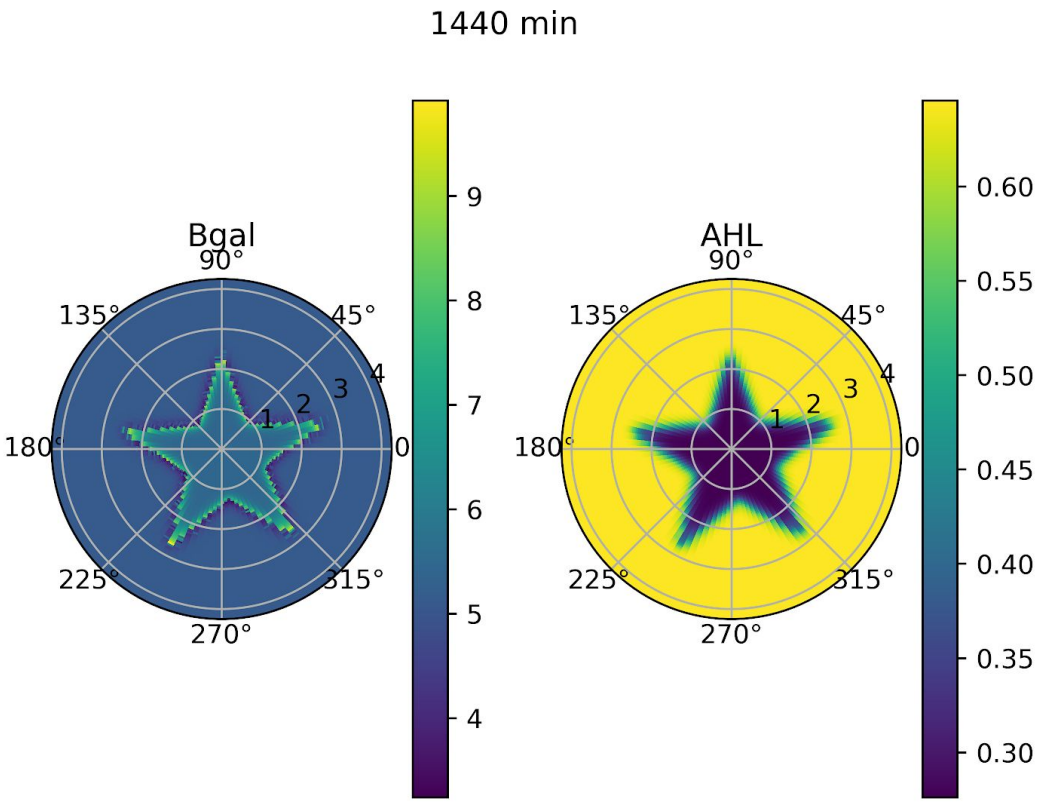

Figure 1: Example edge detection simulation output. *Left*: Beta-galactosidase concentration is highest at the edge of the projected star shape, where cells exposed to light express protein because they receive AHL from cells in darkness but have CI expression silenced by the light sensor *Cph8*. *Right:* AHL concentration is highest where it is being produced in the darkness (outside the star), and decays towards the center of the dish where AHL production is silenced.

Wet-lab preparation

Tabor et al. used Biobrick assembly to build their genetic circuit. This approach was a laborious process, involving separate restriction enzyme reactions for each pair of parts. In an attempt to streamline this process, we explored newer one-pot standards such as Gibson and Golden Gate Assembly. We decided to work with Gibson Assembly, which uses T5 exonuclease to clip off overlapping ends of adjacent parts and create complementary sticky ends. We chose Gibson Assembly for three reasons. First, it requires minimal additional sequences (e.g Golden Gate destination vector) and has the potential to work without introducing any extraneous sequences. Second, it can in principle assemble a large number of fragments (an assembly of up to 20 fragments has been demonstrated). Third, it is conceptually simple to understand, and we felt it would be instructive for students.

