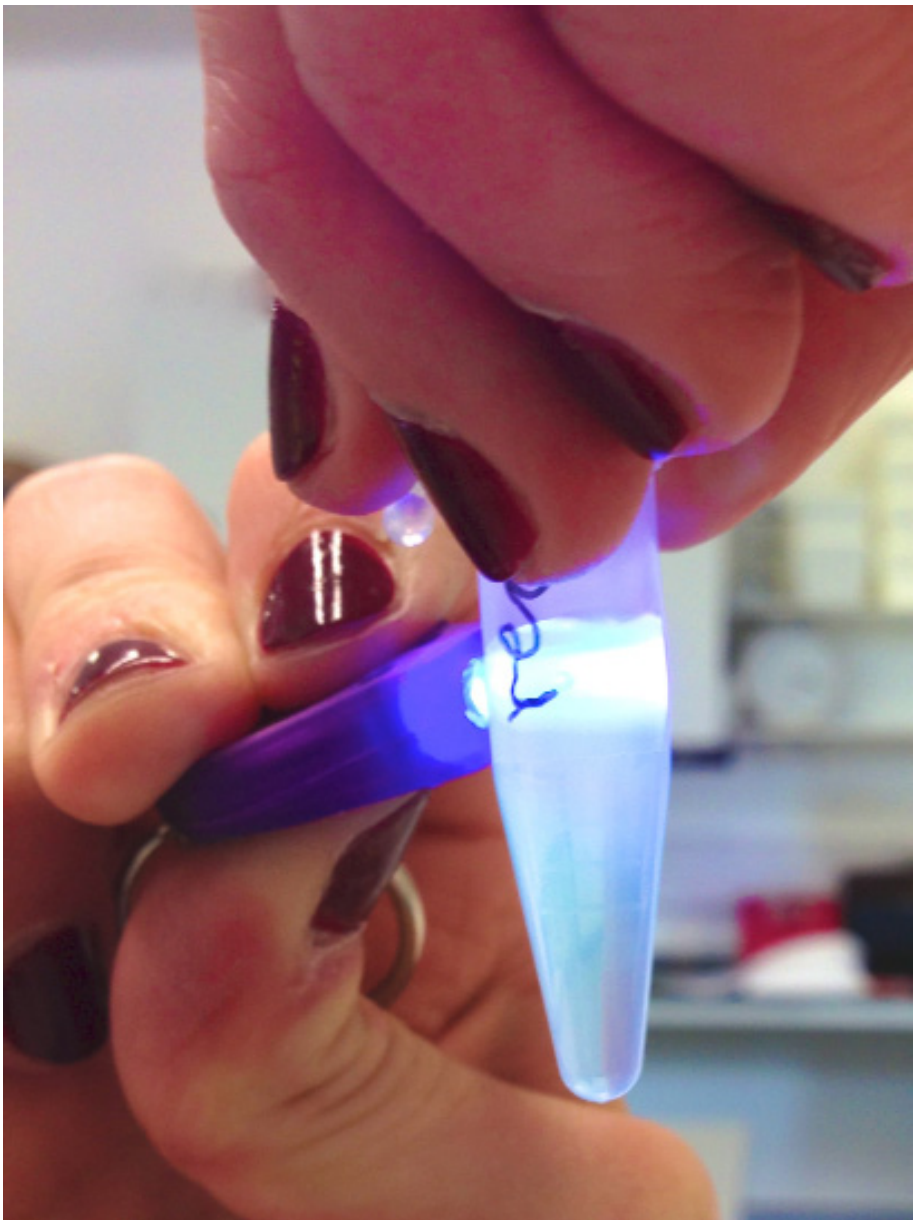
University of Reading

Practical synthetic biology

Report from a workshop for undergraduates

10–21 June 2013



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Introduction

A practical synthetic biology course for undergraduates took place at the University of Reading between 10–21 June 2013. Two groups of students participated in five days of practical experiments involving not only genetic modification of bacteria, but also exercises in electronic engineering and computer simulations. In addition, they discussed some of the of potential societal and ethical impacts of synthetic biology. This report presents an overview and summarises our experiences from the course.

The origin

The course forms a part of the UNIGEMS project, funded by a grant awarded by European Commission to Dr Jarosław (Jarek) Bryk and the National Centre for Biotechnology Education to develop practical resources to facilitate the teaching of synthetic biology at undergraduate level. We researched and developed protocols, techniques and genetic elements to form a draft curriculum in synthetic biology that could be deployed in undergraduate teaching at the University of Reading and elsewhere. The course constituted a vital part of this process, enabling the resources under development to be tested by undergraduates for the first time.

Enrolment

We initially sought to organise a two-week long course for about 20 participants selected in a competitive process. However, soon after announcing the course and despite only very low-profile advertising, we received 54 applications from interested students. We therefore decided to include as many of them as possible and shortened the course to a single week and ran it twice over consecutive weeks between 10–21 June 2013. As the students came from a broad range of backgrounds (from IT to theatre studies, see Figures 1 and 2), which would make fair selection of competitive applications difficult, we simply admitted the first 40 students to the workshops.

Due to illness, etc, only 34 students participated fully in the workshops. Eighty-two percent (28 of 34) of the students were in their first or second years (Figure 4), and 21 of 34 participants (62%) were male. In addition, two members of staff from the School of Systems Engineering took part.

Applicants for the course

The distribution of applicants by undergraduate discipline is shown on Figure 1. Among the 54 applicants (Figure 1), there were students from five University Schools, with 35 applicants (65%) having a biological background, 35% being from scientific and engineering subjects, plus a single student from each of management and theatre arts. This proportion changed slightly for the students who actually participated in the courses (Figure 2), with 25 of 34 (74%) students coming from biological backgrounds.

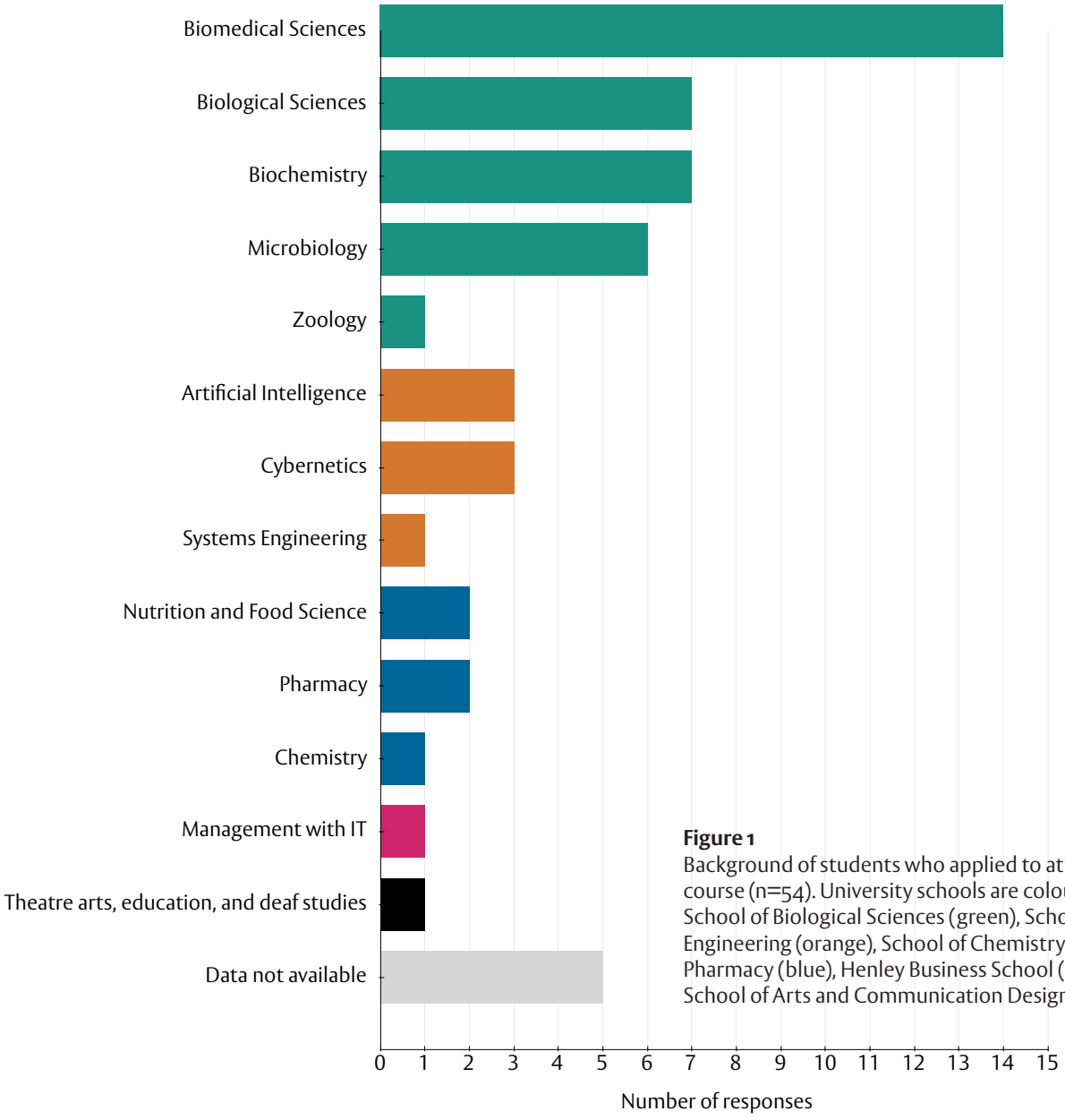


Figure 1
Background of students who applied to attend the course (n=54). University schools are colour-coded: School of Biological Sciences (green), School of Systems Engineering (orange), School of Chemistry, Food and Pharmacy (blue), Henley Business School (purple) and School of Arts and Communication Design (black).

Motivation and previous experience

Before attending the course, students were asked to complete a survey investigating their motivation for attending the course and their previous knowledge related to synthetic biology. Students' motivations are summarised by a 'wordle' (Figure 3, overleaf), created with all of their responses after removing the words 'synthetic biology'.

The answers to other survey questions illustrate the bias towards biologists. Majority of participants had limited or no experience with electronic engineering, but they did have previous experience with biological laboratory work, including eight participants who selected the most confident answer: 'I have enough experience to work independently' (Figures 6 and 7). Virtually all of the participants expressed positive attitudes towards synthetic biology (Figure 5).

