Overcoming Adversities Using DIYBIO and Open Science: Open Lab Equipment and Expression Vector System Validation in Chlamydomonas reinhardtii

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Tags: iGEM report, DIYBio, Spider silk, Chlamydomonas reinhardtii, protein secretion

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Abstract

In this project we propose the use of Do-it-Yourself Biology (DIYBio) and Open Science concepts and tools to characterize a synthetic plasmid construct for protein expression and secretion in Chlamydomonas reinhardtii, exploring the possibilities of this approach in providing solutions to the problems faced by scientists in emerging countries. The project as a whole was inserted in the context of the 2016 International Genetically Engineered Machine (iGEM) competition and is aligned with its effort to promote science democratization.

Financial Disclosure

The project here described was funded by Merck KGaA, Thermo Fisher Scientific, the University of São Paulo, Hardware Livre USP, Integrated DNA Technologies, Lotan, MathWorks, SnapGene, New England Biolabs and through our online public crowdfunding campaign. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests

The authors declare that no competing interests exist.

Ethics Statement

N/A

Data Availability

All data related to the work described in this document is fully available without restrictions at http://2016.igem.org/Team:USP_UNIFESP-Brazil (http://2016.igem.org/Team:USP_UNIFESP-Brazil)

Introduction

It is no well-kept secret that the current way academic science is done across the globe is, to say the least, in deep trouble. Classical peer-review processes seem to be less and less effective, paywalls keep crucial knowledge away from those who need it, and funding problems hamper scientific progress and push towards articles that merely aim to be published, not necessarily contributing to the field. However, science is far from being helplessly doomed. With scientific progress coming faster and faster, new alternatives and solutions to academia have been sprouting everywhere. Open Science, DIYBio, Synthetic Biology, Biohacking and many other independent movements have been taking a different approach at these problems, with the effectiveness of the solutions yet to be determined for such recent changes.

The International Genetically Engineered Machine competition (iGEM) is an annual synthetic biology competition that has been bringing together undergrad and postgrad students from all over the world since its third edition in 2005. It notably stands up for open, accessible and transparent science, which quickly drew our team, USP-UNIFESP Brazil, towards this year’s competition. Hailing from an emerging country and a region ravaged by social inequality, these ideals resonate well with the problems faced in such places by most scientists and provide an opportunity for those discontent with this establishment to try and change it. iGEM is a competition marked by teams striving in the face of adversity and circumventing problems in an unforeseen way in order to accomplish their goals, so it’s only natural that teams from developing regions are a growing presence in every year’s edition.

Our project was conceived as a novel application of the modular characteristics of spider silk, in an attempt to make a biomaterial with immobilized proteins. We decided to use the microalgae Chlamydomonas reinhardtii as an expression vector, and so had to manage to not only express the spider silk proteins, a challenge met by other research groups in the area, but also create a DNA cassette for the expression and secretion of said proteins in a different model organism than usual. The bumpy road of taking part in iGEM 2016 was undoubtedly a huge scientific experience for all of our team members, as we are sure it was for all other teams. However, we think our greatest gain might have been precisely those ideals of open, accessible and transparent science.

Comments

Rozak: I've been working with a high school team in Frederick MD that is trying to synthesize spider silk in E. coli. They initially tried to have the gene synthesized as a gBlock but couldn't get it to work because of the highly repetitive nature of the protein. They've since settled on assembling the repeating subunits via restriction digest

and ligation. Is your team planning to continue its work in this area? If so, it would be great for us to share ideas/strategies!

fabionunesmello: Yes, restriction digest and ligation does seem to be the most used way to deal with those problems, but it has the downside of being so repetitive. We planned on using USER assembly to make this easier, but had trouble assembling the USER cassette so it's not included here - but our wiki has all the info we had, references, notebooks, design and mathematical modeling, check it out! We plan on working more with this topic, just not sure how, but it would definitely be great to share ideas!

teague: What was the rationale for using Chalmydomonas reinhardtii? What are the challenges and benefits associated with that decision?

fabionunesmello: Further explained below in the "Results and Discussion" section! In short, C. reinhardtii deals well with high CG content, which is usually a big problem when expressing spider silk proteins. It's growth is also cost effective, it has low DNA mutations and could possibly process the translated protein better than E. coli, which sometimes has issues with processing these proteins.