We first designed primers to extend our Biobricks with overlapping ends. We used the Gibson Assembly wizard in Benchling, an online app with various tools for molecular biology. The wizard automatically generated primers for each of our constructs that minimised off-target binding and had appropriate overlaps, while allowing incorporation of additional sequences too short for their own part, such as ribosome binding sites. We generated primers for four prospective constructs, each building in complexity up to the final edge detection circuit. In a few parts, the sequence at either end would have caused problems for the PCR, so we manually designed alternate primers. For

example, LuxI, the gene responsible for AHL production, has a strong secondary structure at its 5' end, so we included in the forward primer additional bases upstream of the gene on its backbone.

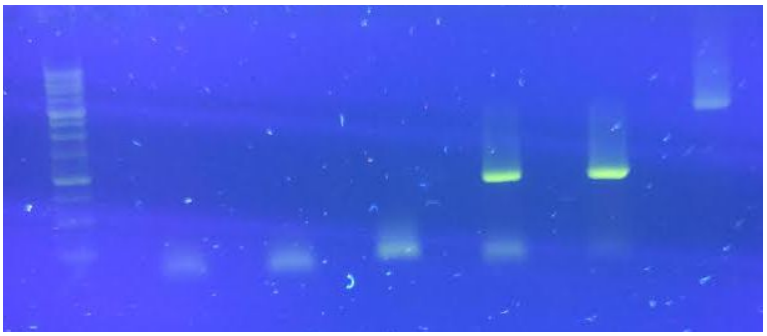

Figure 2: Gel electrophoresis of PCR products for pAZ1. From left to right: 1 kb ladder, strong constitutive promoter (BBa_J23119, 71 bp), first 70bp spacer (BBa_B0040, 106 bp), second 70 bp spacer (BBa_B0040, 106 bp), pcyA (BBa_I15009, 811 bp), ho1 (BBa_I15008, 751 bp), cph8 (BBa_I15010). All bands are located approximately where they are expected, and secondary bands for pcyA and ho1 are expected due to primer dimers.

We then ran the PCRs for each part and verified their success using gel electrophoresis. Due to time and material constraints we elected to only do so for the least- and most-complicated systems, a straightforward bacterial photography system (pCUSBS1) and the full edge detector circuit, in addition to the light-sensing circuit (pAZ1) (Supplementary Figures 1-6, full plasmid sequences in Supplementary Table 1). We used two circuits for the edge detection system, designed by one of our project managers Andre Zylstra, and by the student team (pAZ2 and pCUSBS2 respectively) . The majority of the PCRs appeared to be successful by size validation (Supplementary Figure 7, labels in Supplementary Table 2). There were however two major challenges. First, the light sensor *cph8*, required for both circuits, initially failed to amplify. We attempted to optimize primers and increase the template concentration through bacterial transformation and miniprep. However, we ultimately discovered that the original template from the iGEM distribution was either of poor quality or mislabelled entirely, and obtained an alternative sequence that amplified successfully with our primers. Second, the primers we designed for PCR amplification of iGEM plasmid backbones formed strong primer dimers. We instead obtained the linearized backbones by digest of a BioBrick using restriction endonucleases SpeI and XbaI.

We used a gel extraction kit (NEB Monarch) to purify the amplified fragments and quantified the end DNA concentrations using a nanodrop spectrophotometer. We then attempted to assemble pAZ1 using the NEBuilder HiFi assembly kit. The procedure was unsuccessful; no transformed colonies were observed when plated on chloramphenicol agar. Since the antibiotic resistance gene is contained on the backbone, this result suggests that the plasmid did not circularize successfully. There are several possible reasons for this failure. First, the DNA concentration after gel extraction could have been insufficient (Supplementary Table 3). This is probable as we used a relatively large number of parts, and some of the concentrations indicated by the spectrophotometer after gel extraction were below its detection limit or below the minimum recommended by the kit manufacturer. Second, the DNA sequences might not have been what we believed them to be, and might not have had the required overlaps. The spacers and promoters in particular are very short sequences, making gel electrophoresis a less reliable method of identification. The bands observed could have fairly easily been primer dimers or off-target amplifications. Third, the assembly kit had

already expired by the time we obtained the necessary clearance for bacterial transformation and execution of these experiments. The assembly efficiency could have been reduced by the kit's expiry.

