Open Educational Resources for distributed hands-on teaching in molecular biology

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Abstract

Open Educational Resources (OER), freely accessible learning, teaching and research materials, have been proposed as key enabling tools to achieve inclusive knowledge societies and equitable access to education. Here, we describe novel OER consisting of low cost and locally produced public domain biological reagents, open source hardware and free software collaborative notebooks to teach LAMP DNA amplification, RT-PCR RNA detection, enzyme kinetics and fluorescence imaging. These resources have been distributed nationwide to students' homes as a lab-in-a-box, i.e. remote teaching during the pandemic lockdowns, as well as in the form of personalized learning environments during in-person teaching after the opening of teaching laboratories. All the protocols and design files are available under open source licenses.

Introduction

The COVID-19 pandemic forced many universities around the world to close their facilities and abruptly adopt remote teaching, leading to an urgent need for online teaching resources. In response to this challenge, educators across different fields developed novel teaching resources. For instance, cloud-based tutorials [1] and virtual interactive classroom [2] have been developed for bioinformatics courses in life sciences. However, practical courses that primarily rely on laboratory experimentation faced a more complex scenario as hands-on experiences are difficult to replace by online tools [3]. Experimental practices based on instrument operation, pipetting and experimental design are a vital part of life science education for both theory reinforcement and practical skills acquisition. Replicating these practical experiences at home requires specialized equipment (e.g. microscopes and thermocyclers) and expensive reagents (e.g. enzymes and buffers) that are challenging to deploy [4].

Despite the lack of preparedness for such an unprecedented challenge, pre-pandemic developments such as online programming notebooks (e.g. [5]), low-cost biological resources for advanced practicals in molecular biology [6],[7], [8,9][10][11] [12], and a wide range of open source hardware devices for scientific research [13][13,14][15][16][17] had paved the way for the implementation of remote teaching in molecular biology. Efforts to democratize biotechnology, led mainly by biomakers, open community labs, do-it-yourself biology practitioners and advocates for open biotechnology, have provided resources and information for hands-on practical workshops, and enabled low-cost experimental research outside university laboratories [18][19][20][15][21][22][23]. These initiatives provide not only valuable guidelines for frugal biotechnology development but also stringent biosafety and biosecurity regulations for their deployment [24].

Here, we describe the development and integration of new resources for remote teaching of hands-on practicals in molecular biology. By remote laboratories we do not mean in the form of university infrastructure that is operated remotely (e.g. [25]), but in the deployment of instrumentation and reagents to students' homes. We implemented a lab-in-a-box (Figure S1) that included all the necessary reagents and materials for students to practice molecular biology techniques at home such as RT-PCR, DNA amplification, fluorescence and enzyme kinetics. These resources consist of low cost and locally produced public domain biological reagents, open source hardware and free software collaborative notebooks. These

resources have also allowed a more personalized learning approach during in-person teaching after the aperture of teaching laboratories. All the protocols and design files are available under open source licenses that explicitly grant permission for study, replication and commercialization of the resources.

Results

Lab-in-a-box: a set of laboratory equipment and reagents for remote practicals

The first step was to create a box that could contain and organize the lab equipment and reagents for practicals, as well as withstand transportation from house to house. In collaboration with the School of Design at PUC, we explored cardboard versions of the box considering recycling as a priority; however, the strict sterilization procedures forced us to employ plastic boxes that are more appropriate for sanitization based on spraying and wiping. Thus, we developed a lab-in-a-box based on commercial plastic storage boxes. The lab-in-a-box also contained three smaller plastic boxes inside with all the necessary reagents for each student in the group, along with a set of commonly-used tools (e.g. hardware, sanitizer) (Figure S1). The smaller boxes were chosen with the intention of being able to also act as water baths. Since the main goal for the lab-in-a-box was to enable lab experiences with minimum or no need of household goods, a metallic tray was provided to be used as a sanitizable bench and spillover container. A 3D printed light holder was attached inside the box to attach a white light LED tube and facilitate proper illumination. We created a total of fifteen boxes and split the class into groups of three students (one lab-in-a-box per group). Thus, the lab-in-a-box provided a set of tools for common use for the group, while the smaller boxes provided reagents for each student. The rationale behind this organization was to avoid cross-contaminations among students and consider the three experiments separately as technical replicates in the practicals. The box was delivered from the university to the home of the first student in the group, and later delivered to the second and third student, before being shipped back to the university for thorough sanitization and refilling. Each student followed detailed video and written instructions for the disassembly, sanitization and assembly of the box before passing it to the next home [26] (S1 Appendix).
Since most of the students had no experience in molecular biology, ie. with no pipetting experience, students were instructed to follow a “micro-pipetting” task with food coloring substances (S2 Appendix).

RT-PCR for the detection of SARS-CoV-2:

The first practical was on the reverse transcription polymerase chain reaction (RT-PCR), a technique widely used for converting RNA into DNA and amplifying specific sequences, making it the gold standard for detecting RNA viruses in molecular diagnostics [27–29]. We focus on detecting the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) for two main reasons: first, the students’ motivation for learning about the virus causing the COVID19 pandemic; and second, the availability of low-cost and open-access RT-PCR reagents for SARS-CoV-2 detection, a result of ongoing local initiatives (e.g. [30–34]). The crucial role of SARS-CoV-2 RNA detection in COVID-19 diagnostics has underscored the relevance of this technique. However, its broader implementation in teaching labs, especially

in developing countries, has been limited by the high costs and scarce availability of necessary reagents and equipment.