Materials and Methods

Basic molecular biology techniques

Agarose electrophoresis was performed with 100 mL of 1 g/L agarose and 1X SB buffer. Samples were composed of 4 µL of template DNA, 1 µL 10X Loading Buffer (New England Biolabs, Ipswich, MA) and 1 µL 5X Gel Red dye (New England Biolabs, Ipswich, MA). Samples were run at 80-150 V for 1-1h30. DNA digestions were performed with 10U of desired enzyme(s), 1X CutSmart Buffer (New England Biolabs, Ipswich, MA), 3.5 µg of template DNA and DEPC water to 50 µL. Digestions were performed at 37ºC for 1 hour. Electroporation transformations were performed with a ratio of 100 µL OD600 competent cells to 10-40 pg of template DNA. Electroporations were performed with 0.2 cm gap cuvettes (BIO-RAD, Hercules, CA) at 12.5 KV/cm, 200 Ω (resistor), and 25 µF. DNA extractions and purifications were performed following the protocol recommended for QIAGEN's QIAquick Gel Extraction Kit (cat. nos. 28704 and 28706). DNA ligations were performed with 2 µL 10X T4 DNA Ligase Buffer (New England Biolabs, Ipswich, MA), 0.020 pmol DNA vector, 0.060 pmol DNA insert and DEPC water to 20 µL. Reactions were performed at 16ºC overnight or at room temperature for 2 hours and inactivated at 65ºC for 10 min. Full protocols and lab notes can be found at http://2016.igem.org/Team:USP_UNIFESP-Brazil/Notebook (http://2016.igem.org/Team:USP_UNIFESP-Brazil/Notebook)

Comments

Rozak: Just a stylistic suggestion: You only need to list the location of the company the first time it's referenced. Afterwards, it's enough to simply reference the company by name. I do appreciate the fact that you are so diligent about providing the source of your reagents. It's very helpful!

fabionunesmello: Thank you! Will keep that in mind next time. :-)

teague: I'm a big fan of SB buffer -- it's cheap and works great. Can you reference a protocol?

fabionunesmello: SB buffer just made our lives so much easier in the lab, I'm a fan too. Protocol used (Brody J.R., 2004) added as reference in the reviewed submission.

Screening of best mCherry producing colonies

Colonies were grown on a 96-well plate with TAP media supplemented with 5-10 µg/mL Zeocin and later incubated in 200 µL TAP media per well. Experiments were run twice at 800 RPM, 25ºC and 60 µE/cm2 using different plate shakers, Agitador de MicroPlacas Analógico AM 2.4 AN (INBRAS, Jardinópolis, SP, Brazil) (mCherry, absorbance at 750 nm and chlorophyll measured once every 12 hours) and VWR INCUBATOR SHAKER-508 (Radnor, Pennsylvania, US) (mCherry measured 3 times every 12 hours, absorbance at 750 nm measured 4 times every 12 hours, chlorophyll measured once every 12 hours). Measurements were done with the following setup: mCherry fluorescence [Mode: Fluorescence Top Reading; Excitation Wavelength: 575 nm; Emission Wavelength: 608 nm; Excitation Bandwidth: 9 nm; Emission Bandwidth: 20 nm; Gain: 200 Manual; Number of Flashes: 10; Integration Time: 20 µs; Lag Time: 0 µs; Settle Time: 0 ms; Z-Position (Manual): 18141 µm], Chlamydomonas reinhardtii optical density [Mode: Absorbance; Multiple Reads per Well (Circle (filled)): 2 x 2 ;Multiple Reads per Well (Border): 750 µm; Wavelength: 750 nm; Bandwidth: 9 nm; Number of Flashes: 25; Settle Time: 0], chlorophyll fluorescence [Mode: Fluorescence Top Reading; Excitation Wavelength: 440 nm; Emission Wavelength: 680 nm; Excitation Bandwidth: 9 nm; Emission Bandwidth: 20 nm ;Gain: 100 nm; Number of Flashes: 10; Integration Time: 20 µs; Lag Time: 0 µs; Settle Time: 0 ms; Z-Position (Manual): 18141 µm].