We have addressed the first possible cause of Gibson assembly failure by redesigning the circuits to combine parts using large primers, and aim to reattempt the procedure with these PCRs. The second concern could be solved by sequencing the parts before assembly. While the completion of the assembly with our circuit designs would effectively be a validation of teaching materials that could be used by others, we achieved our desired teaching outcomes for our workshops simply by using the positive control provided with the assembly kit for the Gibson Assembly, and by ordering the completed plasmid from Addgene for the bacterial transformation step.

Workshops

We organised five weekly workshops in October-November 2018. Each workshop contained work in the wet lab of the Biomakespace and short talks going over the theory behind the lab experiments. 55 different individual students aside from our committee engaged with the workshops.

Introductory workshop

Blog post:
http://cusbs.soc.srcf.net/2018/10/31/introductory-workshop-cell-free-expression-14-october-2018/

Our first workshop was an introductory workshop to introduce new participants to synthetic biology and the nature of experiments involved. In this workshop, we demonstrated cell-free gene expression using the myTXTL cell-free kit (Arbor BioSciences). We aimed to express enhanced green fluorescent protein (eGFP) from the σ70 eGFP plasmid included in the kit.

We tested the kit by following the manufacturer's protocol. We used both the extract volume for one full reaction and half of that volume with the plasmid, and used nuclease-free water in place of the plasmid as a control. A colour change was not visible under visible light after 16 hours. However, a green glow was visible under a UV transilluminator for both the full and half reactions.

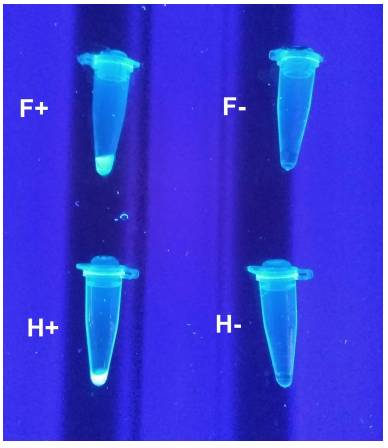

Figure 3: Cell-free expression of eGFP. F and H indicate full-volume and half-volume reactions respectively. The reactions containing plasmid fluoresced, while the negative controls did not.

To amplify the σ70 eGFP plasmid, we transformed NEB DH5α *E. coli* cells with the plasmid using a protocol based on the NEB DH5α high-efficiency transformation protocol (C2987H). Because we lacked ampicillin selection plates at the time, we added ampicillin to Miller LB broth at 37°C, then we inoculated the mixture with *E. coli*+SOC. This created a mixture of every cell that survived in the tube rather than colonies that contain cells that were genetically identical. We decided that genetically

identical colonies were not needed as we were amplifying a plasmid rather than sub-cloning. After overnight incubation, the plasmids were extracted using the QIAprep MIniprep kit, and yielded a 22.1 µg/mL concentration. The miniprep product was tested with the myTXTL cell-free extract and yielded a green glow under UV radiation. Later, the transformed bacteria were plated on ampicillin selection plates. The resulting colonies glowed under UV radiation.

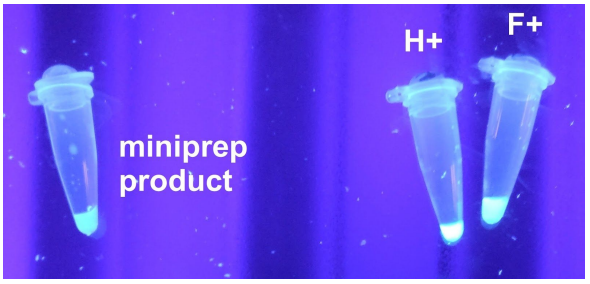

Figure 4: Cell-free expression of miniprep product.

Additionally, we transformed fluorescent proteins and chromoproteins from the 2014 iGEM registry. These included RFP and GFP with varying promoter strengths. This was to test both the effect of colour and promoter strengths, and to illustrate the idea of standardised biological parts. We prepared liquid cultures and miniprepped the plasmids as before.

During the workshop, participants added the plasmids (σ 70 eGFP along with RFP/GFP from the iGEM registry) to full- and half- reaction volumes of the myTXTL cell-free extract. We instructed the use of micropipettes. The results were as expected, and the workshop attracted approximately 25 participants.

The introductory workshop was followed by workshops that aimed to take participants through the techniques in synthetic biology that are required to implement the bacterial photography gene circuit.