We integrated two significant open-source innovations for a cost-effective solution: the PocketPCR and off-patent enzymes. Developed by GaudiLabs, the PocketPCR is a compact, open-source USB-powered thermocycler designed to make PCR technology more affordable and portable (Figure 1) (Figure S2). This open hardware device facilitates PCR reactions through precise, easily adjustable temperature cycles, and can be powered by a standard USB/USB-C charger, making it ideal for use outside traditional laboratory settings. Concurrently, better access to public domain DNA plasmids encoding for diagnostic enzymes has surged during the pandemic [30–34]. For instance, the FreeGenes project and ReClone network have engaged researchers around the globe who share DNA plasmids encoding these off-patent enzymes and protocols for their local production [35]. These collaborative efforts have enabled many laboratories to access molecular diagnostics at a reduced cost.

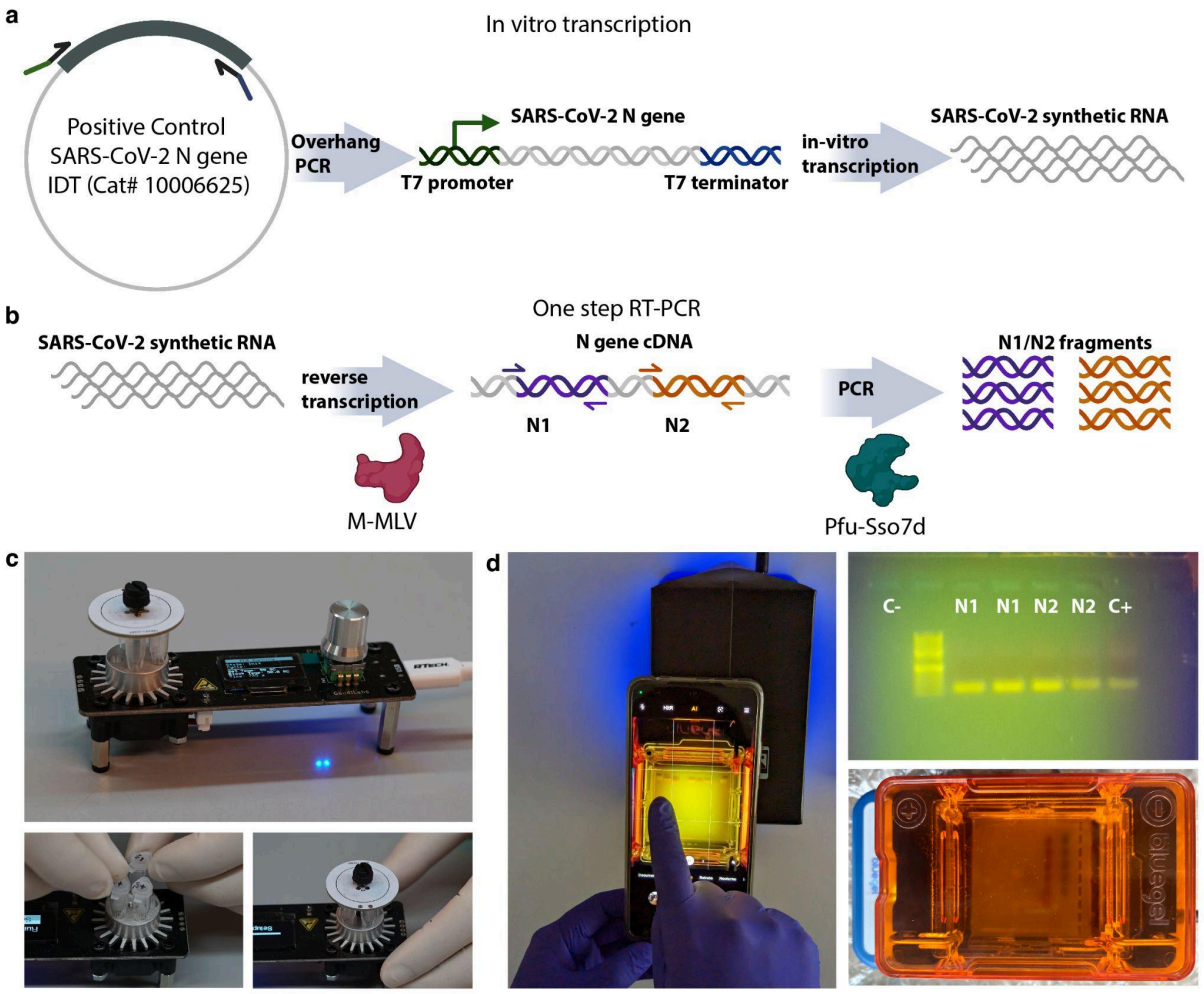

Figure 1: Schematic representation of RT-PCR practical. a) Positive control RNA produced by *in vitro* T7 RNAP transcription from a linear PCR product containing a T7 promoter and terminator. The PCR-amplified linear template was obtained from a plasmid

harboring the “Positive Control SARS-CoV-2 N gene” from IDT (Cat# 10006625). b) Schematic representation of RT-PCR reaction performed during the practical. c) PocketPCR device holding up to five tubes. d) BlueGel™ electrophoresis system showing RT-PCR practical results (top right).

