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Unigems: plasmids and parts to facilitate teaching on assembly, gene expression control and logic in *E. coli*

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Abstract

Synthetic biology enables the creative combination of engineering and molecular biology for exploration of fundamental aspects of biological phenomena. However, there are limited resources available for such applications in the educational context, where straightforward setup, easily measurable phenotypes and extensibility are of particular importance. We developed unigems, a set of ten plasmids that enable classroom-based investigation of gene-expression control and biological logic gates to facilitate teaching synthetic biology and genetic engineering. It is built on a high-copy plasmid backbone and is easily extensible thanks to a common primer set that facilitates Gibson assembly of PCR-generated or synthesized DNA parts into the target vector. It includes two reporter genes with either two constitutive (high- or low-level) or two inducible (lactose- or arabinose-) promoters, as well as a single-plasmid implementation of an AND logic gate. The set can readily be employed in undergraduate teaching settings, during outreach events and for training of iGEM teams. All plasmids have been deposited in Addgene.

DATA SUMMARY

The plasmids have been deposited at Addgene (https://www.addgene.org/Jaroslaw_Bryk/). An accompanying Figshare repository (https://figshare.com/projects/Unigems_paper/114069) contains SnapGene sequences of all constructs as well as EPS and Adobe Illustrator files with the plasmid maps to allow educators create their own high-quality figures of their assemblies.

INTRODUCTION

The development and standardization of biological parts (DNA or RNA fragments) to reliably achieve a predictable output in living organisms has been an often-emphasized aspect of synthetic biology [1]. Remarkable progress has been achieved in characterization of bacterial promoters [2], transcriptional terminators [3], insulators [4], translation optimization [5] and promoter inducibility [6], however advances on a higher level of systems' complexity have not been as forthcoming [7–9] and major synthetic biology applications (e.g. [10–14]) were achieved by a host of sophisticated and custom-developed genetic engineering [15, 16].

Nevertheless, standardized biological parts are particularly well suited to use in an educational context, as they emphasize utility and remove complexities associated with protocol development [17–19]. These advantages are best illustrated by the BioBuilder lessons, which provide detailed protocols that combine lab-based experiments with engineering tasks to demonstrate fundamental principles of biological and electronic circuits [20] (https://biobuilder.org/), and the International Genetically Engineered Machines competition (iGEM), where thousands of students worldwide create synthetic biology designs and constructs based on and inspired by a set of DNA parts provided by the iGEM Foundation [21]. Notably, practical synthetic biology exemplified by building novel biological constructs from compatible genetic parts, favour active learning approaches in teaching, which were shown to improve students' academic attainment [22, 23].

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Keywords: synthetic biology; Gibson assembly; iGEM; plasmids; education.
Abbreviations: GFP, green fluorescent protein; IGEM, International Genetically Engineered Machines; RFP, red fluorescent protein.
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Impact Statement

There is a dearth of practical synthetic biology resources available – plasmids, DNA parts and ways to generate and manipulate them – that could easily be implemented in teaching, outreach events or as foundational constructs for more complex designs. We designed a set of ten plasmids that can be broken down to five functional sections (reporter gene and promoter, origin of replication, antibiotic resistance and repressor) using the same set of overlapping PCR primers, therefore making each section compatible with any plasmid in the set. Each of the sections can also be replaced with synthetic DNA parts and/or via PCR with Gibson assembly. We have been using the system in introductory undergraduate synthetic biology laboratory classes, in student research projects, in iGEM teams training and in outreach events and expect they will become a valuable tool for teachers, university instructors and educators. All plasmids and corresponding source files and illustrations are available through Addgene and Figshare.

To expand the availability of education-friendly resources for synthetic biology we developed unigems, a set of plasmids with simple features such as strong and weak constitutive promoters or single- and dual-inducible promoters and an olfactory construct that can be used directly in laboratory classes. In addition, each plasmid can be split into functional parts that can be exchanged either between the plasmids or replaced with novel parts with PCR and Gibson assembly.

METHODS

Plasmids

All the plasmids in the unigems set have been assembled using Gibson assembly [24] on the backbone of the pJ401 high-copy plasmid obtained from Atum (Newark, CA, USA). The assembled parts were synthesized by IDT DNA (Coralville, IO, USA) and Life Technologies (now Thermo Fisher Scientific, Waltham, MA, USA).

Transformation

All transformations were performed using the Sub-cloning Efficiency DH5α™ Competent cells (Invitrogen, now Thermo Fisher Scientific, Waltham, USA) as per the manufacturer's protocol.

Growth and storage conditions

All cells were cultured using LB Lennox media and agar plates, with incubation at 37°C and agitation at 180 r.p.m. for liquid cultures. Volumes of cultures were 1 or 5 ml, depending on context (smaller for plating transformed cells, sequencing and fluorescence analysis, larger for plasmid isolation). For selective media, both kanamycin and ampicillin (Sigma-Aldrich, now Merck, Darmstadt, Germany) were used at concentration of 50 µg ml^{-1}. Plates and cultures were stored at 4°C or preserved for long-term storage at −80°C with 500 µl of overnight culture suspended in 500 µl of 50% glycerol.

Gibson assembly

Fragments to be inserted were designed with 20–40 bp overlaps of the vector primer binding sites using SnapGene and synthesized by Integrated DNA Technologies. Platinum SuperFi Green PCR Master Mix (Invitrogen) or Q5 High Fidelity Polymerase (New England Biolabs, Ipswich, MA, USA) were used for generation of backbones from p005 and p006 vectors following the manufacturers' protocols. PCR products were purified with GeneJET PCR purification kit (Thermo Fisher Scientific) and concentration measured using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). Homemade Gibson Assembly master mix was prepared and assembly carried out as outlined by [25], using 2-3:1 of molar ratio of the donor parts to target vectors.