2. Gibson design and PCR

Blog post:
http://cusbs.soc.srcf.net/2018/10/31/workshop-1-gibson-design-and-pcr-21-october-2018/

Participants designed primers for Gibson assembly using Benchling.

Subsequently, participants performed PCR to amplify DNA and add overhang sequences that correctly order the parts for circuit assembly. At the time, we lacked suitable DNA templates corresponding to the Gibson constructs as used to assemble the bacterial photography gene circuit. For the purposes of demonstration, we used the iGEM 2014.I.A11, O23, M5, I5 miniprep products that were prepared in the introductory workshop as templates instead. We used the iGEM VR and VF2 as primers. PCR was run on a protocol based on that recommended by the vendor for Q5 High-Fidelity DNA Polymerase (New England Biolabs, M0491S).

We then demonstrated gel electrophoresis as a method to verify PCR products. We prepared a 1% agarose gel with 1x TBE. 2 µL SYBR was added. The PCR products for O23 (1069 bp) and M5 (782 bp) were chosen. Primary bands appeared at expected lengths, but secondary bands were also visible.

3. Gibson assembly and transformation

Blog post:
http://cusbs.soc.srcf.net/2018/10/31/workshop-2-gibson-assembly-and-transformation-28-october-2018/

To prepare for this workshop, we attempted Gibson assembly according to the New England Biolabs protocol (E5510). We used HO1, Cph8, and PcyA and a pSB1C3 backbone. Transformation of the resulting construct into DH5α cells yielded no colonies, suggesting that Gibson assembly failed. To rectify this, we re-designed the Gibson assembly overlap primers, as discussed in the *Wet lab preparation* section.

For the workshop, we demonstrated Gibson assembly using the positive control plasmid included in the kit (NEB E5510). We then demonstrated transformation by having participants follow through the five-minute quick heat-shock transformation protocol by NEB (C2987).

As an optional activity, participants verified PCR products from the week before by quantifying DNA concentrations using a nanodrop spectrophotometer. We demonstrated the use of appropriate controls by including water, a SYBR safe-only solution, a solution containing dNTPs+SYBR, and a solution with a plasmid + dNTPs + SYBR. The PCR product gave a higher absorbance at 280 nm.

4. Plasmid miniprep

Blog post: http://cusbs.soc.srcf.net/2018/11/11/workshop-3-plasmid-miniprep-4-november-2018/

DH5α liquid cultures were prepared with these plasmids before the workshop:

- BBa_K608002 Strong promoter and RBS (14.I.3O)
- BBa_K608351 Temperature sensitive repressor (14.I.6N)
- BBa_J45199 Isoamyl acetate generator (14.IV.5I)
- BBa_K592009 Blue chromoprotein (14.I.19E)
- BBa_K398331 Stress inducible promoter (14.I.1E)
- BBa_K523000 pLac + lacZ (14.I.3E)

We introduced how miniprep kits purify nucleic acids. Participants extracted plasmids from these liquid cultures using miniprep kits.

In an optional session, participants digested the miniprep products with XbaI and SpeI restriction enzymes in green TANGO buffer. The products were analysed on a 0.8% agarose gel, and bands corresponding to the respective DNA lengths were observed.

Additionally, some participants decomposed the plasmids with restriction enzymes and analysed the DNA fragments with gel electrophoresis (see below). Products of restriction digests could not be resolved in our observations, and the results could have been improved by increasing the voltage or run time.

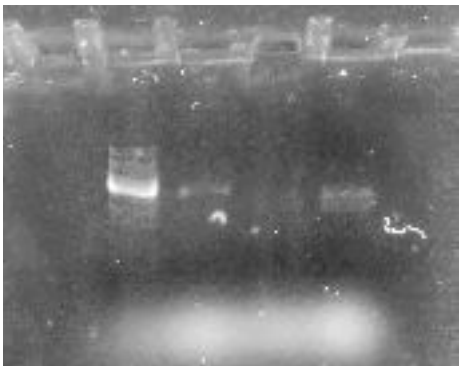

Figure 5: Lanes left to right: (1) marker, brightest band 3 kb; (2) K60831; (3) J45199; (4) K592009. Plasmids are all ~2.8 kb.