To design an experiment that emulates a diagnostic procedure for SARS-CoV-2, we used synthetic RNA that corresponds to the full-length Nucleocapsid (N) gene of approximately 1260 bp of SARS-CoV-2. Additionally, we selected primers used in clinical diagnostics that target different regions of the SARS-CoV-2 N gene (N1 and N2) (Figure 1). The RT-PCR reaction was performed using a master mix containing recombinant M-MLV reverse transcriptase and Pfu-Sso7d polymerase, produced locally [30][31]. The protocols used for the local production of these enzymes are openly accessible at protocols.io [36][37]. In this practical, each student was provided with purified enzymes (M-MLV-RT and Pfu-Sso7d), reaction buffer, primers N1 and N2, a cDNA sample control of SARS-CoV-2 N gene, and two unknown RNA samples ("positive" and "negative" for the presence of the synthetic viral RNA fragment), transported and stored at room temperature. The positive RNA control was generated by *in vitro* transcription from a PCR-amplified linear DNA template obtained with custom-designed primers containing T7 promoter and terminator [31] (Figure 1). To avoid the use of personal refrigerators, the RNA samples, buffers and enzymes were kept at room temperature for the ten days of transit among the three students in each group. Remarkably, the reactions were not significantly affected by the room temperature conditions.

Given the lack of real-time reading capabilities, we avoided the quantitative aspects of the diagnostic process and focused on a qualitative assay, aiming solely for the detection of the presence or absence of synthetic viral RNA verified by the presence of an amplification band on a gel. Although open source and DIY electrophoresis chambers have been developed, we opted for the BlueGel® device that has been CE certified (Amplyus, MA, USA) (Figure 1). This device has an integrated blue LED array for visualization and it has been used for SARS-Cov-2 detection [38].

While this experimental setup does not exactly replicate the conditions encountered in clinical diagnostics, it offers a valuable approximation on the fundamental principles of RNA handling, amplification and analysis. Students were also trained in primer design and applied this knowledge to understand results of this practical.

LAMP reactions for DNA detection

The second practical was focused on DNA amplification via loop-mediated isothermal amplification (LAMP). Although (RT-)PCR remains the gold standard in molecular diagnostics, alternative nucleic acid amplification methods have proliferated in the past two decades to provide faster, simpler and more portable reactions [39]. Among these methods, LAMP (loop-mediated isothermal amplification) has gained popularity due to the use of a polymerase with strand-displacement activity that enables amplification at a constant temperature without the need for thermal cycling [40]. The isothermal nature of this reaction, along with a higher tolerance to reaction inhibitors, makes LAMP reactions attractive for point-of-care applications in resource-limited settings with no specialized instruments and unprocessed samples. These features have put LAMP at the forefront of molecular developments for nucleic acid amplification in the context of point-of-care diagnostics.

Due to the robustness of LAMP, it is feasible to train students on applying this technique to unprocessed samples available at home (e.g. [4][12]). We applied the *GMO Detective* project, an open citizen-science and educational endeavor towards an informed perception of GMOs [41], to detect transgenic DNA from vegetables and processed foods (e.g. veggie burger). *GMO Detective* consists of LAMP reactions that detect the presence of the CaMV 35S promoter, a commonly used sequence in the generation of transgenic plants. This kit applies the Quenching of Unincorporated Amplification Signal Reporters (QUASR) approach for end point determination [42], consisting of a fluorescently-labeled primer and a quencher-bearing probe. Positive LAMP QUASR reactions exhibit a fluorescent signal when the primer is incorporated into the amplified product and no signal when the probe is quenched (no amplification or no specific amplification). Sample preparation consists of grinding and heating a small vegetable or processed food piece (Figure 2a).