Comments

teague: What is 60 uE/cm2? That's not a unit I'm familiar with.

fabionunesmello: Slight typo, sorry! Corrected in the reviewed submission to uE/m^2s, E being Einsteins (mols of photons). It's non-SI, but it tends to give more consistent results when dealing with plants/algae than working with lux or lumen.

mCherry purification

The Fast Protein Liquid Chromatography (FPLC) for mCherry purification was performed with Resource Q (6 mL) columns (GE Healthcare, Little Chalfont, UK), sodium phosphate 50 mM (pH7.5) as buffer A, sodium phosphate 50 mM (pH7.5) + 1M NaCl as buffer B, equilibration of 2 column volumes (CV), injection of 0.5 mL 40X Concentrate supernatant sample, gradient length of 20 CV, flow rate of 5 mL/min and fractionation of 5 mL to unbound and 3 mL to the rest of the method. Another more refined experiment was performed with similar conditions, three steps for gradient length (step 1: 3 CV, step 2: 2 CV, step 3: 5 CV), flow rate of 3 mL/min (for all steps) and fractionation of 5 mL to unbound and 1 mL on step 1, 3 mL on step 2 and 5 mL on step 3. Measurements were taken in an Infinite 200 PRO plate reader (TECAN, Männedorf, Switzerland) in the above mentioned conditions.

Comments

teague: what *kind* of column is a Resource Q? C18? Amino? something else?

fabionunesmello: It's a polyetheretherketone (PEEK) column.

mCherry spectrum analysis

mCherry fluorescence spectrum was analyzed with the above mentioned Infinite 200 PRO plate reader with the following setup: [Mode: Fluorescence Top Reading; Excitation Wavelength Start: 300 nm; Excitation Wavelength End: 600 nm; Excitation Wavelength Step Size: 1 nm; Excitation Scan Number: 301; Emission Wavelength: 640 nm; Bandwidth (Em): 280...850: 20 nm; Bandwidth (Ex) (Range 1): 230...315: 5 nm; Bandwidth (Ex) (Range 2): 316...850: 10 nm; Gain: 200 Manual; Number of Flashes: 10; Integration Time: 20 µs; Lag Time: 0 µs; Settle Time: 0 ms; Z-Position (Manual): 18141 µm]

Secretion assay

mCherry fluorescence in the supernatant was assessed using a stack of 6 Rosco E-Colour #135 light filters and a 532 nm common laser pointer. mCherry presence was also observed with a Confocal Zeiss LSM 780-NLO at 543 nm excitation and spectral detectors set for 610-650 nm range for mCherry, and 405 nm excitation and 680 nm range for chlorophyll.

Microcentrifuge assembly

The microcentrifuge used throughout the project was built with common electronical pieces, an Arduino UNO, 3D-printed pieces and laser-cut MDF pieces. Full instructions can be found here: http://2016.igem.org/Team:USP_UNIFESP-Brazil/Hardware (http://2016.igem.org/Team:USP_UNIFESP-Brazil/Hardware)

Results and Discussion

Our team originated from SynBio Brasil, a group of current and former students focused on spreading synthetic biology and open science, and making projects such as iGEM participations a reality. Many members of the group, some which had participated in the competition before, went on to join forces with enthusiasts from different institutes, campi and universities to comprise a team of 28 undergrad and postgrad students. The team size, large by iGEM standards, stemmed from the ideals held by the SynBio group of democratic and open science, and so we were joined by anyone and everyone that wanted to help, one way or another. We felt that it wasn't right for us to turn our backs to someone who asked to join based on education level, field of study, availability for the project, or really any other reason. If the entire point of the project was to go against the way traditional science is done - elitist, held behind closed doors and with very rigid structure - repeating the same mistakes would lead us nowhere.