5. Testing the genetic circuit

Blog post: http://cusbs.soc.srcf.net/2019/01/03/workshop-4-testing-the-gene-circuit-11-november/

The final workshop aimed to test the final genetic circuit for bacterial photography.

Because Gibson assembly failed earlier, we ordered the plasmids that were used in the Tabor et al. (2009) paper as glycerol stabs via AddGene. These consist of the pJT103 (photography), pJT105, pEDL (edge detection), and pSR44.3r (light sensing) plasmids. We also ordered the JW33670 and JT2 strains that were used in the paper from the Voigt Lab (MIT).

In preparation for this workshop, we prepared ampicillin plates for selection of strains containing pJT103, pJT105, and pEDL. The pSR44.3r plasmids required ampicillin+spectinomycin selection plates, but we prepared ampicillin+streptomycin plates instead as we lacked spectinomycin and reasoned that the two antibiotics had similar properties. After preparing liquid cultures and extracting plasmids by miniprep, we then co-transfected pSR44.3r+pEDL into DH5α and JW33670 cells using heat-shock transformation. We also co-transfected pSR44.3r and pJT103 into JT2 cells using the same method. We observed colony growth for the DH5α transformant, but none for JW33670 or JT2. These results suggested that transformation failed for the latter two strains.

During the workshop, we adapted the methods specified in the supplementary data of Tabor et al. (2009) to create bacterial edge detection plates. In addition to preparing LB agar containing ferric ammonium citrate, we also prepared a variant without this salt. Workshop participants added streptomycin instead of spectinomycin to the molten agarose because of a lack of the latter. Then they inoculated the molten agarose with DH5α transformed with pSR44.3r and pEDL. Finally, the participants incubated the plates with light masks under a red LED lamp.

After 24 hours, all plates regardless of light mask pattern (or whether there is a light mask) turned a uniform black colour. Edge detection was not achieved. We hypothesised that using DH5α instead of JW33670 made the salt concentration in the LB media cause excessive downstream signalling from the EnvZ/OmpR system in the *E. coli* cells (Foo et al. 2015: https://dx.doi.org/10.1016%2Fj.pbiomolbio.2015.04.005), resulting in saturation of pigment production. This is because DH5α is highly sensitive to the salt concentration. However, the presence of pigment indicated that the basic genetic circuit elements were functioning, and that our double transformation was indeed successful.

In this workshop, we also demonstrated our mathematical model, described in the *Modelling* section above. Participants worked with a Jupyter Notebook (based in Python) that broke down the

modelling script into sections. We explained the biological and mathematical theory behind each section.

After the conclusion of the workshops, we started an attempt to reproduce edge detection again. We had prepared glycerol stocks of the JW33670 and JT2 from agar stabs donated to us by the Voigt lab, but had not made them competent. We therefore reasoned that the lack of competence was what caused the transformation to fail. In an attempt to rectify this issue, we prepared electrocompetent JW33670 cells using the protocol used by the Krantz Lab (UC Berkeley) (Baba et al. 2006: http://msb.embopress.org/content/2/1/2006.0008.long).

Logistical Challenges

One of our main challenges was access to laboratory space, equipment, and purchasing. We had anticipated access to Biomakespace (BMS) at the beginning of October 2017, but were only able to begin work in January 2018. The delay of one university term was significant for our undergraduate members, who are only in Cambridge for three such terms in a year. Although we were able to start the wet lab portion of the project in January, we were restricted to conducting PCRs and gel electrophoresis because BMS had not obtained a genetically modified organisms license yet. We helped to expedite the approval process by using our project as the basis for acquisition of this license, but the space did not obtain it until July 2018. This meant that we were unable to do bacterial transformation, obviating the Gibson assembly step. A related source of issues was purchasing - BMS has limited capacity to receive orders, so we set up a cost center in the Department of Plant Sciences. While we were able to obtain the reagents we needed, training, ordering, and transportation presented a time overhead that was sometimes significant and the cause of some delays in the project.