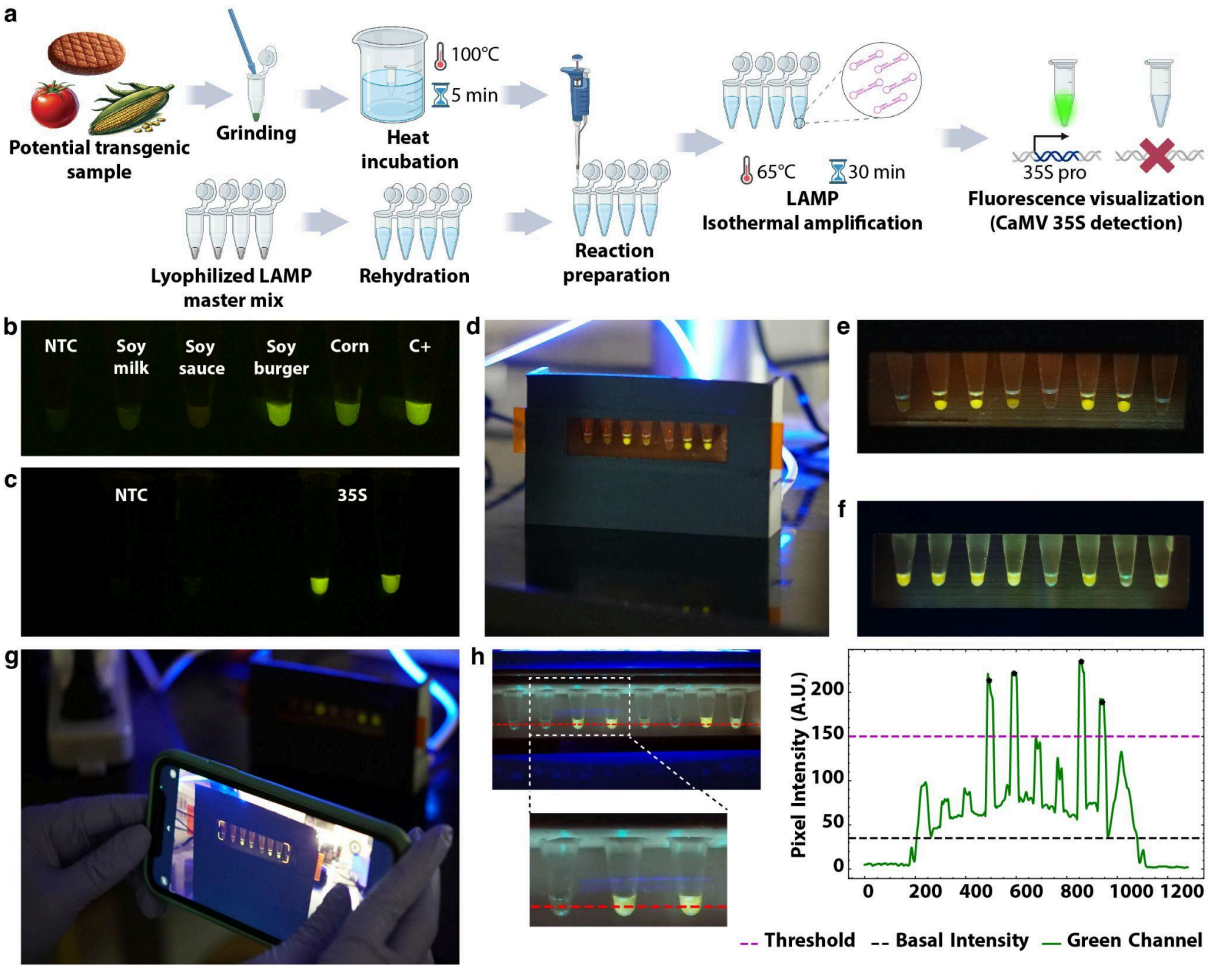

Figure 2: GMO Detective practical. a) Schematic representation of GMO Detective practical. b) Detection of 35S promoter in different soy-based foods. c) Reaction performance of freeze-dried reactions after two months storage at room temperature (two replicates are shown). d) Image of the 3D printed device being used by students. e) and f) Comparison of reaction with (e) and without (f) mineral oil. Mineral oil was added to avoid reaction evaporation and subsequent condensation on the tube lids. g) Example of student taking a picture of the GMO Detective device for image analysis of the results. h) Image processing performed via Google Colab Python code (Jupyter notebooks) applied to phone

pictures. Inset, zoomed in to show the red line at which intensities were quantified. All images are raw images without editing, except for panel b and c, for which linear exposure was enhanced across the whole image for better dynamic range.

The first *GMO Detective* reactions were prepared in France from commercial reagents, lyophilized and shipped to Chile. This logistics is expensive and prone to delays due to importation. Therefore, the local production of *GMO Detective* reactions appears as a more suitable solution. However, this production is limited by the high price of commercial *Bst* polymerase, imported in cold-chain from the northern hemisphere. Moreover, commercial enzymes are delivered in buffers containing glycerol, which affects lyophilization [43]. To overcome this barrier, we developed a local version of *GMO Detective* using homemade reagents and an off-patent *BstLF* polymerase from the openly distributed FreeGenes collection [44,45] (S3 Appendix). We tested these locally-made reactions with a number of soy-based foods (known to contain GMO soy) and positive controls before using them in the course (Figure 2b).

These locally produced LAMP reactions can be prepared with glycerol-free enzymes, lyophilized and stored at room temperature without any apparent loss in activity [46] [44] (Figure 2c). Freeze-drying the reactions without the magnesium nor reaction buffer (ie. keeping them in a liquid format and adding them at the moment of rehydration) permits at least two-month storage at room temperature or at 4 °C (Figure S3). Conversely, including them in the lyophilization step led to signal recovery after two months only if the lyophilized tubes are stored at 4 °C (Figure S3). We also produced 35S and COX1 positive control vectors and tested an alternative quencher that is cheaper to order in Chile (S3 Appendix). We also tested a Texas Red fluorophore (red fluorescence) for the COX1 primer set in order to have dual color QUASR LAMP when used with FAM-labeled 35S primer set (S3 Appendix).

The end-point results of these LAMP reactions can be monitored with a low cost device composed of laser-cut acrylic pieces, a custom-made PCB, low cost lee filters and 5 mm blue LEDs (emitting at 470 nm) [47]. To scale up the number of devices used in this practical, we created a 3D-printed version of the visualization device (Figure S4) (Figure 2d), further optimized by other groups (e.g. UROS project at Hackteria). We also replaced the original *GMO Detective* blue LEDs for 5 mm blue LEDs from SuperBright LEDs (Forward current: 30mA @ 3.3V, Peak forward current: 100mA, Max voltage forward: 3.8V), already used in the IORodeo blue transilluminator [48] and FluoPi fluorescence imaging device [49] for green fluorescence.

The first step in the practical is the preparation of test samples (Figure 2a). For this, 1mm^3 of food samples, e.g. soy burger, were cut, mixed with nuclease-free water and grinded in a 1.5 ml tube using a p1000 tip. These samples were incubated for 5 minutes in a recipient containing 95-100°C water (Figure S5). *GMO Detective* LAMP reactions were performed in standard PCR tubes. These tubes were incubated at 63°C for 35 min and cooled down for 15 min at room temperature before observing the results on the 3D printed device (Figure 2d-f). To avoid evaporation of the reaction and condensation of liquid in the lid, we used mineral oil (Figure 2e, f). Reactions were carried out with two technical replicates and

performed in triplicate, each on a different day and student home. For the 63-65°C incubation step, we used commercially available 12V electronic coffee mugs [50] and/or sous-vide heaters [51] to set a temperature-controlled water bath at home. Some students performed the experiment on water baths by mixing boiling and cold water while using a digital thermometer for temperature control (Figure S5). This water bath was prepared within the small plastic box provided in the lab-in-a-box (Figure S5). Special attention was paid to sealing of tubes to avoid water filtration from the bath. Reactions can also be performed in the PocketPCR (data not shown). Remarkably, no perceivable differences were encountered between these methods (data not shown). Each student was guided to assemble a LED visualization device, prepare the food sample, set up the reactions using different water-bath approaches, and report results.