Comments

Laura_Dress: That's amazing! I think it would be helpful for other's to learn if you add a sentence or two about the value unique/non-traditional members brought to the team, and maybe a challenge. Maybe something like... For example, we had an electrician join our team and he had a really great concept of circuits and engineering that added value to the team, and we really enjoyed teaching him the A,B,Cs of synbio....or something like that. I think it might be useful for readers to hear about a real details about interesting insights the non-traditional folks brought to the team. Congrats on adding this to your project and manuscript!
Rozak: I would also like to know what strategies you used to organize your group. I really like the idea of including whoever wants to participate! However, I know from experience that working with a large group is often difficult... especially if you're in a lab. I'd love to hear more about your group dynamics.
teague: +1 to both @LauraDress (/user/LauraDress) and @Rozak (/user/Rozak)'s comments.
fabionunesmello: Thank you all for the suggestions! A paragraph following this one and talking about both topics has been added in the reviewed submission.

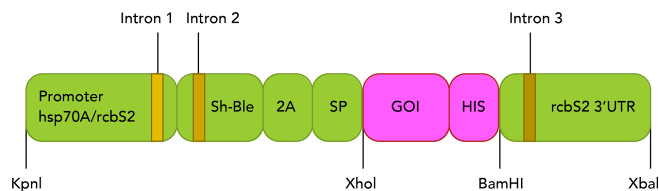
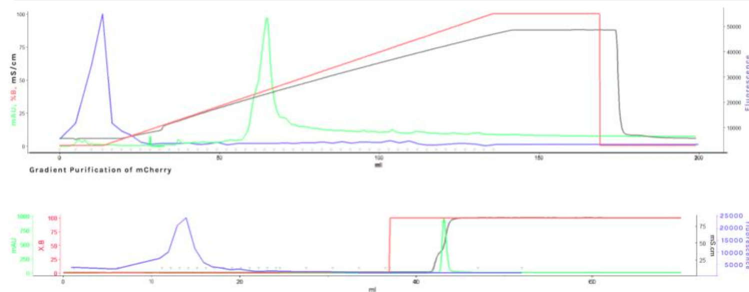


Figure 1. Cassette construction to be inserted in C. reinhardtii nuclear genome. Promoter hsp70A/rbcs2: fusion of the promoters hsp70A and rbcs2. Sh-Ble: resistance gene to Zeomycin. 2A: self-cleavage peptide from Foot and Mouth Disease Virus (FMDV). SP: secretion signal peptide of the gene Ars1. GOI: gene of interest. His: histidine tag. RbcS2 3' UTR: terminal sequence of the gene RbcS2. Introns were added as the figure shows.

Synthetic production of spider silk proteins with molecular biology techniques has been attempted by other groups (including previous iGEM teams), but with limited success. The main difference we thought our project to have was the use of Chlamydomonas reinhardtii as our model organism. Its use for large scale production of biomaterials has been found to be very cost effective, grows rapidly and with stable lineages, with low risk of contamination by DNA-altering factors. But most importantly, the naturally high GC content of its genes indicated C. reinhardtii might be better equipped to deal with the high GC content and very repetitive sequences of our target protein's genes. We then developed a cassette construct (figure 1) to be used for the expression of our desired protein, with a signal peptide for secretion, and proceeded to test it with the reporter protein mCherry, which we codon-optimized for C. reinhardtii, expanding the usage possibilities of this red fluorescent protein from the iGEM registry. The fluorescent protein production was confirmed with fluorescence confocal microscopy, and a Fast Protein Liquid Chromatography (FPLC) (figure 2) was performed in order to purify a sample of mCherry, which then had its excitation and emission spectrums measured as shown (figure 3).