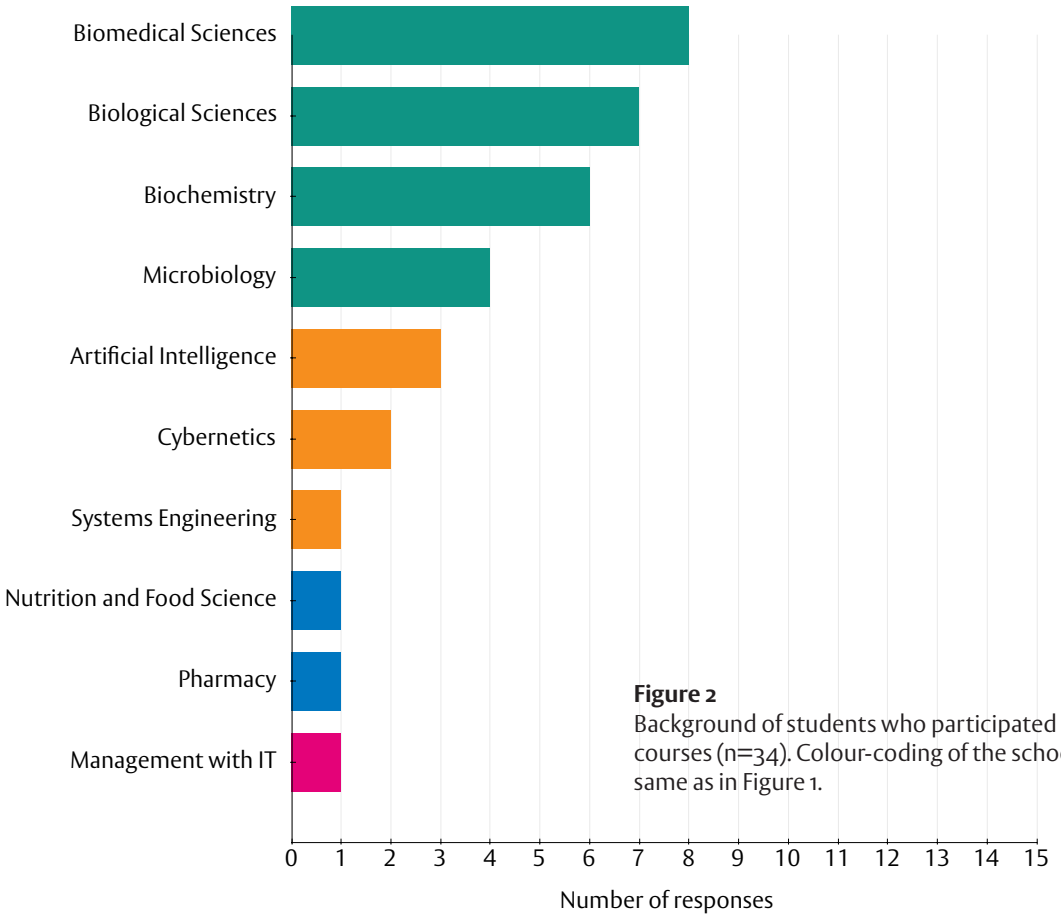


Figure 2
Background of students who participated in the courses (n=34). Colour-coding of the schools is the same as in Figure 1.

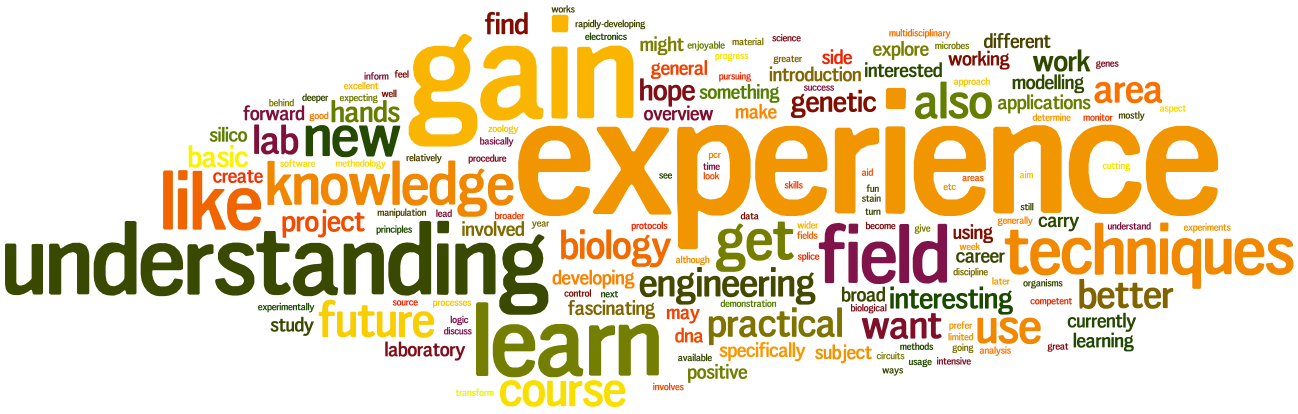


Figure 3
Wordle created from students' answers to the question: *What are your main expectations of this course?*

We also offered students three definitions of synthetic biology and asked which one they most agreed with:

> *Construction of systems and pathways from a set of pre–optimised, pre–characterised and compatible genetic parts, designed according to engineering principles, such as input–output signal standardisation, noise control and characterization in a variety of functional scenarios;*

> *Construction of analogues of electronic circuits, such as logic gates, counters, oscillators and signal propagation networks in living organisms, often used to test theoretical models describing behaviour of complex biological processes and networks;*

> *Modification of microorganisms using advanced DNA recombination methods but without using the interchangeable, pre–characterised parts.*

Twenty-one of 27 participants (78%) chose the first answer, with three students (11%) selecting each of the other definitions. We prefer the first two answers, which are complementary, and designed the workshop to highlight approaches and techniques as defined in this way.

Furthermore, students' prior knowledge was probed with questions about standard techniques and laws in molecular biology and electronic engineering, like PCR or Ohm's Law. However, we did not require students to define them, but only to declare if they were familiar with them. We also asked about knowledge of several topics broadly related to synthetic biology that have recently been reported in the media. Students' answers are summarised in Figures 8 and 9.

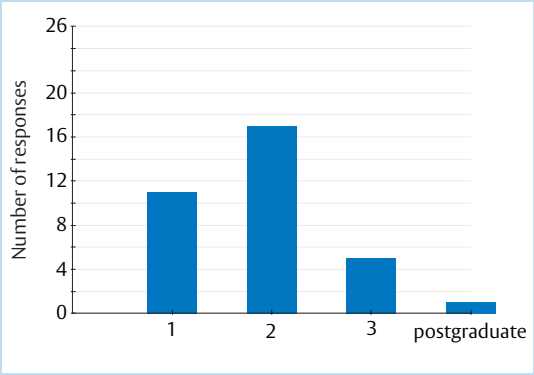


Figure 4
Number of students from each year of study who participated in the course (n=34).

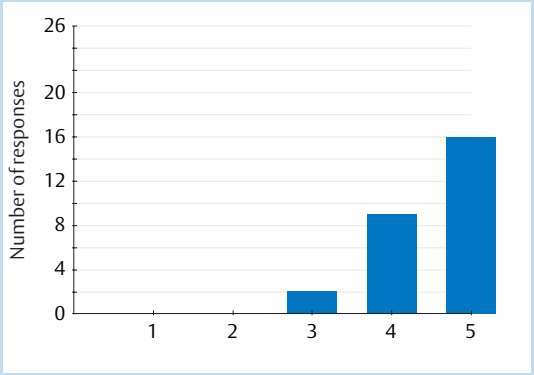


Figure 5
Summary of participants' responses (n=27) to the question: *How would you characterise your attitude towards synthetic biology?*, with a range of answers between 1: *Absolutely opposed to it* and 5: *Completely in favour of it*.

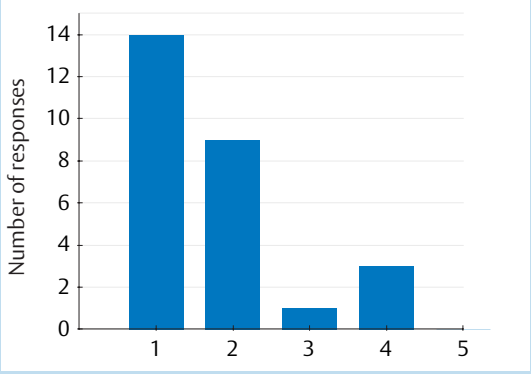


Figure 6
Summary of participants' responses (n=27) to the question: *Have you had any experience with practical electrical engineering work before?*, with a range of answers between 1: *I have no idea about it and have never done it before* and 5: *I have enough experience to work independently.*

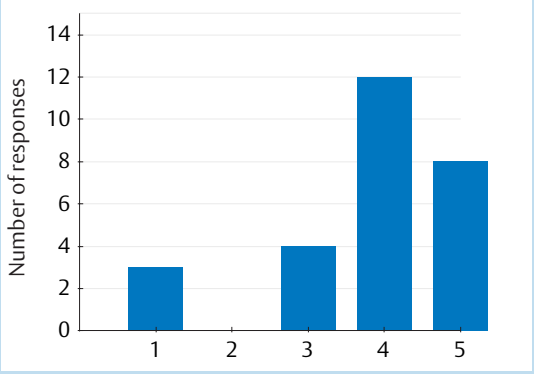


Figure 7
Summary of participants' responses (n=27) to the question: *Have you had any experience with practical biological laboratory work before?*, with a range of answers between 1: *I have no idea about it and have never done it before* and 5: *I have enough experience to work independently.*