Plasmid verification

Following transformation, colony PCR was performed using DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific) as described at https://openwetware.org/wiki/Endy:Colony_PCR. Colony PCR products were verified on 1% agarose gels stained with RedSafe (ChemBio, Oxford, UK) and then purified with GeneJET PCR purification kit (Thermo Fisher Scientific) and sequenced by SourceBioscience (Nottingham, UK).

Analysis of fluorescence

Horiba FluoroMax–4 spectrofluorometer (Horiba, Kyoto, Japan) was used to obtain emission and excitation spectra for green and red fluorescent proteins. We used 1 ml of overnight cultures resuspended in phosphate buffered saline (PBS, Sigma-Aldrich) to an OD600 below 0.120. Guava easyCyte 5HT system flow cytometer (Merck) was used to determine fluorescence of 10000

events of each triplicate culture. A signal from RFP cultures, indicating protein expression, was only detected after a 24 h period of incubation.

Olfactory measurements

The banana smell was identified using the protocol by Dixon and Kuldell [17], following overnight culture at 37°C at 180 r.p.m. to reach stationary growth phase.

RESULTS

Assembled plasmids

The unigems' set includes ten plasmids shown in Table 1.

The promoters and terminators are derived from the BIOFAB collection: pFAB4026, pFAB4282, pFAB4005, pFAB4024 [26] and BBa B0062-R [3], respectively. The pBAD-araC and osmY-ATF1 parts come from IGEM repository (BBa K808000, positions 1:1200 bp) and BBa J45250, while the AND logic gate is based on the D61 clone from [27]. The RFP reporter gene is a synthetic gene (ID 97752) generated by Atum (Newark, CA, USA) by random assembly, but it has a 76% sequence identity to an RFP from a strawberry coral *Corynactis californica*. GFP is a standard reporter gene from *Aquorea victoria*. All plasmids also include the lacI repressor under control of a weak constitutive Amp promoter, even when they do not have T5-pLacO promoter themselves.

The unigems system

The unigems system is built upon a set of six pairs of overlapping PCR primer binding sites (Table 2) that split each plasmid into functional sections that can be replaced or exchanged. Thanks to this arrangement, each plasmid can be a source of parts for other recipient plasmids and new parts can be generated by direct synthesis or PCR with overhanging primers matching the primer binding sites on the unigems plasmids (Fig. 1).

This principle can be illustrated with the following example (Fig. 2). Let our starting point be the p007ampGFP plasmid (Addgene 58535), where a reporter gene (GFP) is under control of the lactose-inducible promoter (T5-pLac$_O$) and the plasmid contains an ampicilin-resistance gene. To replace the lactose-inducible promoter with a constitutive one (for example, a BIOFAB promoter pFAB4026, available in p006-GFP-strong (Addgene 108313)), we would run two PCR reactions: one to generate the recipient vector and one to generate a donor part (p005ampGFP and p006-GFP-strong, respectively).

We would use primers B$_{GFP}$-REV and F-FWD to generate linearized recipient plasmid and primers A$_{GFP}$-FWD and A-REV to generate the donor part. Because the primers B$_{GFP}$-REV and A$_{GFP}$-FWD, as well as F-FWD and A-REV are overlapping, both of their PCR products are directly usable for Gibson assembly. After purification and quantification, they can be mixed in appropriate molar proportions to assemble a new plasmid.

The same principle can be used to exchange or remove the entire reporter gene, antibiotic resistance gene, origin of replication or the repressor gene between the plasmids or with a newly generated or synthesized part. The combination of different promoters, reporters and antibiotic resistances ensures identification of a successful assembly. In our example, the colonies would be grown on ampicillin-containing media with no lactose or IPTG to identify GFP-fluorescent bacteria, indicating correctly assembled plasmids.

Table 1. Unigems plasmids

Plasmidname	*AddgeneID*	*Antibiotic resistance*	*Reporter gene*	*Function*
p006-strongGFP	108313	kanR	GFP	pFAB4026 strong constitutive
p006-weakGFP	108314	kanR	GFP	pFAB4282 weak constitutive
p005-strongRFP	108317	kanR	RFP	pFAB4005 strong constitutive
p005-weakRFP	108316	kanR	RFP	pFAB4024 weak constitutive
p006-pBADGFP	108315	kanR	GFP	pBAD
p006kanGFP	58534	kanR	GFP	T5-pLac$_O$
p005kanRFP	58533	kanR	RFP	T5-pLac$_O$
p007ampGFP	58535	ampR	GFP	T5-pLac$_O$
p006-ANDGFP	112237	kanR	GFP	pLac +pBAD AND logic gate
p006-Banana-Late	112251	kanR	ATFI	osmY

Table 2. Primers for the unigems plasmids

Primer name	Primer sequence (5' → 3')
A-REV	CTCGAAAATAATAAAGGGAAAATCAG
A_{GFP}-FWD	TTCTCCCTCTCCACTGACAG
A_{RFP}-FWD	TACGGTTTGCCTGTACCTTC
B-FWD	CTCAGAAGTGAAACGCCGTA
B_{GFP}-REV	GGGCACAAATTTTCTGTCAG
B_{RFP}-REV	GTACAGGCAAACCGTATGAG
C-FWD	TCACCACCCTGAATTGACTC
C-REV	ACTACCATCGGCGCTACG
D-FWD	CTCACGTTAAGGGATTTTGG
D-REV	CGCCCGGAAGAGAGTC
E-FWD	ACTCAAAGGCGGTAATACGG
E-REV	CCAAAATCCCTTAACGTGAG
F-FWD	CTGATTTTCCCTTTATTATTTTCGAGA
F-REV	CCTGATTCTGTGGATAACCG