To perform quantitative analysis and discrimination of positive from negative reactions across the three replicates, students were provided with custom-made Google Colaboratory Jupyter notebooks that allow users' writing, programming, analyzing and visualizing data via shareable documents, in cloud-based computing environments. The provided notebooks can be executed and edited upon need, allowing students to analyze images taken with their phones and prepare plots for presentations and reports [52] (Figure 2g, h).

Detecting GMO from food samples not only offers a safe procedure, as opposed to a more disease-targeted diagnostic approach, but also engages students in the application of molecular biology concepts to explore food composition and as a jumping board to learn and debate about GMOs.

Teaching the basics of fluorescence readings

The goal of this section was to teach fluorescence measurement from biological samples, particularly, how to appropriately select the best filter combination and illumination wavelengths to obtain high signal-to-background images of different fluorophores. This is a critical task encountered not only in microscopy but also in various other techniques (e.g. plate fluorometry, cell cytometry), hence the use of fluorescence microscopes is not strictly necessary. Therefore, we designed a simple open hardware device called RGBfluor that captures the essence of fluorescence measurement without the unnecessary complications of booking and using an expensive fluorescence microscope. RGBfluor is a 3D printed device that consists of a illumination module controlled by a smartphone through Wi-Fi, a sample holder, and a multiple filter slider (Figure 3). The illumination module consists of a custom-made printed circuit board (PCB) harboring ws2812 RGB LEDs and designed as a shield for the Lolin commercial board based on the ESP8266 microcontroller (S4 Appendix). This module consumes little power (~ 5V/300mA) and can be powered through a USB port on the board using a generic charger (such as those used for smartphones) or a computer USB. The RGB LEDs on the PCB are controlled via a simple web app developed in HTMLI/CCS and hosted on the server through which both the intensity and the color of the device's LEDs are controlled (Figure 3b, c). The web page developed is user-friendly and allows, by horizontal slider-type controls, to control the RGB components of the color of the LEDs, each one represented by an integer between 0 and 255; and the value of the intensity of the LEDs (Figure 3c). The firmware and software to control this PCB are described in supplementary information (S4 Appendix). The filter slider consists of three lee filters, two carefully selected for appropriate green (#105) and red (#113) fluorescence emission with

minimum background and one that was inappropriate for both wavelengths (#122) [53] (Figure 3d). The software as well as editable design files (openSCAD) required to reproduce the device are openly available in a gitlab repository [54] as well as a student guide (S5 Appendix).

RGBfluor is able to work with a wide range of green-to-red wavelength emitting fluorophores. An example of different fluorescent proteins and dyes under long-pass orange emission filter and blue illumination is shown as an example in (Figure 3e, f). In this practical, students learn how to distinguish between two groups of fluorophores, in the red and green spectra. For instance, students are challenged to take an image with minimum background for all the provided proteins, for which the concepts of long pass filtering and long Stokes shift are applied (S5 Appendix). Next, they are asked to selectively image the red fluorescent protein, for which a red filter and green light is preferred to cut off any blue or green light (Figure 3g).