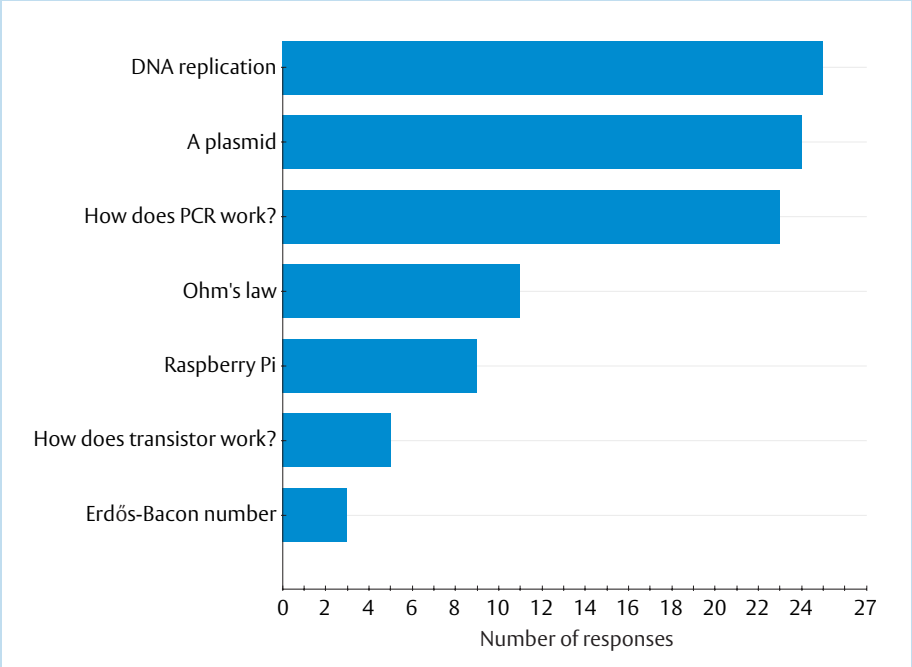


Figure 8
Number of students (n=27) who answered 'yes' when asked: *Are you familiar with the following concepts enough to explain them to a friend?*

The workshops

The course was comprised of three themes: genetic modification, electronic circuits and computer modelling. We first introduced the concept of bacterial transformation by using NCBE's bacterial transformation experimental kit (www.ncbe.reading.ac.uk/transformation) to introduce a plasmid with a gene encoding a green fluorescent protein (GFP) from jellyfish *Aquorea victoria* into various bacterial strains. This exercise familiarised the students with basic microbiological techniques and good microbiology laboratory practice. The students had subsequently to measure and compare the growth of different transformed strains using spectrophotometry.

Next, students chose from several pre-selected genetic parts and assembled them to modify the plasmids they had used before so that the production of GFP was either constitutive or switchable. They also had the option of swapping the GFP for a red fluorescent protein from a coral *Obelia sp.*. These constructs were also sequenced to check whether they had been assembled correctly. This practical work constituted the core synthetic biology approach: modification of living organisms from pre-characterised parts, akin to building from *Lego*® bricks or electric components. It was followed by a series of measurements to investigate the performance of the novel GFP-producing plasmids in induced and non-induced state, using both fluorimetry and flow cytometry. Throughout the 'genetic modification' part of the course, students used the DNA assembly method developed in 2011 by Daniel Gibson and colleagues to assemble a completely synthetic genome of *Mycobacterium*, coupled with a simple bacterial

Gibson DG, Young L, Chuang R-Y, et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods*, 6, 343–345.

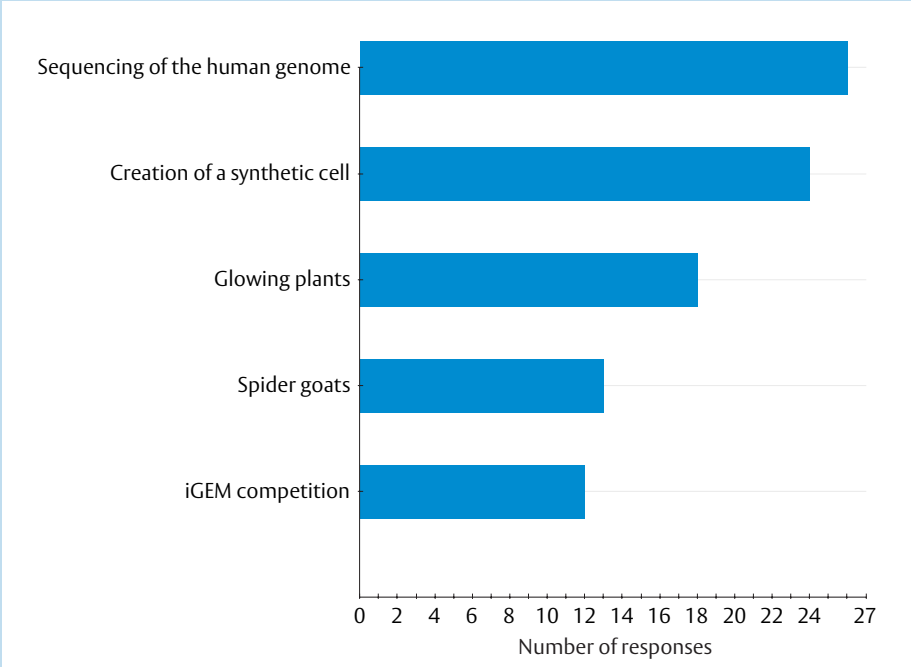


Figure 9
Number of students (n=27) who answered 'yes' when asked: *Have you heard about the following before you decided to take part in the course?*

transformation method developed in 1989 by C. T. Chung and colleagues and optimised in NCBE for the UNIGEMS project. These methods allow for the construction of PCR-derived parts and transformation of bacteria in a few hours. Design of the novel plasmids was facilitated by the *SnapGene* software (www.snapgene.com), whose owners kindly prepared and donated a special licence to be used by students during the course.

The second major part of the workshop was practical electronic engineering. We chose this subject because it provides a useful analogy to what synthetic biology aspires to and contrasts the relative ease and reliability of electronic circuit assembly with the bespoke, time-consuming and complex nature of genetic modification of microorganisms. In addition, it familiarises students with an engineering approach to the design and construction of complex systems from simple components. In this exercise, students built several circuits of increasing complexity using schematics with various parts of the circuits missing. Some of the circuits were electronic logic gates, which were then used to challenge students to designing biological analogues of such gates *in silico*. This part of the course was made possible by support and enthusiasm of Prof. Nasuto from the Department of Cybernetics, who also played a key role in attracting engineering students to the workshop, and his graduate student Ioannis Zoulias, who prepared and led the electrical engineering exercises (while also being a participant in the course).

The last part of the course involved use of computer software to model the properties and behaviour of synthetic biological circuits, one of the major tools and applications

Chung CT, Niemela SL, Miller RH (1989) One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. *PNAS*, 86, 2172–2175.

of synthetic biology. For this part, we chose the Genetic Engineering of Cells Simulator (GEC, http://research.microsoft.com/en-us/projects/gec/) software, developed by Andrew Philipps from Microsoft Research, Cambridge. It allows relatively simple input and computer-assisted assembly of various genetic parts and simulation of their behaviour. Drs Philipps and Boyan Yordanov prepared an extended version of GEC for our students to allow modelling of spatial interactions of two populations of bacteria on a Petri dish, as students followed one of the milestone publications in synthetic biology concerning synthetic circuits employing bacterial communication systems based on quorum sensing molecules (Basu S. *et al.* 2005).

During the course, Prof. Bob Rastall from the Department of Food and Nutritional Sciences and Dr Dean Madden from NCBE gave talks which put synthetic biology in social and legal perspective regarding public attitudes and legal frameworks applied to genetic modification of organisms and controversies often associated with them. Their talks provided students with necessary background to tackle two case studies in synthetic biology we had prepared: one involving a fictitious bacteria-based device to measure level of contamination in the Whiteknights Lake and the other much-publicised announcement of the crowd-funded (*Kickstarter*) creation and commercialisation of fluorescent plants (http://goo.gl/P7Zc1W).

Lastly, two invited guest speakers from University College, London, Philipp Böing and Bethan Wolfenden from the UCL 2011 iGEM team, presented the concept and practice of the International Genetically Engineered Machines (iGEM) contest and shared their experience of being part of this initiative.

The detailed programme of the workshop is presented in Appendix 1. One of the students' takes on the *Kickstarter* case study is presented in Appendix 2.

Students' feedback

We collected students' feedback after the course using an online form similar to the one used for a pre-course information gathering, asking about their experience and judgement about various elements of the course, as well as their suggestions for improvement. Students' ratings are summarised on Figures 8–13.

The majority of the 27 students who provided feedback gave the highest or second highest marks to all elements of the course, with laboratory exercises given almost unequivocally the highest mark, followed by lectures and then electrical engineering, computer modelling and 'instructions and flow of experiments' given similar but slightly lower ratings (Figures 8–13). Overall, students gave the course the highest mark, 'great', with an average score of over 4.8 on the 1–5 scale (Figure 11).

Apart from these numerical ratings, we also asked students about things they liked and didn't like about the course, and suggestions for improvement in the future. Below are excerpts from the students' answers: all of the data is given in Appendix 3.

Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R (2005) A synthetic multicellular system for programmed pattern formation. *Nature*, 434, 1130–1134.

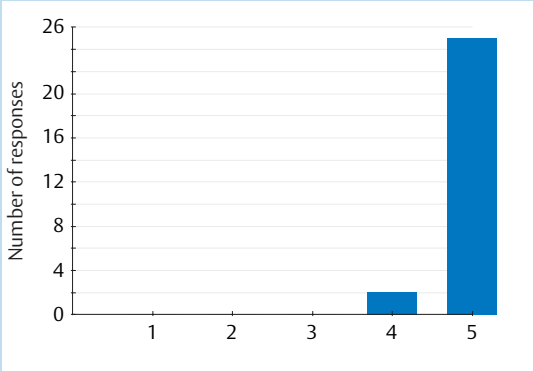


Figure 8
Students' answers (n=27) to the question: *How would you rate the laboratory, experimental parts of the course?* on a scale from 1: *I didn't like them at all* to 5: *They were very good.*

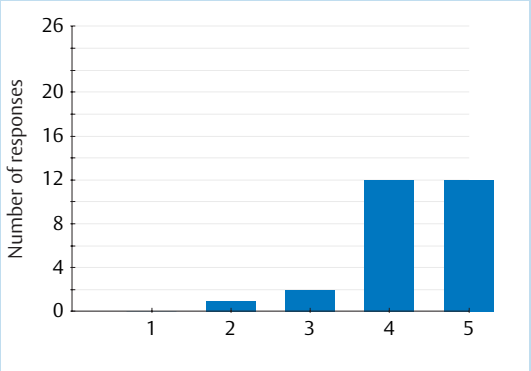


Figure 9
Students' answers (n=27) to the question: *How would you rate the electronic engineering part of the course?* on a scale from 1: *I didn't like it at all* to 5: *It was very good.*