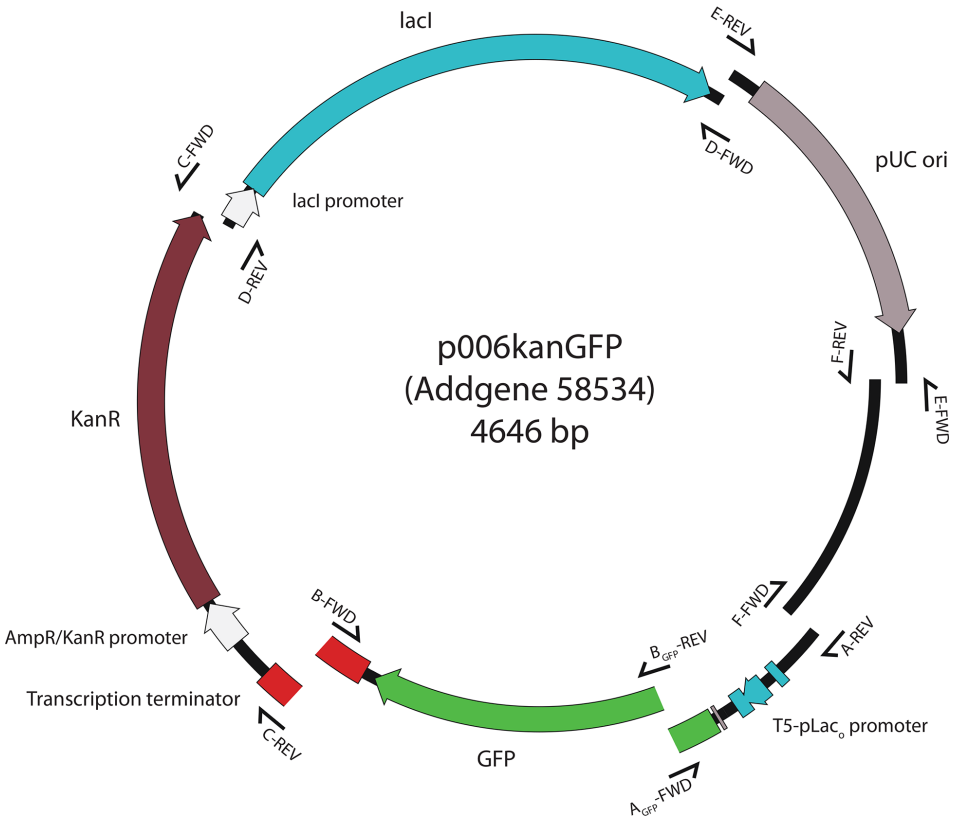

Fig. 1. Building sections of the standard unigems plasmid, with the location of each pair of overlapping primer binding sites: primers F-FWD and A-REV, A_{GFP}-FWD and B_{GFP}-REV, B-FWD and C-REV, C-FWD and D-REV, D-FWD and E-REV, E-FWD and F-REV overlap such that PCR products made with them can be directly used in Gibson assembly. Note that all primer pairs except A_{GFP}-FWD and A_{GFP}-REV are identical in all plasmids.