Comments

VeroniqueKiermer: As part of this work, you have created amazing resources for the community. Congratulations! you should make sure that you list the new materials/reagents created and how they can be obtained or rebuilt. I think it is well worth a specific section in your Materials & Methods, as others will greatly appreciate the resources.
fabionunesmello: Well, we sure hope it can help others as much as it helped us! We've expanded the DIY section of our Materials & Methods, with all the step-by-step instructions for everything fully available at our team wiki.
teague: Nicely done - the expression cassette is quite a resource. I would really like to see a deeper discussion of its design -- for example, why the introns? In the context of open science, your *process* may be even more important than your *result* -- it lets others apply the same process to their own problems.
fabionunesmello: Thank you for your question! A discussion on its design has been added in the reviewed submission, as well as all the references used for the different functional parts of the cassette.
teague: What data did your confocal experiment give you? I don't see any micrographs.)-: If it didn't work, why might that be?

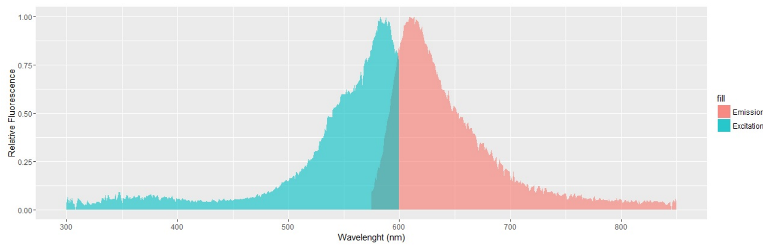


Comments

teague: This figure is a little small -- I can't read the axis or legend labels.

fabionunesmello: The bottom axis was a little low resolution, but we fixed it for the reviewed submission. Other than that, for some reason I don't quite understand the page formatting hasn't been kind to our graphs, but they do show up in full resolution when opened in a different tab! (Same for the bottom one, too)

Figure 2. Above: Chromatogram of gradient mCherry purification. Below: Chromatogram of step gradient mCherry purification. Green line (-) is the UV sensor reading. Red line (-) is buffer B percentage in the mixture. Black line (-) is the conductivity measurement. Blue line (-) is the fluorescence measurement of fractionated samples.



Comments

teague: Same here. +1 for using ggplot, though. (-:

Figure 3: Excitation/Emission spectrum of mCherry produced and purified from the supernatant of a transformed Chlamydomonas culture.

A major part of our team's project this year revolved around overcoming basic adversities, mostly related to equipment, reactants and research budget. To put it bluntly, it basically revolved around not having a lab. Our team was very generously taken in by Professor João C. M. de Carvalho (Faculty of Pharmaceutical Sciences, University of São Paulo) in his lab, where some of us had undergrad and postgrad internships. The problem was, Professor Carvalho's lab is focused on microalgae cultivation and optimization of biomass production processes, and lacked equipment and structure for molecular biology experiments. In fact, pretty much all of the resources we did have at our lab were either previously introduced by team members in their own academia-related projects, or equipment we were given access to in another lab, based off of the immense generosity of others. We had limited access to microcentrifuges, thermocyclers, transilluminators, electrophoresis cells, electroporation systems, and many other lab materials, fundamental for molecular biology and synthetic biology experiments. The project - which was initially focused solely on the expression of spider silk-immobilized endolysins - quickly became about making solutions for our problems and holding up the idea that, despite the lack of resources, we could still do science. By the end, our project and Jamboree presentation were very heavily focused on our open hardware projects and DIY alternatives for our needed equipment, reactants and protocols (figure 4). Those jerry-rigged solutions, initially meant to be just a "quick-fix" to get basic experiments going, quickly became more and more important each step of the way. So much so, that team members themselves started dedicating more and more time to finding, testing and refining these alternatives, as well as spreading this new-found knowledge outside of our own lab, in hopes to help someone else in the same situation.