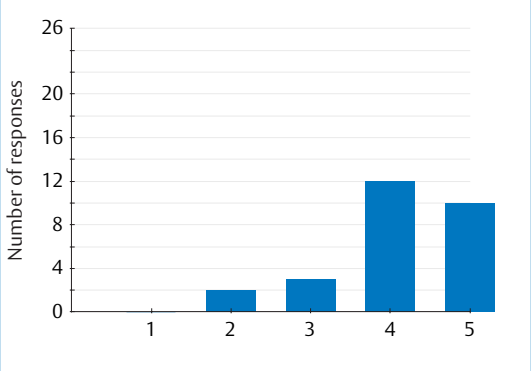


Figure 10
Students answers (n=27) to the question: *How would you rate the computer modelling part of the course?* on a scale from 1: *I didn't like it at all* to 5: *It was very good.*

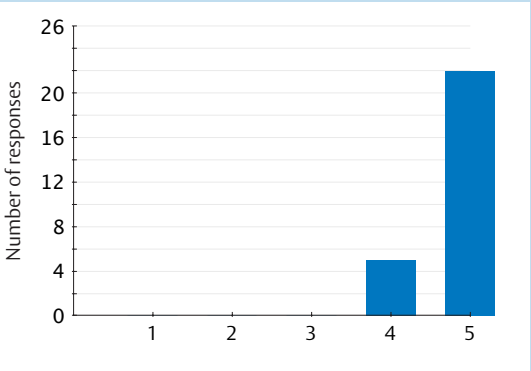
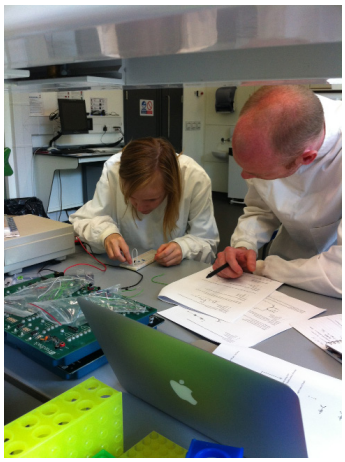


Figure 11
Students' answers (n=27) to the question: *Overall, how would you rate this course?* on a scale from 1: *It was horrible* to 5: *It was great!*



What was the best thing you personally enjoyed about this course?

Lab time, I personally don't think we get enough lab time at Reading University and it was nice to go over all the basics in aseptic technique and pcr etc as well as learning new techniques (Gibson assembly).

Designing new E. coli *using SnapGene, when we substituted the Red protein for the Green fluorescent protein and actually grew those bacteria. Also being able to see the results of our work when the bacteria were fluorescing the next day under UV light.*

I liked the way the course is taught and the fact that it is an interdisciplinary where teams collaborate to achieve the ultimate goal which is to get the best out of the course.

Understanding the structures of plasmids, fragments and vectors using SnapGene and what construct we would be trying to form during the practical. Overall, learning the theory and then putting it into practice.

Please write down three things you liked best about the course

Carrying out PCR and growing fluorescent bacteria. Learning how to build a basic circuit using transistors, resistors etc. Learning how to use snapgene to swap and delete genes.

Combination of theory and practical work in silico approaches, very good protocols—easy to understand, diversity of techniques used.

Wet lab work, Circuit practical, Lectures

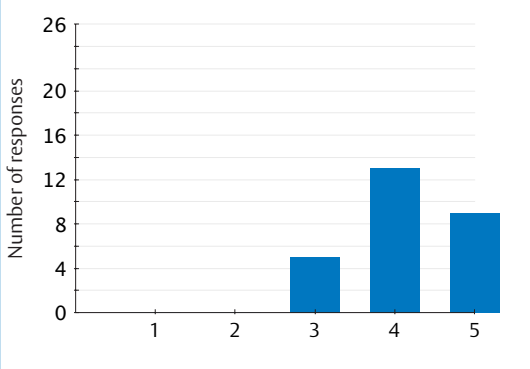


Figure 12
Students' answers (n=27) to the question: *Do you think the instrictions and the flow of the experiments were easy to follow?* on a scale from 1: *No, not at all* to 5: *Yes, I had no problems with that.*

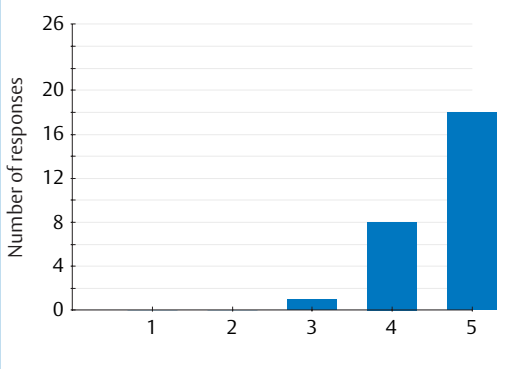


Figure 13
Students' answers (n=27) to the question: *How would you rate lectures and explanations throughout the course?* on a scale from 1: *I didn't like them at all* to 5: *They were very good.*

Please write down three things you didn't like about the course

The time constraint, it would have been better if it was stretched over two weeks. Early start, Lack of information on the specifics of what to do in the experiments.

The electrical engineering, I didn't feel that it was explained as well as it could have been, probably due to the limited time. Also the GEC, I understood how it was relevant but again, it seemed a little too short. Finally , the fact the course wasn't over 2 weeks, it would've probably been the solution to the first 2 problems, meaning more time to get to grips with the concepts that I felt I struggled with.

If you were to improve this course for other undergraduates, what would be the single thing you would change or introduce?

Extra time to allow for deeper understanding and more complex constructs If budget allows, create multiples of each biological reaction to make the process more robust if one culture does not grow.

Make the course longer to allow students to apply the basic techniques they have learnt to solve a problem. I think this would help students to really understand what they've learnt as application is always a good way to teach new topics.

A binder which clearly (step by step) explains the methodology of all the practicals including the underlying science or theory. I think this would be helpful to people who were not sure what needed to be done for a task.

If there was one thing you had to remove or limit in the course, what would that be?

Long breaks with not much to do, could have been given worksheets to back up the knowledge we had learnt

I wouldn't like to remove or limit anything in this course. I only wish that it could of been a week longer. As I feel there was some extreme cramming of content involved.

I thought all the course was useful, the only parts that didn't benefit me was the material and information aimed at those not from a biological sciences background, which although not useful for me was necessary for other students.

If i had to limit one thing it would be the modelling aspect of the course using GEC.

Things to improve

While the course was a great experience for us and we consider it a success, there are several things we believe would make such workshop much better for the participants. Here we briefly mention the most important ones (some of which reassuringly were also mentioned by the students):

We would like to make the course longer. The two-week course we had initially planned would allow two extra experiments which were not performed in the course's current format. Firstly, the students would be able to build various logic gates in bacteria following their electrical engineering practice, and measure their performance. Secondly, they would be able to recreate the bacterial communication system they modelled in the computer simulation exercise. They would also be able to manipulate the parameters of the circuit *in silico* and then test it *in vivo* in the system they had created. These two elements: bacterial computing *via* logic gates and bacterial communication *via* quorum sensing arguably constitute major modifications currently applied in various synthetic biology designs.

Furthermore, we believe that it would be even more beneficial if the course was turned into a practical, term-long module. Not only this would allow a much more comprehensive presentation and practice of various methods and issues in synthetic biology, but would also enable the design, synthesis and delivery of novel genetic parts of the students' own design and invention.

We also need to develop a full set of students' and teachers' guides to the course. Due to the preliminary nature of many of the course elements, we haven't provided students with appropriate guides to the protocols and procedures yet. These materials will be developed by the project's end.

The course provided evidence that Gibson assembly coupled with TSS transformation does not yet produce sufficiently high number of transformants — only about half of the students achieved a successful assembly and transformation. We will further optimise the protocol to make it more robust and reliable.

Very importantly, we need to develop comprehensive evaluation and assessment methods for the course. Only with appropriate assessment we can accurately judge whether the course resulted in improved knowledge and understanding of the issues and procedures discussed and practised during the workshop. For now, we recognise that without appropriate data on students' learning we cannot judge this course's effectiveness in teaching, except in the cases where understanding of the procedures were explicitly demonstrated, i.e. by successful design and creation of transformed bacteria or the description and justification of students' decisions in case studies. As an example, we provide a sample of the lab report each team had to prepare during the course in Appendix 4.

In addition, we believe that development of the assessment methods for the course should considerably facilitate the uptake of the course design and framework by other educators, as well as its introduction into a full-module format within a university course.

Future plans

Perhaps the most apparent legacy of the course is students' initiative regarding the International Genetically Engineering Machines competition. In the pre-course survey, 13 of 27 students (48%) said that they heard about the iGEM competition before learning about the course. In the post-course survey, 26 participants said that they would be interested in joining a University of Reading iGEM team next year, with one reply left unchecked and not the negative. Following the course, a group of students—alumni of the workshop—took part in a large meeting organised by University College London's Synthetic Biology Society for all present, past and potential UK iGEM teams. The students also took the initiative to establish the SynBioSoc at the University of Reading and start the iGEM team for the 2014 competition. The SynBioSoc application is under consideration by the Students' Union at the time of writing and we will suport students' efforts to establish a synthetic biology community in Reading.

We also were encouraged by students and members of staff to repeat the course in the next academic year, which we are very happy to do, funds permitting. Should there be an iGEM team in Reading next year, such a course may form a selection event or introductory workshop for team members. Nevertheless, we believe that extending our course into a 2nd or 3rd year synthetic biology module would be a very valuable addition to the students' curriculum and we will be exploring this option in the following months.

Acknowledgements

First of all, we would like to thank all the students who participated in the course. They were inquisitive, smart, interesting and funny and we really enjoyed working with them. We greatly appreciate that they took their time and joined us on this little adventure.

This course wouldn't be possible without help and support from many members of staff and research community at the University of Reading and elsewhere, who provided equipment for us to use, helped us run some of the experiments, disseminated course announcement and encouraged students to participate. In no particular order, we would like to thank:

Sławomir Nasuto, Bob Rastall, Sheila MacIntyre, Simon Andrews, Liam McGuffin, Michael Fry, Julian Park, Hilary Loxton, Sandra Tejero, Adele Constabile, Christopher Humphrey, Andreas Karatzas, Yoshikatsu Hayashi, Ioannis Zoulias and members of the NCBE staff at the University of Reading;

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Jim Haseloff, Fernan Federici and Paul Grant from University of Cambridge;

Aline Glick and Michael Scott from SnapGene.com.