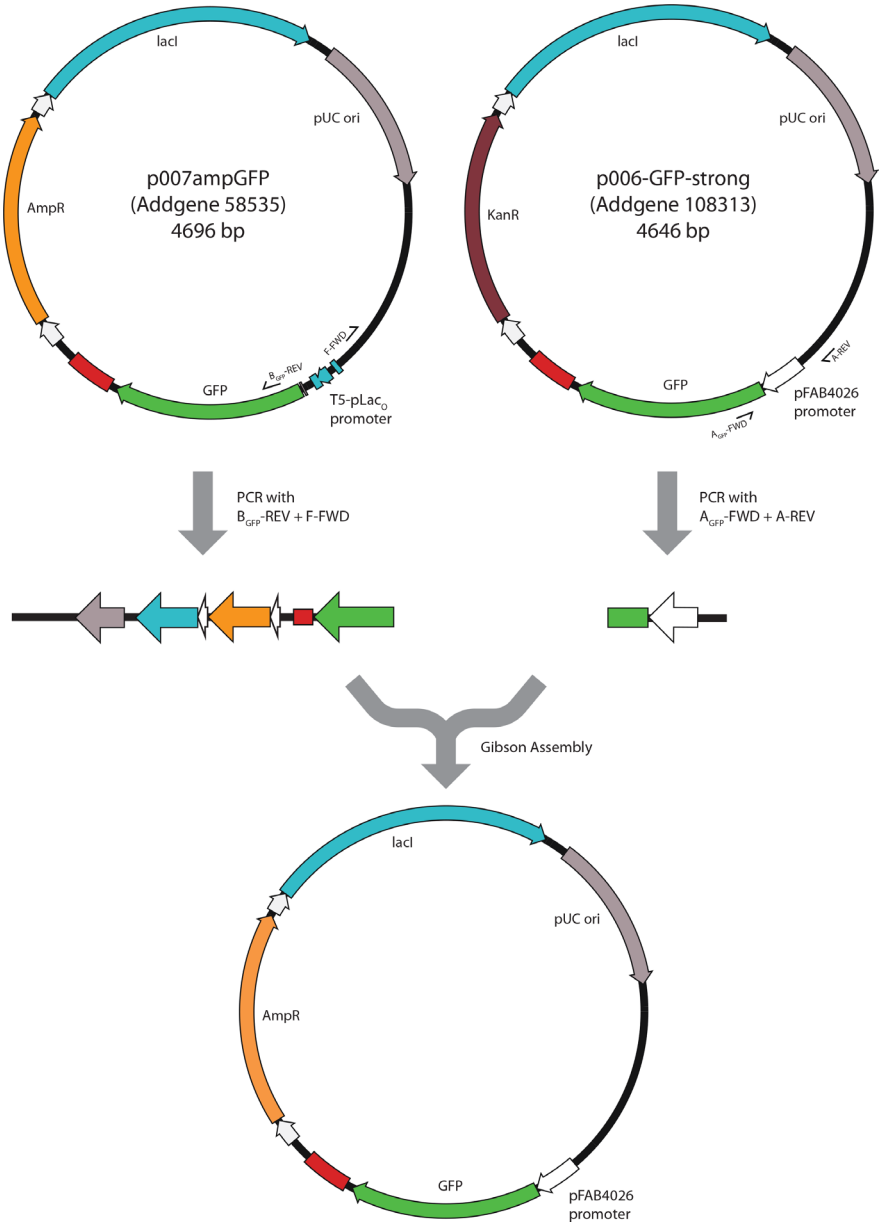

Fig. 2. Example of novel plasmid assembly using existing unigems plasmids as sources of vector and insert parts with PCR and Gibson assembly. See text for details.

Because primers A_{GFP}-FWD and B_{GFP}-REV are located inside the ORFs of the reporter genes, they are specific to these genes. However, change of the promoter could also be achieved by inserting the entire ORF of the reporter gene combined with the promoter and part of the terminator. In this case, primers A-REV and B-FWD would be used and they would be identical for every reporter-promoter combination to be exchanged in any of the unigems plasmids.

Following assembly, we experimentally verified the characteristics of the plasmids.

Constitutive promoters

GFP and RFP reporter genes, placed under the control of either a strong or a weak promoter (pFAB4026 and pFAB4282 for GFP and pFAB4005 and pFAB4024 for RFP, respectively) (BIOFAB collection [26]) were analysed using a flow cytometer on three replicate samples each (separate colonies from the same transformation event). To characterize the properties of the fluorescent proteins, we used a spectrofluorometer to test a range of excitation wavelengths for both GFP and RFP. We found that the GFP can be excited between 350 to 420 nm (with a peak at 395 nm) for emission at 506 nm. The RFP can be excited between 480 and

505 nm (with a peak at 505 nm) for emission at 560 nm. The fluorescence levels of the reporter genes were measured using the Guava easyCyte 5HT, with cells grown in liquid culture (Fig. 3).

Inducible promoters and logic gate

To demonstrate the inducibility of the pBAD and T5-pLacO promoters, we measured fluorescence of GFP with an increasing concentration of inducers, arabinose (0–5%) and IPTG (0–5 mM). We observed clear activation depending on the inducer. pBAD exhibits a binary-like induction, where already at 0.1% concentration of arabinose it produces 90% of fluorescence observed at 5% arabinose. In contrast, T5-pLacO produces a more dose-dependent pattern of induction (Fig. 4).

We also used combined lactose- and arabinose-inducible promoter based on design by Cox and colleagues [27]. This promoter acts as a biological AND gate, where both inputs (IPTG and arabinose) are necessary to activate the output – expression of the GFP. We tested the performance of the logic gate with arabinose only, IPTG only and both, at increasing concentrations. GFP fluorescence does not increase significantly in the presence of either IPTG or arabinose alone, but is clearly inducible in the presence of both inducers (Fig. 5).

Olfactory construct

The p006-Banana-Late construct is identical to the *Eau de Smell* described by Dixon and colleagues [17] and produces ATFI enzyme (alcohol acetyltransferase I) that converts isoamyl alcohol to isoamyl acetate, which has a strong banana odour. ATFI production is controlled by the osmY stationary phase promoter, therefore the banana odour can only be detected once the cells reach the stationary growth phase. The construct was tested in broth culture.

DISCUSSION

We have assembled and characterized a set of plasmids that enable out-of-the-box investigations of gene expression control and straightforward extensibility with PCR and Gibson assembly. The phenotypes presented are clearly distinguishable and interpretable in an educational context rather than being designed and tested to produce precise quantitative output. For instance, Mutalik and colleagues reported (2013) that the relative difference in GFP fluorescence driven by BIOFAB promoters pFAB4026