Figure 4. Left: Transilluminator "Tabajara" as an UV transilluminator system. It can also be used as a photo documentation system (not shown). Right: Assembled DIY centrifuge "Seletora", with 8 microtube rotor and silicon anti-vibration base.

Comments

Rozak: The schools I work with also struggle to get the equipment needed for their experiments. I'm interested in learning more about the equipment you've shown here. Is it commercially available? If so, from where? And finally, how did it work for you? Would you recommend it to other groups?

fabionunesmello: We've added a paragraph centered on our experience with it in our reviewed submission! As an open science project, we did not make it commercially available but instead publicly shared the assembly instructions (on our team wiki). Honestly, I cannot recommend it enough. Our centrifuge, for example, worked on par with expensive company-bought ones if not better, and the freedom we had to alter, improve and fix it when needed were amazing. And the cost was ridiculously low, too, as we basically used an Arduino, basic electric components (switches, buttons, copper wires and such), 3D printings and a laser-cut exterior - both of which you can have access to in a community hackerspace or FabLab, no need to own the machine.

A very relevant example of such new-found solutions was the DIY method developed to detect the secretion of mCherry by our C. reinhardtii cassette. Based off of the excitation/emission spectra measured, we used a stack of 6 photografic red filters and a common green laser pointer to screen for fluorescence in the supernatant, after centrifugation of the samples. The stacked filtered paper's absorption spectrum was measured (figure 5) to make sure it blocked the wavelength of the laser, but not the measured wavelength of the mCherry emission. So, by shining the laser through the sample and placing the filter in front, we would be able to isolate the desired fluorescence, as shown (figure 6). This allowed us to confirm that the signal peptide of our construct worked as expected, while being faster and cheaper than traditional methods and without sacrificing reliability. In interest of a double confirmation, confocal fluorescence microscopy was also used to detect mCherry fluorescence (figure 7).

Figure 5. Measured absorption for the Rosco E-Colour #135, superimposed with mCherry excitation and emission spectrum and laser spectrum.

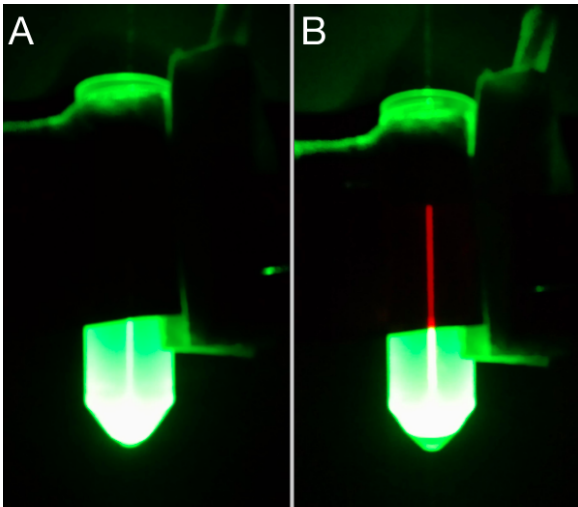


Figure 6. Visual results of the DIY method for detecting secreted mCherry. Left: Supernatant of a wild type culture sample. Right: Supernatant of a culture sample with plasmid construct containing the mCherry-coding sequence.