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The evaluation of the course, as well as consumables, synthesis and sequencing services used on the course, were funded by Society for General Microbiology Education Development Fund Practical Teaching Aids (TA13-2) 'Practical synthetic biology: an inquiry-based, interdisciplinary workshop for undergraduates'.

Jarosław Bryk

17 September 2013

Appendix 1

Programme of the course

Recommended reading before Day 1

Gibson Assembly using SnapGene (instructions and animation)
Polymerase chain reaction (DNA Learning Center animation)

Day 1

9:00	Welcome and introduction: why this course, how different experiments and activities tie together, brief introduction to DNA, PCR and bacterial transformation
9:45	Short break
10:00	Practical introduction to good laboratory practice and basic microbiology techniques using NCBE's transformation kit by John Schollar,
11:30	Introduction to Gibson assembly using SnapGene, including primer design
12:00	Setting up the PCR
13:00	Lunch break
14:00	Gibson assembly of the chosen constructs
15:15	Coffee break
15:45	Transformation and plating of bacteria

Recommended reading before Day 2

Synthetic Biology: public dialogue on synthetic biology (chapters 4–6)
An Evidence Review of Public Attitudes to Emerging Food Technologies (chapter 3.1, 3.6)
Repression of the lac Operon (blog post by Larry Moran)

Day 2

9:00	Talk: GM food fiasco of the '90s by Bob Rastall
9:45	Short break
10:00	Setting up liquid cultures to measure bacterial growth of the cultures made the day before
11:00	Introduction to synthetic biology and bacterial gene regulation
11:45	Short break
12:00	Setting up Gibson assembly for the gene regulation modifications from pre–made parts (Ara, Lac, Tet, constitutive)
13:00	Lunch break
14:00	Talk: Legal aspects of work on genetically modified organisms by Dean Madden
14:30	Short break

14:45 Transformation and plating of bacteria
16:00 Short break
16:15 Analysis of the growth of cells and introduction of the case studies on potential societal and ethical impact of synthetic biology

Recommended reading before Day 3:

Basic logic gates and functions (University of Surrey)

Day 3

9:00 Setting up liquid cultures to measure fluorescence and to isolate plasmids for sequencing of the constructs made the day before
10:00 Introduction to electrical engineering by Ioannis Zoulias
11:15 Coffee break
11:45 Practical electrical engineering, continued
13:00 Lunch break
14:00 Practical electrical engineering, continued
14:45 Short break
14:30 Introduction to fluorescence and flow cytometry
15:00 Isolation of plasmid DNA and setting up the PCR for sequencing of the constructs, measuring fluorescence of the bacteria grown throughout the day

Recommended reading before Day 4:

A Programming Language for Genetic Engineering of Living Cells

Day 4

9:00 Clean up, concentration measurement and dilution of the PCR products for sequencing
10:30 Coffee break
11:00 Introduction to GEC and modelling of bacterial patterning by Boyan Yordanov / Andrew Phillips
13:00 Lunch break
14:00 Introduction to GEC and modelling of bacterial patterning, continued
15:00 Short break
15:15 Discussion and design of biological logic gates

Recommended reading before Day 5:

Synthetic biology students win gold at iGEM Europe, now on their way to world championships at MIT (article about Philipp Böing's team at last year's iGEM with links to follow for more)

Day 5

9:30	Analysis of the sequenced products
10:15	Short break
10:30	Talk: State of the art in synthetic biology
11:30	Preparation of the reports: Gibson calculations (from Mon and Tue), bacterial growth (from Tue), fluorescence (from Wed), sequencing (from Fri) and including constructs from Mon, Tue and Wed as SnapGene files
13:00	Lunch break
14:00	Talk: The iGEM experience by Philipp Böing / Bethan Wolfenden, UCL
15:00	PIMMS and snacks to facilitate further discussions

Links to recommended reading and other resources are available on the course website.

Appendix 2

Clean lake project case study

A group of biology and engineering students at Reading University for their summer iGEM project came up with a synthetic biology system to measure and monitor contamination of water in the Whiteknights lake. The system consists of genetically modified *E. coli* that output red dye depending on the amount of a particular contaminant in the water, and a small pump that forces water from the lake to flow through bacterial culture. The device is permanently attached to one of the bridges on the lake and all passer-byes can read out the output on the provided detector. Please write (no more than 500 words) what potential obstacles would you anticipate assuming you were to work on this project. Please consider potential legal, social and/or technical issues that might influence successful execution of the idea. You are free to assume other technically and scientifically realistic parameters of the project/device/bacteria than what I described above, but if you do that, please describe the relevant portions of the system.

The glowing plants Kickstarter project case study

Five days ago a Kickstarter project that attempts to create plants constitutively expressing firefly luciferase that would allow them to glow, was successfully funded. Please present your opinion, in no more than 500 words, on this project. Is it interesting? Is it desired? Is it dangerous? Is it controversial? Is it legal? If you were to decide on whether to allow this project to happen, would you do it? Why? Why not? Below are several links which provide a broader perspective on the controversy:
http://www.kickstarter.com/projects/antonyevans/glowing-plants-natural-lighting-with-no-electricit
http://splasho.com/blog/2013/04/27/crowd-funding-avatar/zz
http://blogs.scientificamerican.com/oscillator/2013/06/03/glowing-futures/
http://www.nature.com/news/glowing-plants-spark-debate-1.13131
http://www.etcgroup.org/kickstopper

Irrespective on what case study you want to consider, you might find the presentation 'Responsible conduct in synthetic biology' helpful.

Students' analysis of the 'Kickstarter' case study (example)

Is this project interesting?

This project is interesting, even if the idea may not be completely new. I found the website for the project a little unsettling as it seems to be focused on selling t-shirts and other merchandise, rather than explaining how the money is spent and where the funds are going. There are even testimonials, which give a very commercial impression. Is it likely that the public would put more trust into something run by an educational establishment? Would they prefer to fund something that seems a little less frivolous? Some people may be unwilling to spend this amount of money on a bioluminescent plant, when there is no guarantee that there will be any major outcome from the project.

Desirable?

The idea of lighting areas naturally is desirable, however what would the practical impact of saving this much electricity be? There could be more beneficial projects that need funding. Will this group be able to manufacture trees which are bright enough to light areas? So far the plant arabidopsis thaliana has been used. Creating enough light for practical purposes would use a lot of energy, would a large plant survive?

Dangerous?

Introducing a genetically engineered organism into the wild population could hold many risks. If bioluminescent plants were to escape and breed with wild-type organisms, this could lead to light pollution in many areas, which could cause harm to nocturnal animals. Would the introduction of bioluminescent plants into the wild effect the animals which live in and around these plants? It has been suggested that seeds could be manufactured which create trees that only survive when a certain nutritional mix is added to them. This would create a cost and would possibly give whoever is creating the trees a monopoly of the supply of this product. We can never be sure how plant lines are going to evolve, so there may be future repercussions that we are unaware of. Could any mutations be dangerous?

Controversial?

For some people, most aspects of synthetic are controversial. However, as this is such a radical idea, it is likely that it would be met with a significant amount of backlash from many areas of society. I don't think that Kickstarters are helping themselves by suggesting the film 'Avatar' as a source of inspiration, this could scare those members of the public who are already wary of synthetic biology. The idea of bioluminescent plants would likely cause issues for certain pressure groups and farmers, who would be worried about the possibility of their crops being effected.

Legal?

In America it is possible to create and use synthetic organisms freely as long as they are not pathogens. In the UK however, it is very unlikely that this kind of project would go ahead, considering the fact that genetically altered crops are still not allowed here, even after decades of use elsewhere. There are issues surrounding patents and genetic products which need to be addressed, especially considering that there are other groups which currently hold patents on bioluminescent plants.

Would I back it?

I would back this project, as the science is very interesting and it is unknown what other applications the technology in question could have. However, I find it unlikely that one day bioluminescent trees will line our roads. There are many issues that would need to be addressed before getting to this stage, most importantly; the effect that bioluminescent trees would have on the natural ecosystem. If this were to be attempted and fail or cause major environmental issues, it could create problems for future synthetic biology projects which may hold more promise. It would be a shame to tarnish synthetic biology at such a young stage in its progression, especially on a project which may only reduce carbon emissions by a small amount.

Appendix 3

Students' feedback on the course

All students' comments are presented as they were written in the feedback form.

What was the best thing you personally enjoyed about this course?

Probably the practical involving adding ATC in various concentrations to see the degree to which the colonies started to fluoresce.

The experiments were interesting and fun to do.

The talks were interesting and opened up the idea of synthetic biology to me.

Being able to see and use new equipment and software.

I have enjoyed meeting a wide variety of people and I liked being able to gain experience of different types of work rather than just pure biology.

Personally, I enjoyed how interactive the course was as this is a huge difference to the normal classes during the term.

Designing new *E.coli* using SnapGene, when we substituted the Red protein for the Green fluorescent protien and actually grew those bacteria. Also being able to see the results of our work when the bacteria were fluorescing the next day under UV light.

I liked the way the course is taught and the fact that it is an interdisciplinary where teams collaborate to achieve the ultimate goal which is to get the best out of the course.

Discovering the world of synthetic biology, through first hand experience. Working in the lab for the first time and using some very cool pieces of equipment. Learning a great deal a very short time and advancing me field of knowledge and opening up the door for a possible new field to journey. Seeing engineering from a new perspective, and opening my mind up to new concepts.

The integration of biology and engineering to attempt to alter bacterial gene expression.

Your enthusiasm has inspired me to look further into synthetic biology and possible career pathways which before this week I knew very little about.

Everything seemed to equally contribute into giving a good insight into the field. It worked as a whole; it is hard to pinpoint one thing I liked best. I enjoyed being introduced to some techniques / equipment that I had not come across before in my undergraduate degree (Biomedical science).

Coming from an non-bio background, all manipulation of bacterial DNA was incredibly enjoyable.

The practical aspect, was not only learning about concepts in synthetic biology but was actually given the means and instruction to be able to create genetic constructs.

The transforming of and culturing of bacteria.

Working in a team to tackle challenges.

Understanding the structures of plasmids, fragments and vectors using SnapGene and what construct we would be trying to form during the practical. Overall, learning the theory and then putting it into practice.

I enjoyed all the lab work that we did and also the interesting concepts put forward through the work and the guests who talked to us.

Experience of working in a real modern lab.

Getting to do the electrical stuff, as I am a biology student and we do not get to do any of that. Also meeting engineering students was nice to build up contacts.