The participation structure for our project also hindered its progress. Our aim was to welcome all students by being inclusive and not explicitly asking for commitment. However, this strategy additionally implied that there was some discontinuity between project sessions and that engagement was less strong. Work was often left up to committee members and progress might not have been as quick or efficient as it could have been. We have attempted to remedy this problem in the 2018-2019 academic year. The first term consisted of low-commitment workshops that applied the educational output of our project to new students, teaching them skills and improving engagement. At the end of the term, the participating students came together to develop a new project to work on, and we hope that future work will be more independent and involved due to this higher level of ownership.

Changes to team

Please include here a note if there have been any changes to the team so that we can make sure the information on the www.biomaker.org website is up to date. If additional team members have joined, please provide a photo together with their name, job role and affiliation.

In keeping with our position as a student society at the University of Cambridge, our committee has changed by by democratic election since the last project update:

President
Bill Jia
Part IIb Engineering Tripos

Vice-President
Camillo Moschner
PhD Candidate, BBSRC DTP in Biological Sciences

Treasurer
Stefan Grossfurthner
PhD Candidate, Plant Sciences

Secretary
Andre Zylstra
PhD Candidate, Babraham Institute

Project Manager, Biological
Jarrod Shilts
PhD Candidate, Wellcome Trust Sanger Institute

Project Manager, Biological
Arin Wongprommoon
Part II Biochemistry, Natural Sciences Tripos

Project Manager, Biological
Dr. Eugene Park
Postdoctoral Fellow, Ringshausen Lab (MRC Stem Cell Institute)

Project Manager, Hardware
Joshua Lawrence
PhD Candidate, BBSRC DTP in Biological Sciences

Webmaster/Social Media Officer
Erica Lee
Part IIa Manufacturing Engineering Tripos

As in previous years, the project team itself is flexible and as such we do not keep strict records of team membership. X individual students have been involved in the project in the past year.

Expenditure

Category	**Amount (GBP)**
Admin	295.80
Speaker event	55.15
Consumables	118.47
Reagents	1,155.08
Hardware	269.58
Biomakespace fees	500.00
Total	**2,394.08**

Would you like to claim the £1,000 follow-on fund?

Yes, we would like to claim the £1,000 follow-on fund to be used as described below.

Follow on Plans

A short description of your plans for follow-on work with a breakdown of how you will spend the additional £1000 (if requested) and any remaining funding from the initial £4000. Please include timings as it is expected that all funds will be spent within six months of this report, after which point a brief report from the follow-on activities and return of the remaining funding will be requested.

We hope to accomplish three goals with the remaining money from the OpenPlant Fund.

1. **Successfully assemble our designed constructs to complete our set of resources for running synthetic biology workshops.** We have successfully executed other steps of the workshops (design, transformation, testing). We only need to validate our construct assembly before we can share our work as a reliable set of materials for others to replicate. We expect this to cost £1000, to cover the purchase of assembly kits, primers, and sequencing.
2. **Continue to run our workshops in the next academic year**. We found that these workshops were an effective way to engage students with little to no experience in synthetic biology while teaching them practical lab skills. We think it would be valuable to continue to hold these workshops, and that doing so would be in line with the initial goals of the OpenPlant grant. We expect this to cost £1000, including the required reagents and Biomakespace fees.
3. **Assist in purchasing reagents and Biomakespace memberships for our next project.** The work will involve engineering defined spatial interactions between pairs of bacterial strains that interact with each other (see project proposal linked below). Specifically, pairs of bacteria that are symbiotic, competitive, or commensal will be engineered to adhere to each other or themselves. We hope that this will allow insights into the evolution of spatial structure in microbial communities, and into the formation of complex patterns in development. This topic was voted on by current society members. It will be structured as a multi-year research project involving independent work on the part of society members, with assistance and leadership from experienced project managers. Our goal is to provide a level of engagement in research beyond the technical lab skills taught by our workshops, and to allow students to ask deeper questions in synthetic biology with a higher level of ownership. An additional benefit to this structure is the possibility of producing novel research output in addition to new educational materials. We believe that this level of continuity is now feasible because the Biomakespace is now open and because we have strong engagement from first and second-year students who we expect to continue participating in the society's work. We ask for the remaining £500 from the follow-on fund to help subsidize reagents as we get started on this project.

Project Proposal:
https://docs.google.com/document/d/1J0pqvED7fiAp2KaPw3ScOHViLgVj-ALVgXEbb6nPdnc/edit