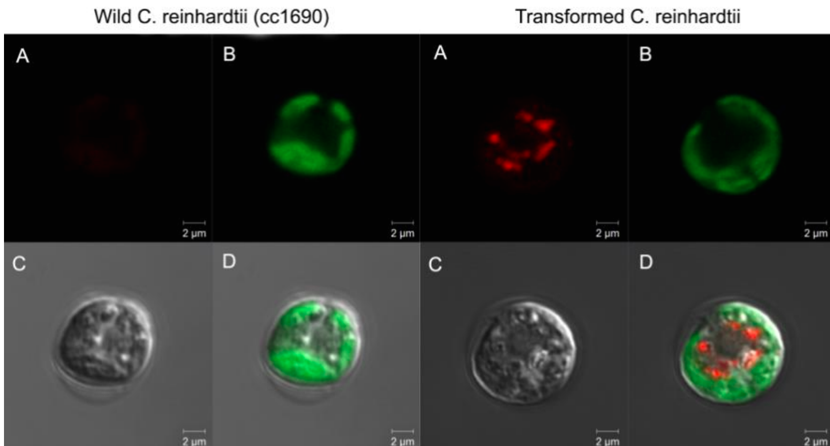


Figure 7. Left: Wild-type C. reinhardtii, viewed with confocal microscopy. Right: C. reinhardtii transformed with the mCherry producing plasmid construct, viewed with confocal microscopy.

Comments

teague: Ah. There are the micrographs. (-:
fabionunesmello: Our pride and joy, couldn't leave them out :)

Conclusion

The synthetic production of spider silk proteins as a biomaterial for immobilized enzymes remains, unfortunately, unachieved by the end of our 2016 project. As shown, the Chlamydomonas reinhardtii model holds potential for a future project along this line, now helped by the construction and characterization of our DNA cassette for protein expression and secretion.

Following iGEM's lead, all of our completed DIY-projects - both original designs and projects adapted from other sources and rightfully credited - have been fully uploaded in our iGEM wiki with accessible instructions for anyone who may need it in the future. Furthermore, we took part in the Brazilian FabLearn Conference in São Paulo, as well as, presented our hardware projects at FAZ, a maker festival organized by Red Bull, and even held our own public hackathon at the University of São Paulo, with the aim to spread the word about open hardware and collaborative science. After all, we would do nothing to solve the problems we encountered if we kept the solution locked away. With the rise of the community labs such as GenSpace, and movements like biohacking or DIYBio, this kind of open access low-cost equipment may prove itself to be an affordable and reliable option, with an added benefit: due to its decentralized and collaborative nature, these equipment can be a massive instrument to tear down the divisions between academia and non-academia, bringing scientific knowledge and tools to the public.

After our iGEM Jamboree experience, and meeting the other two Brazilian teams which participated this year, an idea to make our own regional Jamboree in 2017 sprouted among us. Brazil, as many other countries in Latin America, is still stuck with the same old scientific scenario, which is not only exclusionary but also limiting of itself. Scientific progress is done in tiny steps in our country, and we believe that supporting projects such as iGEM participations is crucial to change that. We realized that many teams in Brazil are not formed due to the present adversities, despite having interested students and researchers, and so we would all like to organize an event to help foster new ideas for new teams, and help each other to keep going. As of now, our own regional Jamboree is still being planned, but by bringing Brazilian iGEM team members and enthusiasts together, it is bound to lead to great things in the future.

Comments

teague: Love it! You may wish to talk to other teams that have organized similar events -- for example, the Boston University team has run regional get-togethers for the last few years and is putting on a mini-Jamboree next month.

fabionunesmello: We'll definitely want to hear from them, and other teams organizing such events worldwide. Thank you!