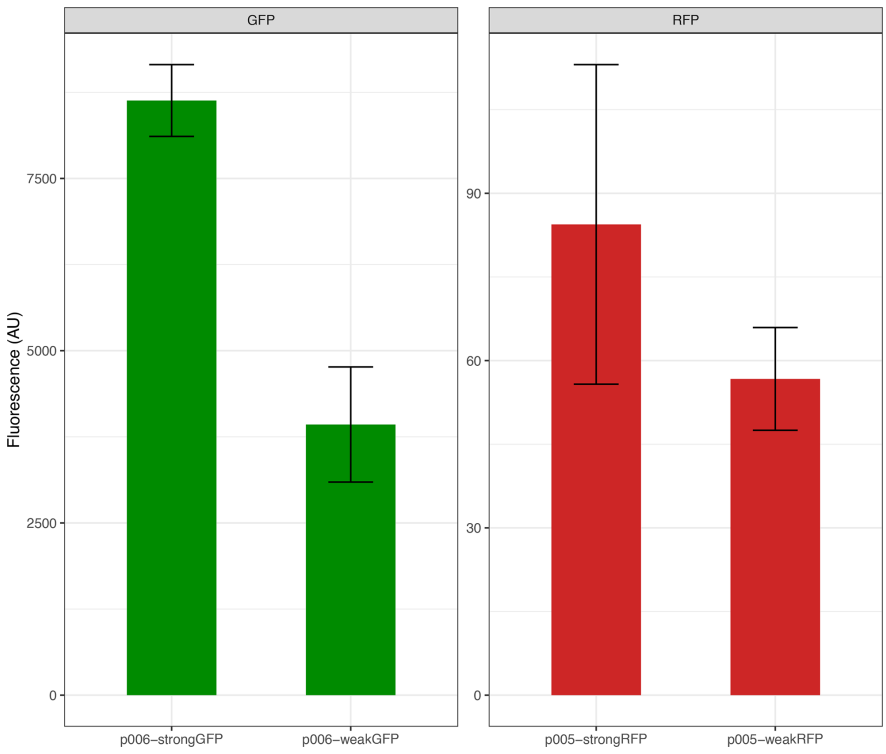

Fig. 3. GFP fluorescence of three replicates of each p006-strongGFP and p006-weakGFP and RFP yellow fluorescence of p005-strongRFP and p005-weakRFP. Note the difference in scale in GFP vs RFP: the lack of differences between strong and weak RFP fluorescence is due to a mismatch between its optimal excitation wavelength (550 nm) and the 488 nm excitation laser in the flow cytometer (see Discussion).

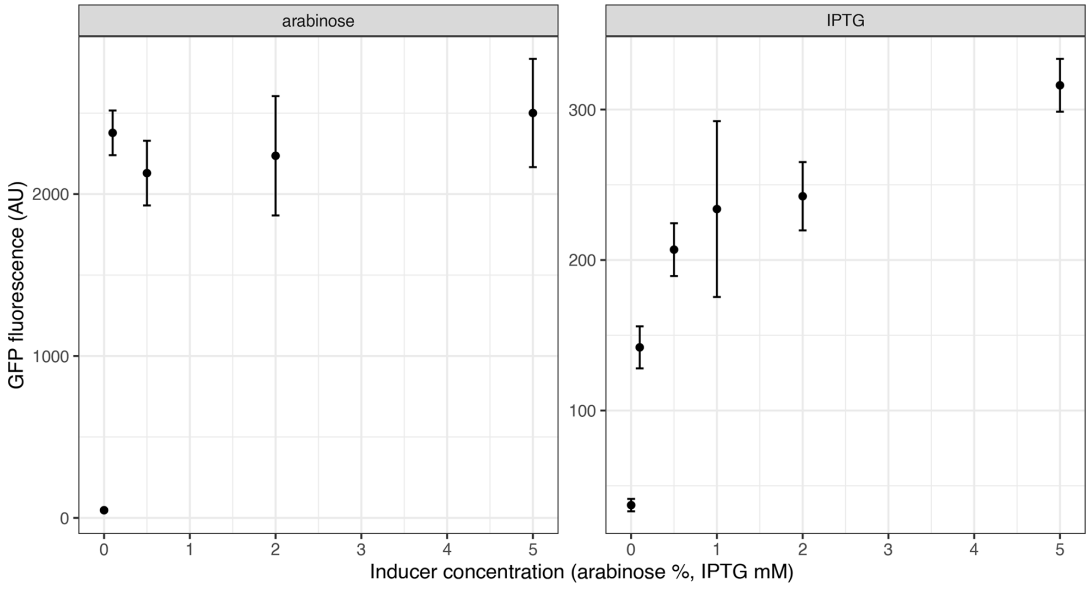

Fig. 4. GFP fluorescence of three replicates of p006-strongGFP under arabinose-inducible p006-pBADGFP or IPTG-inducible p006kanGFP.