The multidisciplinary environment and the enthusiasm of instructors/lecturers.

I enjoyed learning about how simple systems using inducible promoters could be used to create bacterial logic gates. I also enjoyed building the logic gates out of electrical components as this helped reinforce the concepts underlying the bacterial logic gates.

The amount of practical we were able to engage in was fantastic. The fact that we were able to make our own decisions and choices in the experiments rather than just being given instructions to follow was great, and I felt this allowed us to develop our skills a lot more efficiently.

I really enjoyed learning some basic electronics, as I have never been exposed to it before. We used this knowledge to think about the possibilities of building these systems within cells and the applications that this could be the base of. This is a concept I find very exciting and am very interested in doing similar work in the future.

I very much enjoyed swapping in the two fluorescing proteins into the *E. coli* as this was an immediately visible way to see the concept working, and it is an easy way to explain to a lay person what I had been doing in the workshop with a photo of the RFP and GFP expressed [in the bacteria].

I really enjoyed doing the different aspects of electronic engineering and then applying it to biological ways. Also really enjoyed the mix of wet lab and computational lab work that was included in the week and was genuinely disappointed how quick it felt!

Lab time, I personally don't think we get enough lab time at Reading University and it was nice to go over all the basics in aseptic technique and PCR e.t.c as well as learning new techniques (Gibson assembly). Jarek was very friendly and easy to approach which makes a difference if you don't know exactly whats going on.

Very well organised workshop with informative and interesting material. I gained a lot of practical experience which I am sure will be useful for me in the future. The quality of information provided was at the very high standard and easy to follow and understand. I am looking forward to more of the similar workshops in the future!

I really enjoyed using SnapGene and making computational models of synthetic systems. This was something I had not done before and I learnt a lot from the course.

Please write down three things you liked best about the course

Learning about biological logic gates , learning to use SnapGene and attempting to isolate plasmid DNA.

Good rapport with lecturers.

Learning how to use SnapGene

The electronics workshop.

Using equipment used in industry.'

The teachers (v. interactive and encouraging, easy to understand).

The oportunity to learn so many new things in such a short period of time (electrical circuits, logic gates, synthetic biology).

The opportunity to meet many people which otherwise I would not have met!

Carrying out PCR and growing fluorescent bacteria.

Learning how to build a basic circuit using transistors, resistors etc.

Learning how to use snapgene to swap, remove and delete genes.

Team work.

The way the course is designed and the the systemic flow of information.

The lectures and presentations delivered by the various speakers.

Learning all about DNA and how create our own new life, in the crazy world of synthetic biology.

Using snap Gene and being able to analyse DNA samples and to modify them. Also well as to being able to create biological logic gates.

Working in the lab and using some very cool machines. Gaining a better understanding on how they all work. As well as see engineer in a practical use and from a different field.

Good teamworking course.

Large emphasis on the practical side of biology.

Learning what synthetic biology is and all the various approaches - SnapGene, electrical circuits - AND and OR gates in combination with laboratory work to reinforce.

The bringing of various people and backgrounds ie Biology / biomedical science with engineers/ cybernetics. At presented at a standard for all to understand.

Friendly and approachable academics which can explain things clearly and with detail.

The diversity of the subject matter, tying together computer science with biology, opened my mind to an area of biology that I would never have come across on my degree course - It was challenging and thought provoking, and highly academic.

The course is well adapted for university students and felt free of compromise - it was well funded, well staffed, good lab, and we were more than provided for; it felt like no corners were cut.

The academics were great help, knew their stuff and were keen to help you understand the subject matter at hand. Their enthusiasm and attitude towards the subject rubbed off on me and made me more eager to understand the material.

The instruction was very good; it felt like I was making progress for myself and the instructors were just helping along the way, rather than being told exactly what to do.
Learning about fields that are not my own.
Making a genetic construct on SnapGene and then transforming a bacterial culture.
Learning something about electrical engineering of which I knew nothing before.
The range of people who gave interesting talks.
Performing a range of experimental procedures which I have read about but never had the opportunity to do myself.
Learning to use different software especially the Gibson assembly in SnapGene, modelling the bacteria in GEC and learning about the different aspects of synthetic biology, from GM crops to examples of previous iGEM projects.
Interesting, challenging, practical.
Wet lab work, circuit practical, lectures.
Gibson assembly.
GM lecture.
Learning modern techniques like Gibson assembly.
The combination of practicals and lectures, which was well balanced.
I thought the course was well taught and introduced the concepts in an manner that was easy to understand.
The various lectures on issues such as regulation of GMO, GM food scares etc were interesting and informative.
I enjoyed getting to try and create genetically modified organisms (even though it didn't work all the time), i thought it was particularly good that all the systems we built were very simple and we could see the results very simply, because although all we did was either introduce different colours or change the promoters the concepts used to create more complex systems are essentially the same.
The enthusiasm and attitude of the teachers and students created an incredibly relaxed environment where I felt able to ask for help. I felt like we were working as a team as opposed to merely being talked at and given instructions to fulfill, which I think helped me to learn more and engage better in the material.
The variety of the course was great as it wasn't just biology but incorporated physics, engineering and computer science also. I felt like this opened my eyes to realizing that synthetic biology, and even general biology involve a complexity of disciplines. The course ignited an interest in physics and computer science for me!
I thoroughly enjoyed the numerous talks that we were given, and appreciated the fact that our lecturers were experts in their field and came from afar and other companies etc to talk to us.
Performing a Gibson assembly, using SnapGene. A very useful software and an experiment that I have never performed before.

The GEC talk, this helped to link the electronics we had learnt with practical applications and experiments that had been carried out recently.
The teaching style was very inclusive and engaging.'
Swapping GFP and RFP.
The talks from the various contributors from Microsoft, UCL, Food department etc.
The fact that I could be part of something that is so cutting edge and thoroughly fascinating.
Free Pimms!!!
Computational work - using all the different programs and especially the new coding language.
Using different techniques like the Gibson assembly.
The Electronic Engineering work.
Manipulating DNA.
Learing in more detail about genitic modification and state of the art synthetic biology.
Learning (I learnt so much this week its crazy!).
All of the speakers were very interesting especially the man from Microsoft and the girl from iGEM.
Being in the lab.
Combination of theory and practical work *in silico* approaches.
Very good protocols-easy to understand.
Diversity of techniques used.
A lot of practical work.
Speakers from outside the university.
High quality and easy to understand material.
Making new constructs and transforming bacteria.
Learning to use a computational model.
Using SnapGene.

Please write down three things you didn't like about the course

The electrical engineering, I didn't feel that it was explained as well as it could have been, probably due to the limited time. Also the GEC, I understood how it was relevant but again, it seemed a little too short. Finally , the fact the course wasn't over 2 weeks, it would've probably been the solution to the first 2 problems, meaning more time to get to grips with the concepts that I felt I struggled with.

Could have done with a protocol to follow methods as we forgot some specific details as we went on, although suggesting to take notes when talking about the protocol is also an idea.

A lot of talking - was sometimes hard to stay focused.

Wasn't always clear what was being asked - instructions would have helped.

Mixing the class up a bit more so you got to meet more people would have been good.

The seating arrangements during presentations could have been better (not across two benches as sometimes you couldn't see the lecturer)

Only one week :(

Can't think of anything else!

At times it was hard to follow what was going on, I think some of the people that came in to talk to us assumed we knew a lot more than some of us did since we were a very varied group.

Maybe a prepared a booklet with instructions for some days would be helpful to avoid confusion.

The time constrain, it would have been better if it was stretched over two week.

Being lost for a great deal of time, though this is unavoidable when being involved in a project like this having to prior background in biology.

I didn't enjoy the computer modelling part much though did find it very useful to the field and see it as a very important element which could save a lot of time and money.

The talks about the biological theory seemed to go on for longer than the talks for engineering (maybe it just seemed longer as I've done it all already).

Broken pipette (not a lot you can do about that though).

Could possible do with a step by step sheet for the Snapgene and GEC in addition to lecture.

More people to assist in electronics workshop as us biologists struggled a lot.

Some of the systems biology and computer science lectures were completely alien areas for some of the people on the course. As a frightened biologist, expecting people like me to try to piece together circuits after a short one hour lecture was a bit much. saying that, I did like the challenge and it forced me to think - i feel motivated to go away and try and get my head around it. Maybe extending the lecture to two hours and explaining it a lot slower, stopping to check we all on the same page at the end of each slide would have helped - or maybe provide some reading material about the basics of circuits to

read before the day. It felt like something I would have had to go away and digest before understanding it (I'm sure that as soon as i get my head around it, i will find it perfectly simple). I had a similar problem with the GEC programme - although sleep deprivation and an inability to concentrate may have factored in on that one - this is why i think extra work-sheets with explanations on would be useful - the information would always be there for us to read over and get our heads around.

Need to wash/spray benches with virusolve after every experiment if going on to use laptops or just at end of day.

There were a few minor things about the general protocol that could have be improved - worksheets containing details of the theory of each experiment followed by step-by-step protocols for all experiments would have helped greatly and cut down a lot of the time waiting for a supervisor to be free. Also, after working with bacteria, it is good practice to wipe down the bench afterwards (this should have been part of the compulsory protocol). *(please note the students were told to do that after experiments)*

Using the logic tutor was difficult and there wasn't a great deal of guidance in that respect

Some of the GEC work seemed a little abstract, might consider dedicating more time to that.

I found the computer modelling confusing and, while interesting, needed more time and instruction on using it.

I would have liked more instruction on how to properly use SnapGene.

I felt there could have been better instruction on how to construct circuits when doing the electrical engineering part of the course.'

I really enjoyed the workshop as a whole and I would not change anything apart from maybe make the course longer.

Difficult, some lectures were hard to follow.

Lack of information on the specifics of what to do in the experiments

The programming was quite hard, could have done with more explanation for commands.

The course ending!!

Not long enough.

Early start.

Programming was good, but a manual of commands would have been useful to quickly refer to during the class. (I can't think of two more).

I would have liked the opportunity to build some more complex plasmids to put into bacteria, such as creating bacteria containing systems such as AND and OR gates to see these function in vitro, rather than just as videos or computer simulations.

I would have liked more time spent on explaining the GEC software and modelling software as i didn't feel like i could use it that well after the crash course we were given.

It wasn't long enough! It was so enjoyable I wish it was longer!