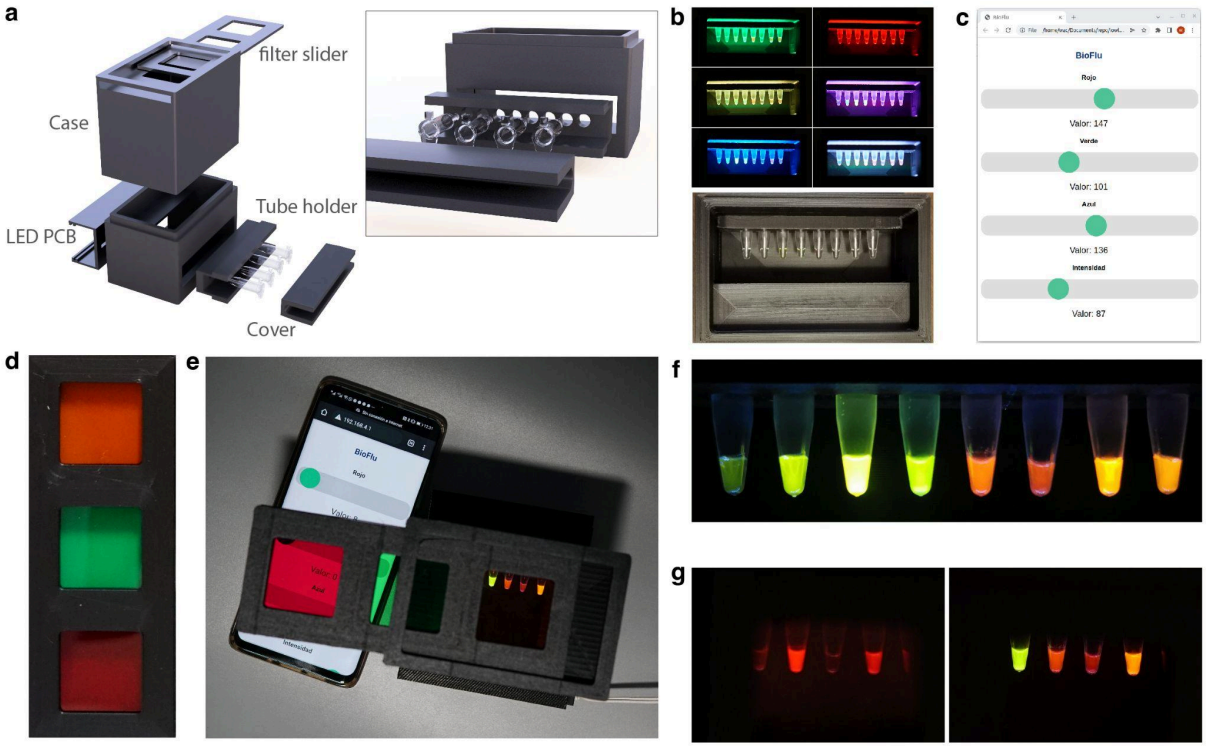

Figure 3: RGBfluor. a) Schematic representation of the 3D printed components of RGBfluor. b) Examples of illumination wavelengths (filter slider removed for clarity). c) User interface to control the LED-based RGB illumination from a mobile phone or external computer. d) 3D printed filter slider with low cost lee filters. e) Image of the RGBfluor device in use. f) Blue illumination and orange filter setup for different fluorophores, from left to right: 0.01μM FITC, 1μM FITC, 10μM FITC, sfGFP, mScarlet-I, mBeRFP, CyOFP and RRvT. g) Comparison of sfGFP, mScarlet-I, mBeRFP, and CyOFP fluorescence, imaged with green light and red filter (left) and blue light and orange filter (right). Raw images without editing.

Practical on enzyme kinetics

Our course also consists of a module dedicated to enzyme kinetics, a subject that conveys concepts from different fields (e.g. physical chemistry, biology and mathematics)[55].

Motivated by the positive response of students to the use of open hardware in personal laboratory experiences both at home and in the classroom (see questionnaire in S6 Appendix), we also developed a hands-on practical on enzyme kinetics using low cost and locally-produced resources. In order to study the Michaelis-Menten-like kinetics of an enzyme, we use the beta-galactosidase enzyme from *E. coli* and o-nitrophenyl-beta-D-galactopyranoside (ONPG) substrate in order to produce o-nitrophenol as a product, a chemical compound quantifiable by absorbance at 420 nm (Figure 4). The beta-galactosidase enzyme used in our practice was produced using a cell-free transcription-translation coupled (Tx-Tl) reaction [56]. This allows its production at low cost as a GMO-free substrate that can be lyophilized and transported at room temperature until its use by rehydration. Users start from the freeze-dried reactions, rehydrate and aliquot them in tubes containing different amounts of substrate ONPG. Incubate the reactions for 12 min at 37°C and quantify the yellow product using one of three methods: computer analysis of tube images, absorbance assay with an open source colorimeter or a standard spectrophotometer set at 420 nm (Figure 4) (S7 Appendix). For the image processing approach, we use custom-made Google Colaboratory Jupyter notebooks that filter the images and quantify intensities in digital transects across the tubes (FIgure 4b) [52]. Starting from two images: the first one consisting of a collection of serial dilutions of o-nitrophenol to build a calibration curve from the image intensities, and the second consisting of a collection of tubes containing the beta-galactosidase enzyme and OPNG at different concentrations. Both images were processed to remove the background and noise, facilitating the quantification of intensity. After selecting a transect for both images, it was possible to fit a linear regression to the calibration curve, and a non-linear Michaelis-Menten function to the saturation curve, obtaining parameters within the known range [57] (FIgure 4c, d).

For the method based on an open source colorimeter, we used the “reGOSH Colorimeter” [58] (Figure S6). This device integrates a TCS3200 sensor and an RGB LED or a near-UV LED (depending on the application) directly onto its printed circuit board (PCB). Components were chosen based on their local availability and low cost. The firmware and the software for Windows is from IORodeo [59], whereas the software for GNU/LINUX has been custom-made. Additionally, a GUI for Android has been developed by CoSensores group [60] for its operation from a mobile phone via Bluetooth. Commercially available LEDs generally do not come with their technical specifications. So, in order to verify the dominant wavelength of the LED, we developed a 3D printed adaptor to measure the emission spectrum of LEDs using a commercial spectrometer (Figure S7)[61]. After running the samples through the colorimeter and an expensive spectrophotometer we obtained near perfect correlation (Pearson`s r=0.997, Figure 4e).