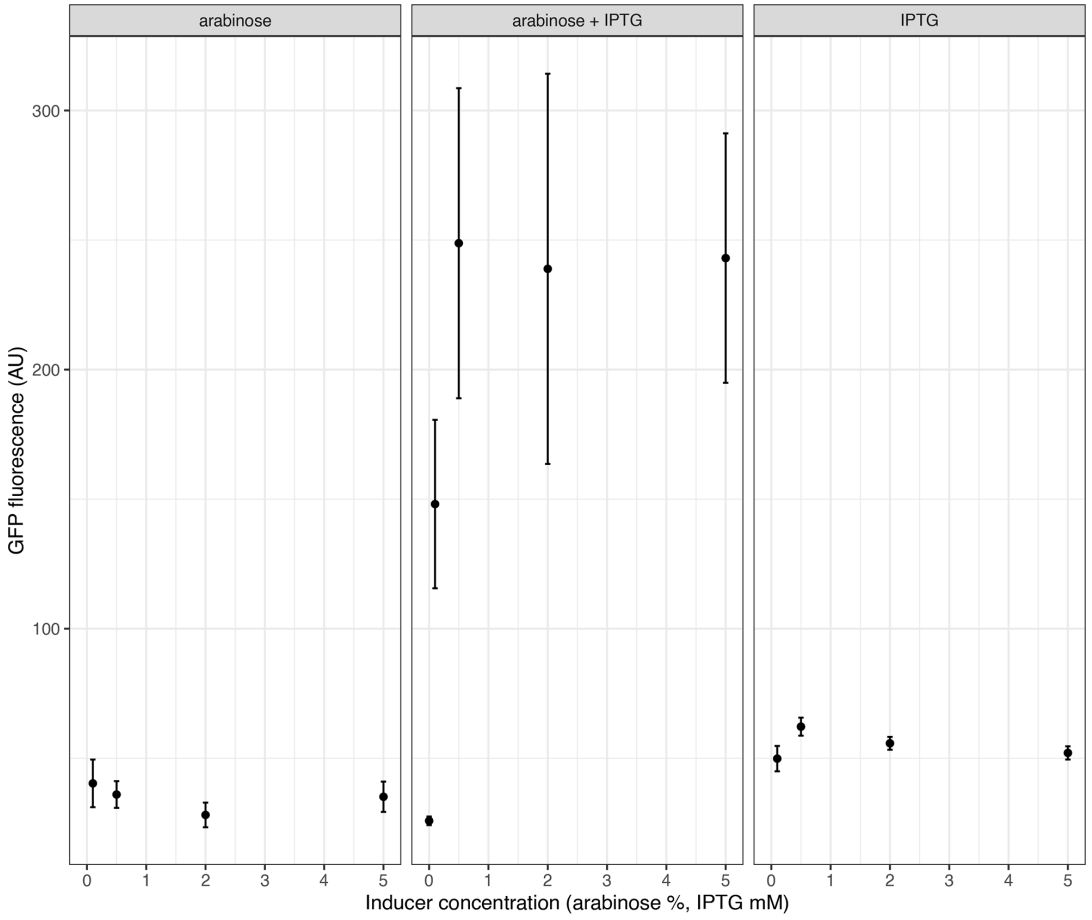

Fig. 5. GFP fluorescence of three replicates of p006-GFP-Logic-AND induced by individual inducers (arabinose or IPTG) or by both inducers.

and pFAB4282 is sevenfold vs threefold in our characterization (Fig. 3), the discrepancy that can reasonably be attributed to differences in bacterial chassis, fluorescent marker sequence and culture conditions. The minimal signal from RFP fluorescence shown on Fig. 3 is due to a mismatch between its optimal excitation wavelength (550 nm) and the 488 nm excitation laser in the flow cytometer. The GFP's excitation and emission reported here are similar to those reported by Heim and Tsien [28] for an unmodified protein [28]. The RFP's excitation and emission spectra behave similarly to that quantified by Baird *et al.* [29]. Overall, the performance of the plasmids in the K12 *E. coli* strain DH5alpha is suitable for demonstrating the quantitative differences between various promoters and inducers, including those in the bi-inducible promoter.

A standard benchtop UV transilluminator or a keyring UV torch, with excitation at ~400 nm, is the only equipment required to verify the expression of the reporter genes. Expression of RFP is also visible in daylight without any equipment (Fig. 6). Fluorometer or flow cytometer are only needed to quantify the expression level of GFP or RFP.

We (AS, JB, JS, DM) have been using the unigems plasmids in a variety of contexts, ranging from outreach events, where participants transform *E. coli* with p006kanGFP or p005kanRFP using a 15 min protocol to observe a spectacular fluorescence after an overnight incubation to undergraduate research projects, where students have to design a new compatible part (e.g. a NOT gate, or a quorum-sensing system) and characterize its functions following a successful assembly. The plasmids themselves were first tested in two synthetic biology workshops run for prospective members of iGEM teams. Students' feedback from this course is included in the report available in the Figshare repository (https://figshare.com/articles/media/Unigems_report_from_a_synthetic_biology_workshop_for_undergraduates/14627211). Since deposition in Addgene, Unigems plasmids have enjoyed a stable stream of requests.

The availability and use of GMOs is regulated in almost all jurisdictions [30–32]. In the UK, at the time of writing, the use of the unigems plasmids needs to comply with the UK's Health and Safety Authority's Genetically Modified Organisms (Contained Use) Regulations 2014 (https://www.hse.gov.uk/pubns/books/l29.htm). These guidelines allow for exemptions if the constructs and parts have a long history of safe use, but they are still restrictive compared to the US regulations, which only require that constructs do not pose an unreasonable risk (Toxic Substances Control Act, Environmental Protection Agency, https://www.epa.gov/laws-regulations/summary-toxic-substances-control-act). Therefore, use of the unigems system within high school classrooms would be possible in some jurisdictions, but it would pose an administrative burden in most. On the other hand, as most undergraduate biology teaching facilities already comply with GMO containment requirements, unigems can be straightforwardly employed at higher education institutions.

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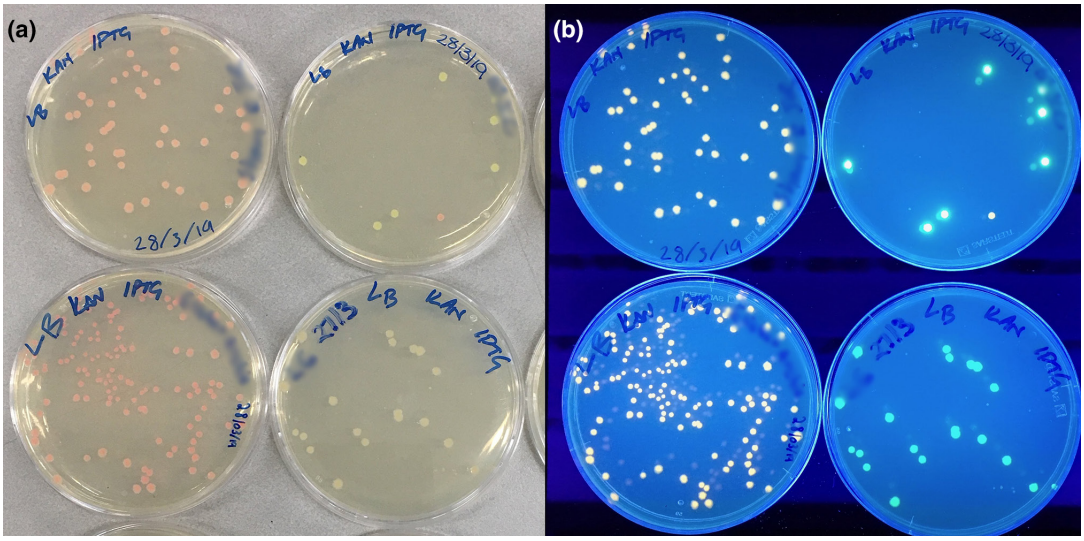

Fig. 6. Images of p005-kan-RFP (left column) and p006-kan-GFP (right column) in daylight (a) and on a benchtop UV transilluminator (excitation wavelength 395 nm) (b). Transformed DH5α cells were grown with 50 µg ml^{-1} of kanamycin and induced with 100 mM IPTG. The single RFP-expressing colony on the top right plate is due to a mix of plasmids used by the students in this transformation. Photographs were taken with a mobile phone by JB.