Some of our experiments did not work, however this was through no fault of the course.
I found the physics quite hard, however this is just due to the fact that I have little experience in the field.
I would have loved the course to have been longer.
As a 1st year from the food department, I did occasionally feel a little out of my depth but team members with more experience and knowledge are always there to help
A week is too short! It would be great to do a second to use the newfound knowledge
A bit more on the electronics would be good.
Can't think of anything! Was a great course to do and would recommend it to anyone!
Not enough time to do more complex biological circuits.
Going home.
The course ending.
The breaks (lunch was great but the 10 minute breaks were not so good)
overall there was a lot of waiting around between most experiments/talks
lectures in the lab.
Quite short course.
Some of the practical work was at a lower level than expected and a bit too easy for me. However, it was well aimed for first year undergraduates.
I'd have liked to have maybe had a go at using what we'd learnt to solve a small problem, maybe even in SnapGene if we didn't have time to make it in the lab.

If you were to improve this course for other undergraduates, what would be the single thing you would change or introduce?

Make the course longer, allowing more time to get to grips with the variety of concepts and software introduced.

Having instructions in the booklet of more of the steps.

Make the course longer :)

Do something one day that is more related to the iGEM competition, most people find that very interesting and is generally what attracted people to this course.

Just to stretch the course over a longer period of time.

I thought that the electronic practicals for the course maybe have been to complicated for first hand users possible make the worksheets easier.

It would have been useful to have a more structured practical booklet, some of the time it was confusing what we were meant to be doing.

Longer time spent and more academics to help out on the GEC programme lecture and electronics. Those who have limited understanding of computing/electronics who do not do a degree in this area find it very confusing/there is a lot of detail within a few hours.

Worksheets providing more step-by-step instructions for some of the practicals, as-well as explanations of the theory behind them (underlining the main points of the lectures perhaps)

Allow for more time for the computer modelling part of the course. That was the aspect I personally found most difficult and I feel I could have become more competent with it if I had been given more time.

A binder which clearly (step by step) explains the methodology of all the practicals including the underlying science or theory. I think this would be helpful to people who were not sure what needed to be done for a task.

I would spend more time on the electrical engineering as I feel it was tricky and that way we would have time to design logic gates using SnapGene. And I felt we had limited knowledge about modelling bacteria so spending more time on this would be beneficial.

Overall I think the university should encourage the course so that it can be opened up to more students as, I gained a lot from it and feel it should be run as module in its self and this way more time could be allocated to the field.

Add more details to the lab procedures.

Make the overall point of the reason we were learning about the software and GM foods more known.

Make a big booklet that goes through from start to end, with more detailed instructions. this would especially be really good for the modelling etc.

More detailed instructions, namely for the visualGEC section and the lab work. While instructions were fairly clear, we had to constantly ask staff if we forgot the protocol, so more detailed would make the classes run more smoothly

I would want to include more of the biological practical side of the course, especially the Gibson assembly, construction of plasmids and transformations of bacteria and the building of more complex systems. I think this would give the opportunity to get lots more practice and also it would give people the opportunity to come up with different systems that they could build with the various components available.

I would perhaps introduce a challenge for the teams in which they would compete against each other; in effect a mini iGEM but purely based on creating theories. I feel like this would encourage team work and give students a drive to actually compete in iGEM.

I would introduce a group discussion about the case studies, so that we could think about the issues as a group.

Bring in some analogies to use for genes to make the concept more accessible to non-biologists as terms such as repressor/promoter and lacI AHL TetR etc. can be very confusing even for me with my 1st year bio modules.

Maybe a more detailed protocol that flowed with the week? I found that when doing the write up at the end it was difficult to bring together the large amount of experiments we did, as they were all quite similar and it got a touch confusing!

Extra time to allow for deeper understanding and more complex constructs. If budget allows, create multiples of each biological reaction to make the process more robust if one culture does not grow.

I would make it longer. But I know due to the number of applicants that may not be possible. I would also include an activities book for students to do whilst they have nothing to do. It would also be good if students interested could then be referred on to work experience in a lab for a week to help people that actually carry out this work.

I would recommend to not have a too diverse group as it might be a bit patronizing for biologists learning to use pipettes.

I would extend the logic gate explanation section in order to improve the understanding of this field between people with biology background.

Make the course longer to allow students to apply the basic techniques they have learnt to solve a problem. I think this would help students to really understand what they've learnt as application is always a good way to teach new topics.

If there was one thing you had to remove or limit in the course, what would that be?

Electrical engineering. I understood the concept of biological logic gates without having to know too much about the electrical engineering. For example, I would have understood biological logic gates with only having been taught about OR/AND/NAND/NOR gates.

I would limit the breaks.

The computer section was quite long and I didn't really have any idea what I was doing!!

Limit the amount of breaks we get :)

Don't need so many breaks, it is almost better to work continually and that way finish earlier as it makes the day very long if not.

I wouldn't like to remove or limit anything in this course. I only wish that it could of been a week longer. As I feel there was some extreme cramming of content involved.

If i had to limit one thing it would be the modelling aspect of the course using GEC. maybe fewer talks? (although I've already gone through it in my course; it may have been more useful for the system engineers).

Nothing - everything led to a greater understanding of the topic and helped reinforce one another.

Nothing, the content appeared to all be necessary and provide a good insight into the field.

Note - I want to give this course a 5, it IS a really good course and i really enjoyed being on it! But it still has potential to improve in small areas.

Would remove the pipette challenge. Whilst it was a fun exercise a lot of time was dedicated to it and I feel it was a fairly easy task to begin with.

I think the computer modelling part of the course needs to be given a lot more time than it was to make it worthwhile otherwise it should be removed or limited to an hour lecture.

I feel all aspects of the course were beneficial and rightly included so I don't think anything should be removed.

Can't think of anything.

Instructions to the biology students about how you do basic things.

Long breaks with not much to do, could have been given worksheets to back up the knowledge we had learnt.

There were some long breaks in the course while waiting for results - it would be nice if stuff was planned to do while we were waiting.

I thought all the course was useful, the only parts that didn't benefit me was the material and information aimed at those not from a biological sciences background, which although not useful for me was necessary for other students.

Being a biologist I have done a lot of bacterial growth curves already....

I can't think of anything I would want to remove or limit about the course!

Overall, I felt that all aspects of this course were necessary. I really enjoyed it and wish that we had a module within this university that included similar topics. It was well taught and very engaging.

Wouldn't remove anything.

I think SnapGene should be explained more clearly. As it seemed really interesting software but I still don't really know how to use it.

I do not think anything should be removed from the course. It was thoroughly enjoyable and I learnt a lot from it.

Appendix 4.

Summary of Results

1) **Gene Constructs**
2) **Gibson Assembly**
3) **Bacterial Growth Curve**
4) **Sequencing Results**

1) Gene Constructs

Figure 1a. RFP Construct Plasmid. The aim was to swap the GFP gene for an RFP gene. Using SnapGene the construct was designed using pNCBE_kan_avGFP_p006 (p006) as a vector plasmid and the obRFP gene from pNCBE_kan_obRFP_p005 (p005). It was determined using this software that the primers pTR and pTF were needed to amplify only the obRFP gene from p005 producing a product of 1102 bp in length, and that the primers needed to amplify all but the avGFP gene from p006 were pTR-rev and pTF-rev producing a product of 3548 bp in length.

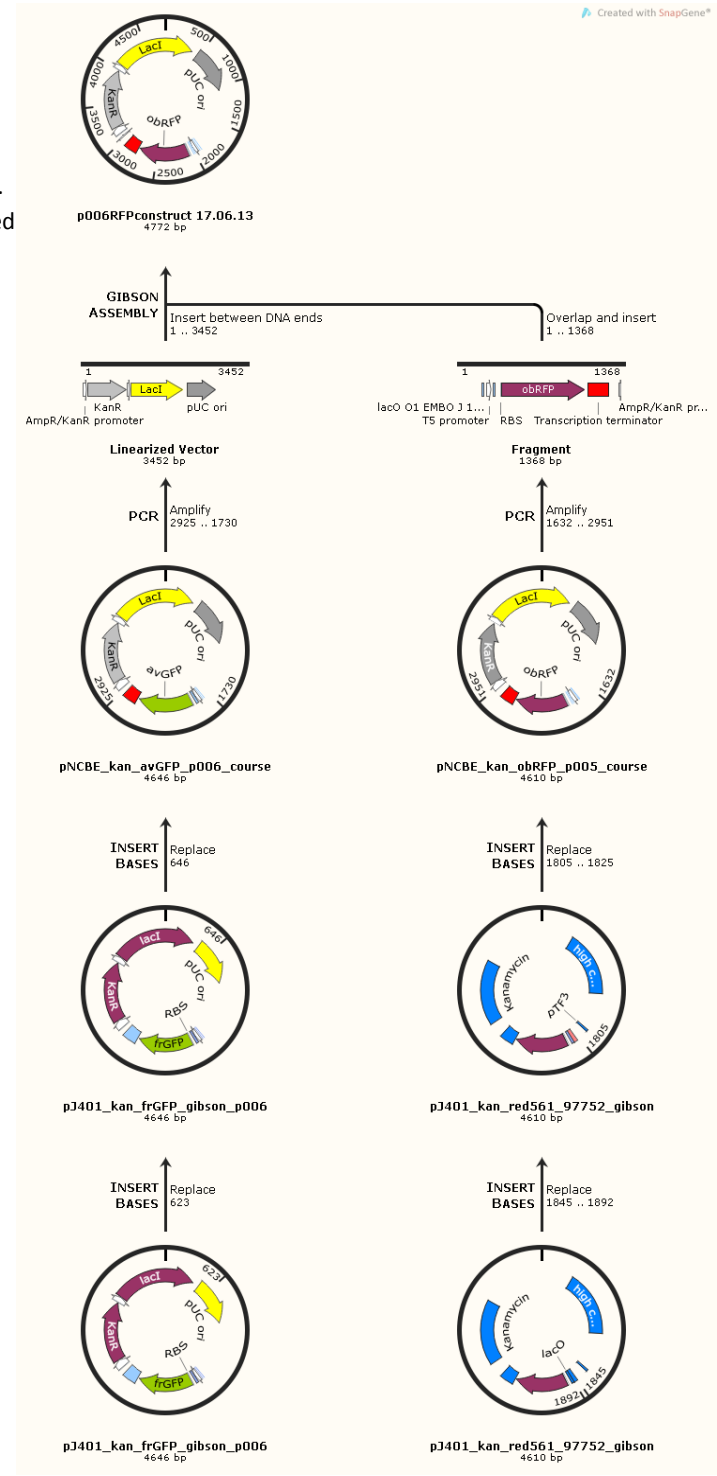
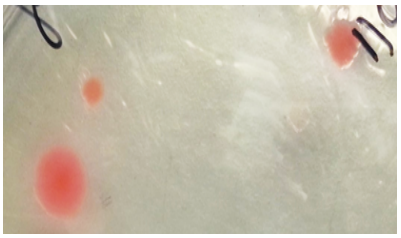


Figure 1b. Transformed RFP insert E.coli. Image of the E.coli with GFP removed and RFP inserted.