Acknowledgments

We would like to thank the Professors, friends, companies and institutes that helped making this project possible. We are thankful to Lukasz Kowalik (Merck KGaA), Marlene Lunardi (Thermo Fisher Scientific), Otto Heringer and Danilo Zampronio (Lotan), Research Ideas and Outcomes (Journal RIO), São Paulo Research Foundation (FAPESP), Núcleo de Empreendedorismo da USP (NEU), Professors Ricardo Pinheiro de Souza Oliveira, Ph.D., Gisele Monteiro, Ph.D., Mario Hiroyuki Hirata, Ph.D. and Professor Adalberto Pessoa Junior, Ph.D. (University of São Paulo/Pharmacy School), Professors Luiziana Ferreira da Silva, Ph.D., Professor José Gregório Cabrera Gomez, Ph.D., Professor Márcio Bertacine Dias, Ph.D. and Mario Costa Cruz, M.Sc. (University of São Paulo/Biomedical Sciences Institute), Professors Alexandre Bruni-Cardoso, Ph.D. and William Cenens, Ph.D. (University of São Paulo/Institute of Chemistry), Professor Eduardo de Senzi Zancul, Ph.D. (University of São Paulo/Polytechnic School), Professor Stephen Patrick Mayfield, Ph.D. (University of California, San Diego/Biological Sciences Division), Professors Edislane B. de Souza, Ph.D., Dario A. Palmieri, Ph.D., Pedro O. Neto, Ph.D. (São Paulo State University, Assis/Dep. of Biotechnology), Professor Ana Maria Pires, Ph.D. and Airton Germano Bispo Júnior, M.Sc. (São Paulo State University, Presidente Prudente/Dep. of Chemistry and Biochemistry), Ph.D. and Elibio Leopoldo Rech Filho, Ph.D. (Brazilian Agricultural Research Corporation (EMBRAPA - Genetic Resources & Biotechnology)) and Professor Carlos Gustavo Nunes da Silva, Ph.D. (Federal University of Amazonia UFAM).

Comments

Rozak: This is amazing that you were able to establish such a broad network of collaborators. It's nice of you to acknowledge all the support you received for this effort.

fabionunesmello: The support given by third-parties to this project was massive, and it's no exaggeration to say we couldn't even have done half of it without them.

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Comments

Guilherme: I would like to congratulate the team by the efforts towards a more open and accessible way to do science. It is not easy, but greatly regarding, to overcome funding and equipment access problems, creating a pathway to other teams in similar conditions. The DIY method to detect mCherry is a mix of deep scientific knowledge and creativity. However, in my opinion, a deeper analysis of both potential and limitations of the developed protocol would be interesting. I think it would be good to explain why mCherry was chosen instead of other fluorescent reporter. Also, you could propose and discuss what kind of adaptations in the protocol would be necessary when using other fluorescent molecules. Finally, I would like to highlight the courage to utilise an unusual organism in the project and how this can inspire and give important clues to future iGEM teams.

fabionunesmello: Thank you very much! We have included in our reviewed submission a paragraph discussing the DIY detection technique, and really hope it can be of use to other researchers. We - unfortunately - didn't have the time to fully explore it and play with it, but there's definitely room for improvements and adaptations.

VeroniqueKiermer: Congratulations on upholding the principles of openness in your science and in the way you approached the problem. I agree with the previous commentator that further discussion of potential and limitations of the protocol will be very helpful. They can help others to develop similar ideas in other systems and

may lead to collaboration.

fabionunesmello: Thank you for the input! As mentioned in the previous comment, we added a deeper analysis to the reviewed submission.

PLOSFeedback: Hi @fabionunesmello (/user/fabionunesmello) Thanks for your submission to the PLOS iGEM Project. Our editorial team have reviewed your article and have the following feedback: This piece is a good source of motivation for other young scientists in poor resourced areas facing hurdles to scientific access. The authors are keen on open science and cooperation. As this is not a research article per se, but an account of the authors' journey through iGEM 2016, this would probably be a better fit as a blog. It will make an excellent contribution to the PLOS iGEM 2016 Collection and is a great example for other researchers. If we can provide any more guidance or support please don't hesitate to contact us - collections@plos.org

teague: I enjoyed your account of your iGEM experience and the resources you developed to help future teams "stand on your shoulders," as it were. I'd love to see the C. reinhardtii silk project work!

fabionunesmello: Thank you! And we would most definitely love to see it work too (and plan on making it so, if all goes well)