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The authors wish to thank all members of the University of Reading 2013 and 2014 iGEM teams and University of Huddersfield 2015–2019 project students for help in testing the parts and plasmids. JB would like to dedicate this manuscript to Dr. Dean R. Madden, Director of the National Centre for Biotechnology Education, University of Reading.

Author contributions
A.S., A.A.W. investigation, A.S. formal analysis, writing – original draft, writing – review & editing, J.S. formal analysis (digital illustration), J.A.D. formal analysis (regulation), writing – review & editing, D.M., JSchollar. resources, D.M., JSchollar, J.B. conceptualization, funding acquisition, methodology, supervision, project administration, J.B. investigation, formal analysis, validation, writing – original draft, writing – review & editing.

Conflicts of interest
The author(s) declare that there are no conflicts of interest.

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Peer review history

VERSION 2

Editor recommendation and comments

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Georgios Efthimiou; University of Hull, Biomedical Science, Hardy Building, Cottingham Road, UNITED KINGDOM, Hull

Date report received: 04 August 2023
Recommendation: Accept

Comments: The work presented is clear and the arguments well formed. This study would be a valuable contribution to the existing literature. This is a study that would be of interest to the field and community. All comments were satisfactorily addressed.

SciScore report

https://doi.org/10.1099/acmi.0.000596.v2.1

iThenticate report

https://doi.org/10.1099/acmi.0.000596.v2.2

Author response to reviewers to Version 1

Dear Reviewers,

Thank you very much for the positive comments and suggestions on improving the manuscript. Below we detail changes that we introduced to the manuscript following the review (and we not some discrepancy with line numbering – we provide current line numbers where our changes are located).

Reviewer 1 Comments to Author: This paper describes the development of a set of plasmids that can be used to teach synthetic biology and gene regulation using fluorescent and olfactory reporters. The strengths of the system are the easy ability to manipulate and alter the plasmids allowing for the creation of new constructs and the variety of established promoters and reporters being used. Overall the paper is well written and the methods and results well described and I have no doubt that the Unigems plasmid system will be an incredibly useful resource for educators and those involved in outreach alike. I have made a few comments below which I think would be useful to provide a little more context for those interested in using the system and a little more clarity to some sections but overall I think the paper gives a nice detailed overview of a very useful set of molecular tools for teaching and outreach purposes.

1) Line 51 - I couldn't get the "addgene" link to work. Could the authors check this is active (and apologies if this is a limitation on my part).

Link corrected (was missing an underscore)

2) Line 70 - Should this be "which provide" rather than "which provides"?

(Line 76) Corrected.

3) Line 84 - Should the plasmid p007KanGFP not be p007AmpGFP?

(Line 96) Corrected.

4) Line 137 - Should this be "specific to this gene" rather than "specific to these genes"?

(Line 148) We used plural earlier in the sentence as there are two ORFs (GFP and RFP) in the current system.

5) Line 160 - Figure 3 - The lack of a significant difference between the strong and weak promoter using RFP is mentioned in the discussion to be due to the readings being taken at a non-optimal wavelength. I think the authors need to clarify this in the results section and mention this limitation for the data shown at the time the data is shown.

(Line 177) Added description in situ of where the data is reported. Caption is now extended with: "Note the difference in scale in GFP vs RFP: the lack of differences between strong and weak RFP fluorescence is due to a mismatch between its optimal excitation wavelength (550nm) and the 488nm excitation laser in the flow cytometer (see Discussion)."

6) Line 171 - Figure 4 - This figure shows inducibility of the system

using arabinose and IPTG. The arabinose was tested from 0-5% and the IPTG from 0-5mM. The authors comment "pBAD exhibiting much more prominent inducibility than T5-pLacO" is a little vague. It might be useful to point out that the T5-pLacO produces a much more dose dependent and gradual response than the pBAD in this system. I would have expected the pBAD to be better for dose dependent induction but either way this is a useful difference between the two systems and would be an important consideration for those choosing which inducible system to use for a specific teaching or outreach application. I think a few more lines on the difference in inducibility between the two systems would be helpful.

(Line 185) Agreed - we added a better description of the two patterns of induction. Line 185 now reads: "We observed clear activation depending on the inducer. pBAD exhibits a binary-like induction, where already at 0.1% concentration of arabinose it produces 90% of fluorescence observed at 5% arabinose. In contrast, T5-pLacO produces a more dose-dependent pattern of induction (Fig. 4)."

7) Line 181 - the phrase "is clearly visible and in the presence of both inducers" is a little confusing. I would suggest "is clearly inducible in the presence of both inducers" or another alternative version.

(Line 199) Corrected.

8) Line 188 - The section on the olfactory construct - The inclusion of an olfactory construct is a nice addition to this toolkit. Its clear this is expressed at stationary phase but it would be useful to clarify whether the odour is detectable in plate as well as broth culture.

(Line 212) We clarify that our construct was tested in broth culture.

9) Line 196-198 - This sentence in whole reads a little strange. I would suggest replacing the final phrase "rather than deliver precise quantitative output" with "rather than being designed to deliver a precise quantitative output"

(Line 218) We rephrased is slightly differently. The line now reads: "The phenotypes presented are clearly distinguishable and interpretable in an educational context rather than being designed and tested to produce precise quantitative output."