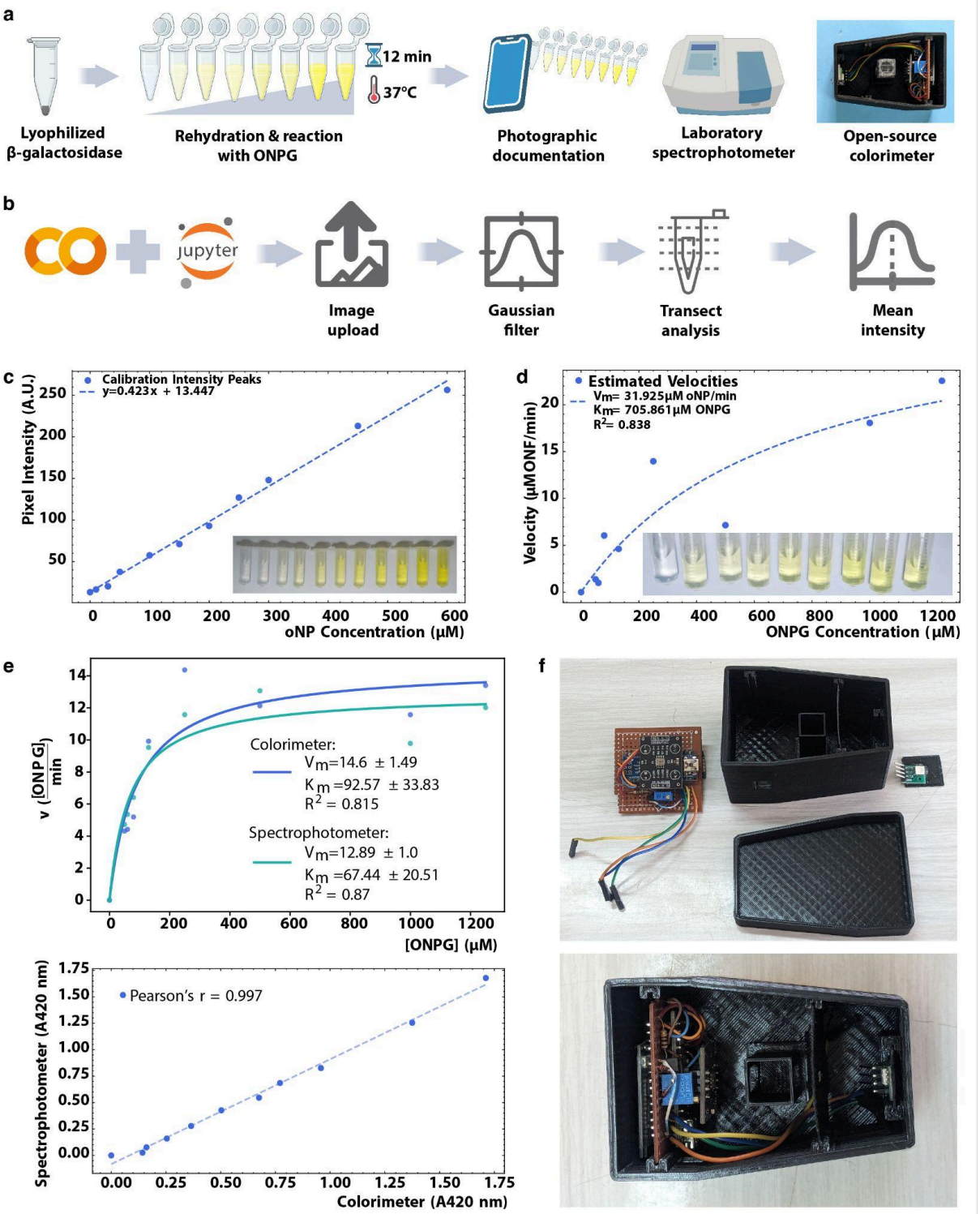

Figure 4: Enzyme kinetic assays. a) Schematic representation of the practical on enzyme kinetics, using a lyophilized and cell-free produced enzyme and two different measurement methods: phone image analysis and absorbance measured on an open source colorimeter, both compared to commercial spectrophotometer. b) Workflow of image processing with jupyter notebooks in the google collaborative platform. c) Calibration curve: ONP intensity peaks vs concentration. Inset, image of tubes containing a serial dilution of ONP used to build the calibration curve. d) Estimated velocities vs ONPG concentration Michaelis-Menten curve. Inset, image of tubes used for processing. e) Enzyme kinetic assay with an open

source colorimeter in comparison to a commercial spectrophotometer. Top, michaelis-menten model fitting. Bottom, correlation analysis between the two approaches. f) Open source colorimeter composed of an arduino nano, bluetooth module, LED, TCS3200, a LED constant current regulator and a 3D printed case.

By quantifying the amount of product per time as a function of the amount of initial substrate, users are able to extract the kinetic parameters of interest (in this case, the relative maximum velocity of the reaction Vmax and the Michaelis constant Km). These parameters are obtained by using custom-made Google Colaboratory Jupyter notebooks that allow a lively interaction with the data to apply enzyme kinetic concepts and visualize enzyme properties [52].

Discussion

The advance of new techniques in synthetic and molecular biology are pushing the boundaries not only for diagnostics but also for hands-on educational training (e.g. [62–64]). However, most advanced teaching resources are still challenging to implement in LMIC due to the need for importation of molecular biology reagents and the use of equipment that is either inexistent or inaccessible due to a high demand for research (e.g. fluorescence microscopes and thermocyclers). The progress of frugal biotechnology (e.g. [65][65,66]), DIYbio movements [18,19][67], standardized genetic resources [6], open scientific hardware (e.g. [14, 62]), and ip-free resources for the local production of reagents [10, 45] are facilitating a more distributed implementation of molecular biology practical training [74]. The local production of both hardware and reagents significantly lower the costs of teaching. For instance, the homemade production of cell free reactions, RT-PCR and LAMP has resulted in cost reductions of one to two orders of magnitude for our institution [68][32]. These reductions have enabled new hands-on modules in three other courses as well as training workshops for high school teachers. Further reductions can be achieved, for instance with the use of cellular reagents [10,69] to replace enzyme purification protocols.