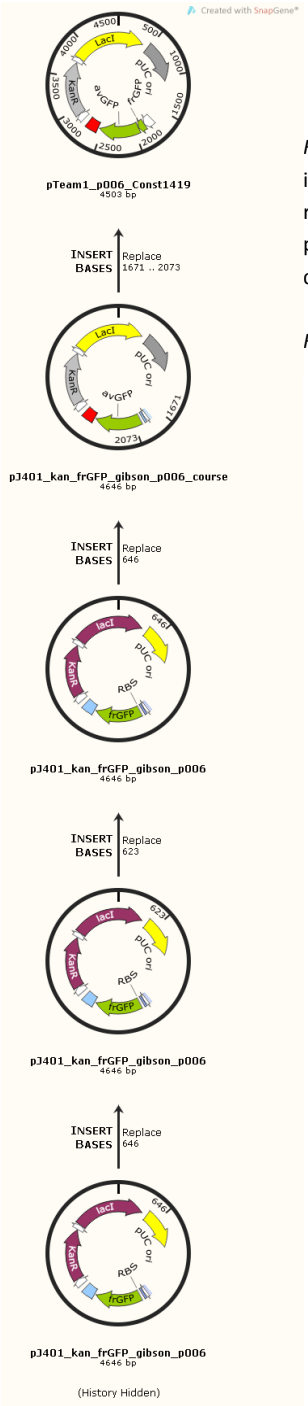
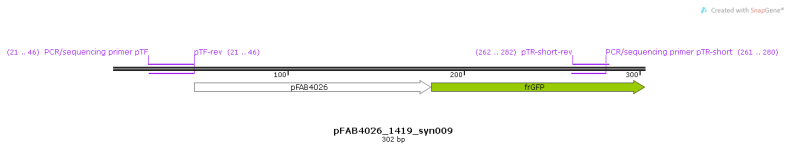


Figure 2a. Constitutively Active Promoter Construct. The aim was to insert a constitutively active promoter before the GFP coding region.Using SnapGene the construct was designed using pNCBE_kan_avGFP_p006 (p006) as a vector plasmid and the constitutively active promoter syn009 (see Fig. 2b).

Figure 2b. Inserted Constitutively Active Promoter Syn009.



1

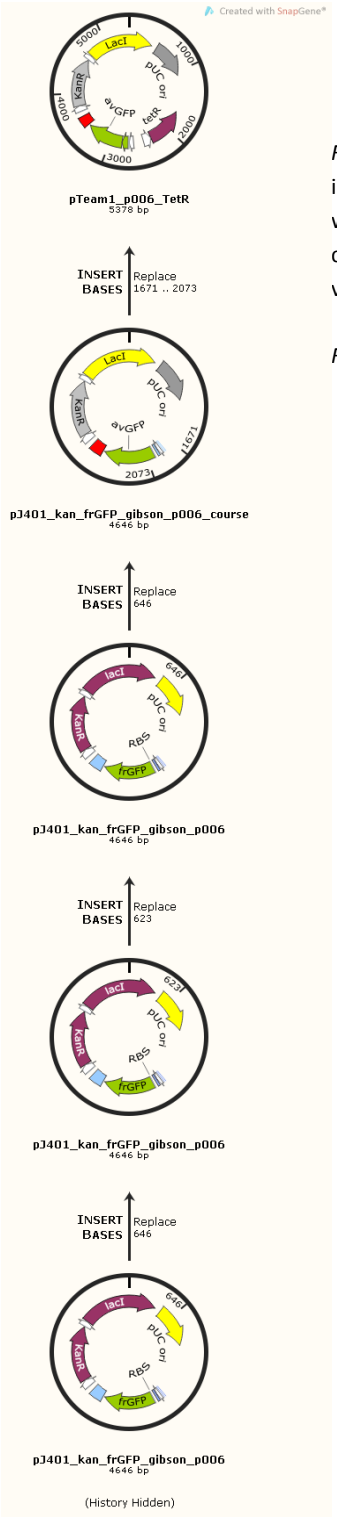
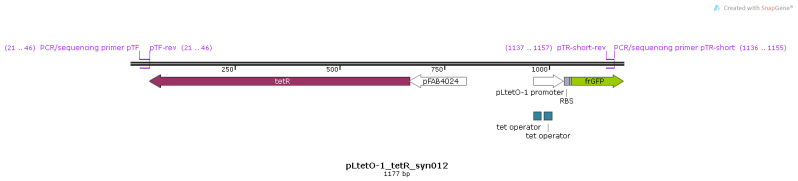


Figure 3a. TetR Inducible Promoter Construct. The aim was to insert an inducible promoter before the GFP coding region. The promoter chosen was inducible by a Tetracylcin analogue ATC. Using SnapGene the construct was designed using pNCBE_kan_avGFP_p006 (p006) as a vector plasmid and the inducible promoter TetR (see Fig. 3b).

Figure 3b.TetR Inducible Promoter



2

2) Gibson Assembly

These constructs were made using the Gibson Assembly method. A ratio of 1:1 vector to gene/promoter sequence to be inserted was used.

Gibson Calculations to determine pmol of DNA fragments

pmols= mass of DNA (ng) x 1000 / (fragment length (bp) x 650)

1) GFP replaced by RFP
Aim: to replace GFP gene in plasmid 006 with RFP from plasmid 005

	DNA mass (ng/µL)	**Fragment (bp)**	**pmol**
RFP	19.9	1102	0.0278
Vector 006	21.4	3548	0.0093

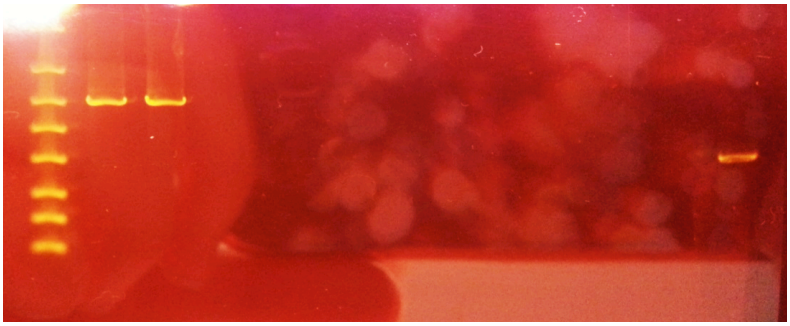
Ratio of **what** to **what**:
~3:1

2) Constituitive (1419 **units?)** vs TetR
Aim: compare promoters on GFP expression (inducible cf. constitutive)

	DNA mass (ng/µL)	**Fragment (bp)**	**pmol**
Const1419	10	260	0.0592
TetR	30	1135	0.0406
Vector 006	162	4288	0.058

Ratio of insert to vector:
Vector:Const1419 ~3:2
Vector:TetR ~1:1

Gel Electrophoresis of PCR Products
Constitutive Lac (Promoter) Plasmid Fluorescence Results



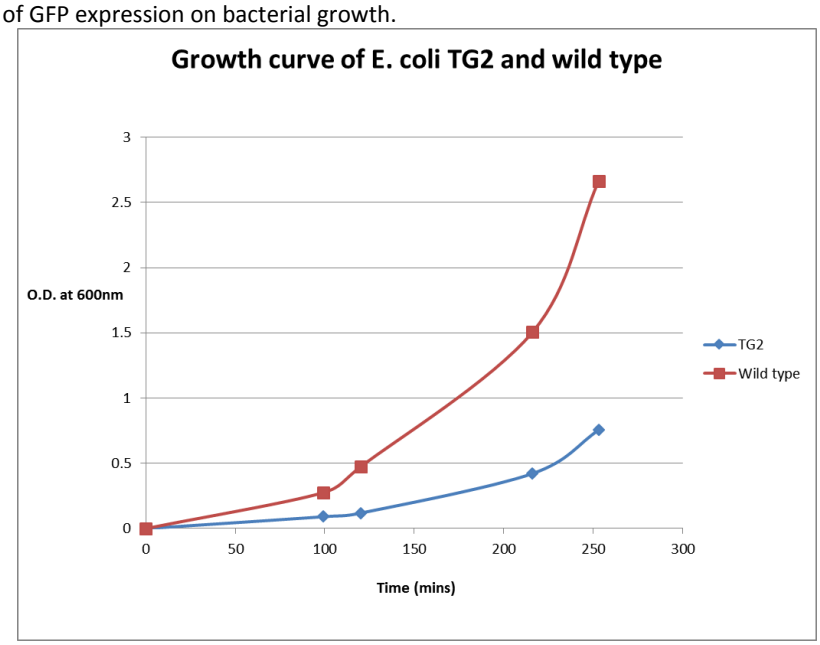
3) Bacterial Growth Curve

A bacterial growth curve was carried out to determine the effects of GFP expression on E.coli growth. Wild type E.coli growth was compared to GFP expressing E.coli T2R in duplicate. Colonies of each were added to 10 mL media (and for TG2 strain 10 µL of Kanamycin was added). Cultures were incubated at 37°C and shaken at 150 RPM. Measurements of turbidity were taken hourly using a spectrophotometer at 600 nm.

Table 1. Table of results of turbidity measurements of wild type and TG2 E.coli over time.

	Optical density (600nm)		
Time (mins)	**TG2 (1)**	**TG2 (2)**	**Wild type**
0	0	0	0
99	0.091	0.076	0.275
120	0.119	0.044	0.474
216	0.4215	0.077	1.505
253	0.755	0.109	2.666

Figure 4. Graph of optical density (turbidity) of E.coli cultures over time to determine the effects of GFP expression on bacterial growth.



It is clear from the graph above that the expression of GFP affects bacterial growth and slows down the logarithmic phase of growth. However, the stationary phase was not reached due to the limited time the measurements were taken for.

4) Sequencing Results