10) Discussion - The authors are describing an incredibly useful toolkit and have mentioned that it could be used for outreach as well as educational purposes. It would be very useful to include a paragraph clearly outlining the equipment requirements for detection of fluorescence from this system and the overall tractability of the system which I suspect is one of its strengths. Might be useful to factor in here the whether the RFP or GFP is more tractable and the limitations depending on whether you an excite at a specific wavelength. They mention some of these reporters are visible by the naked eye and it would also be useful to mention here whether for outreach purposes how visible they would be on plates. Effectively a little more information on the tractability of the system for the end user would be very useful

(Line 232) We added a sentence to state equipment requirements to visualise the expression of the reporter genes and included a set of 2 photographs of the same GFP and RFP expressing plates in daylight and on a UV-transilluminator (new Fig. 6).

11) Line 241 - Can the authors also include the conc of ampicillin used as well as they do describe a plasmid with an amp cassette.

(Line 285) Information added.

Please rate the manuscript for methodological rigour

Reviewer 2: Very good

Please rate the quality of the presentation and structure of the manuscript

Reviewer 2: Very good

To what extent are the conclusions supported by the data?

Reviewer 2: Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?

Reviewer 2: No:

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?

Reviewer 2: Yes:

Reviewer 2 Comments to Author: This is a very interesting manuscript focused on use of plasmids and parts for teaching microbiology. Overall, the paper is well-written, with sound methodology and the potential for strong impact in the teaching community. Some minor corrections:

Introduction: briefly describe how hands-on learning can improve learning efficiency and student enthusiasm.

(Line 82) Added a sentence about active learning approaches improving students perfomance: "Notably, practical synthetic biology exemplified by building novel biological constructs from compatible genetic parts, favour active learning approaches in teaching, which were shown to improve students' academic attainment (Freeman et al., 2014, Theobald et al., 2020)."

Conclusions: did you have any feedback from the students? (optional) Has it helped boosting enthusiasm and performance?

(Line 252) Added a link to a report with students' feedback from a workshop with undergraduates at the beginning of the development of the Unigems plasmids.

Section 8.3: what was the volume of the liquid cultures?

(Line 281) It now reads: "Volumes of cultures were 1ml or 5ml, depending on context (smaller for plating transformed cells, sequencing and fluorescence analysis, larger for plasmid isolation)."

Line 276: rpm not RPM, be consistent

(Line 281) Corrected (also in line 314).

VERSION 1

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000596.v1.5

Georgios Efthimiou; University of Hull, Biomedical Science, Hardy Building, Cottingham Road, UNITED KINGDOM, Hull

Date report received: 25 May 2023
Recommendation: Minor Amendment

Comments: The work presented is clear and the arguments well formed. This study would be a valuable contribution to the existing literature. This is a study that would be of interest to the field and community. The reviewers have highlighted minor concerns with the work presented. Please ensure that you address their comments.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000596.v1.3

Gilbert Miller; University of Cape Town, SOUTH AFRICA

Date report received: 25 May 2023
Recommendation: Minor Amendment

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No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000596.v1.4

Tadhg O Croinin; University College Dublin, School of Biomolecular and Biomedical Science, Room C140, Health Sciences Building, Belfield, Dublin, IRELAND
https://orcid.org/0000-0003-1966-204X

Date report received: 07 April 2023
Recommendation: Minor Amendment

Comments: This paper describes the development of a set of plasmids that can be used to teach synthetic biology and gene regulation using fluorescent and olfactory reporters. The strengths of the system are the easy ability to manipulate and alter the plasmids allowing for the creation of new constructs and the variety of established promoters and reporters being used. Overall the paper is well written and the methods and results well described and I have no doubt that the Unigems plasmid system will be an incredibly useful resource for educators and those involved in outreach alike. I have made a few comments below which I think would be useful to provide a little more context for those interested in using the system and a little more clarity to some sections but overall I think the paper gives a nice detailed overview of a very useful set of molecular tools for teaching and outreach purposes. 1) Line 51 - I couldn't get the "addgene" link to work. Could the authors check this is active (and apologies if this is a limitation on my part). 2) Line 70 - Should this be "which provide" rather than "which provides"? 3) Line 84 - Should the plasmid p007KanGFP not be p007AmpGFP? 4) Line 137 - Should this be "specific to this gene" rather than "specific to these genes"? 5) Line 160 - Figure 3 - The lack of a significant difference between the strong and weak promoter using RFP is mentioned in the discussion to be due to the readings being taken at a non-optimal wavelength. I think the authors need to clarify this in the results section and mention this limitation for the data shown at the time the data is shown. 6) Line 171 - Figure 4 - This figure shows inducibility of the system using arabinose and IPTG. The arabinose was tested from 0-5% and the IPTG from 0-5mM. The authors comment "pBAD exhibiting much more prominent inducibility than T5-pLacO" is a little vague. It might be useful to point out that the T5-pLacO produces a much more dose dependent and gradual response than the pBAD in this system. I would have expected the pBAD to be better for dose dependent induction but either way this is a useful difference between the two systems and would be an important consideration for those choosing which inducible system to use for a specific teaching or outreach application. I think a few more lines on the difference in inducibility between the two systems would be helpful. 7) Line 181 - the phrase "is clearly visible and in the presence of both inducers" is a little confusing. I would suggest "is clearly inducible in the presence of both inducers" or another alternative version. 8) Line 188 - The section on the olfactory construct - The inclusion of an olfactory construct is a nice addition to this toolkit. Its clear this is expressed at stationary

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If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

SciScore report

https://doi.org/10.1099/acmi.0.000596.v1.1

iThenticate report

https://doi.org/10.1099/acmi.0.000596.v1.2