Although remote teaching has existed for a long time [3], the lockdowns to mitigate SARS-CoV-2 spread led to an unprecedented growth of at-home teaching resources. These resources can now be applied to other necessities where remote access to teaching materials is still needed, e.g. disease- and/or climate-driven mobility impediments for students. Additionally, these resources for remote training also enabled the creation of minilabs for personal learning experiences once lockdowns were lifted, which has been welcomed by students as reflected by a questionnaire to 25 students (S6 Appendix). Operating open source hardware (OSH) leads to a more transparent and better understanding of the inner workings of machines and principles in place. We are working towards expanding the use of OSH in our courses, for instance to include devices for DNA assembly [49,70], fluorescence microscopy [49], optogenetics (e.g. [71–73] and integrated incubation & real time reading from reactions [74]. We also aim to implement the Airflow microreactor for isothermal DNA amplification [75] as well as ultra low cost devices for isothermal incubation (e.g. [76]).

Fostering better access to educational resources contributes directly to the Sustainable Development Goal 4 (SDG4), focused on ensuring inclusive and equitable quality education and learning opportunities for all [77]. In the same way community-driven efforts on open

source hardware have proven effective for emergency response during the COVID-19 pandemic [78], this approach can be employed to achieve more equitable, inclusive and effective access to educational resources. Although the adoption of open tools is rapidly growing in academia and is being proposed as an important component of the UNESCO Open Science Recommendation [79], its introduction in the curricula and implementation in university courses remains scarce. The use of open hardware, free software and free-to-use reagents aims to engage students into open source technology and discussions on how this approach can help achieve a paradigm shift towards a global collaborative science and truly open innovation ecosystem [80]. A future scenario in which researchers create, share and build their own instruments upon other designs requires nurturing future generations by addressing the topic of OSH in the curriculum [81].

The use of free software in the form of shareable notebooks introduces students not only to a growing trend in data analysis and programming [82] but also to the relevance of good practices in software documentation to enable reproducibility. Reproducibility represents one of the pillars of Open Science [79]; and open access computational methods such as jupyter notebooks that allows others to be able to recreate your results [83] become key for this task. Jupyter Notebooks foster reproducibility by integrating executable code, documentation text and analysis output in a single place that can be read and re-run by other users. We have used Jupyter notebooks in classrooms both as a standalone simulation tool and to interact with imported data [49]. As these notebooks become readily available upon opening and do not require time-demanding installations of software libraries and dependencies, their use leads to more fluid class experiences [1].

During this work, the use of open resources has also triggered new collaborations with emerging open hardware companies, such as IORodeo, GaudiLabs and IOWlabs, as well as academic initiatives such as GMO detective and members of Gathering for Open Science Hardware (GOSH). The collaboration with IOWlabs has led to the co-design of devices that are scaled and sold to the university while they remain free to be studied, adapted and commercialized by third parties. The collaboration with GMO detective has led to a contribution to their design, later improved by members of the Hackteria network. This open interaction between academic laboratories and the private sector has given rise to a very efficient and fluid collaboration that has been key for a rapid response to these lockdowns (i.e. approximately 4-5 months to obtain all the resources for the whole course).

Complying with Open Science [79], OER [84] and open hardware recommendations [85], we provide detailed instructions, schematics, bill of materials and editable design files in openly accessible online repositories with version control (git). These resources are released under open licenses that grant students and teachers the freedom to not only replicate these resources but also to build upon this work. Protocols for the production of buffers and enzymes were deposited in protocols.io.

Materials and methods

Reagents preparation

RT-PCR for SARS-CoV-2 Detection: The positive RNA control for the RT-PCR assay was synthesized using a standardized protocol based on the procedures detailed in [31]. Initially, the pT7_N_amplicon template, derived from the SARS-CoV-2 N gene positive control vector (IDT, Cat # 10006625), was amplified using the *NT_Fw* and *T7_Nter_Rv* primers. The resultant PCR products were subsequently subjected to purification through agarose gel electrophoresis employing the Wizard Sv Gel Clean-up system (Promega). Following purification, the products were used to generate RNA transcripts using the HiScribe T7 In Vitro Transcription Kit (NEB, E2040S) according to the manufacturer's instructions. Post-transcription, the DNA template was removed by treatment with DNase I (NEB), followed by purification of the RNA transcripts using the RNeasy Kit (Qiagen) as per the supplied protocol. This procedure yielded viral N RNA suitable for use as a positive control in RT-PCR assays (~50 µL at 1,000 ng/µL). The construction of vectors encoding the enzymes used in this study has been previously described [31]. Additionally, all proteins expressed in this work were purified following detailed protocols, which have been optimized and documented. These protocols are publicly accessible on the protocols.io platform. Specific links for each protocol are as follows: for Pfu-Sso7d, visit https://dx.doi.org/10.17504/protocols.io.bzusp6we; for M-MLV RT, refer to https://dx.doi.org/10.17504/protocols.io.bsernbd6. RT-PCR was conducted with N1 and N2 primers for the SARS-CoV-2 N gene (Table I), as recommended by the CDC's Research Use Only primers list [86]. Reactions were performed using the previously described BEARmix reaction buffer [30]. Each RT-PCR reaction mix was prepared in a total volume of 20 µL, consisting of the following components: 1 µL Pfu-Sso7d (0.3 mg/mL), 0.125 µL M-MLV RT (0.02 mg/mL), 2 µL DTT (100 mM), 2 µL each of forward and reverse primers (3 µM, N1 or N2 as appropriate), 4 µL 5X BEARmix Buffer, 2 µL dNTPs (4 mM), 3.875 µL nuclease-free water, and 5 µL RNA sample. To each tube, 10 µL of mineral oil was added without mixing to prevent evaporation of the reaction mixture. The reactions were then incubated in the PocketPCR thermocycler (https://gaudi.ch/PocketPCR/) following a program set at 50°C for 15 m, followed by 30 cycles of 98°C for 10 s, 56°C for 20 s, and 72°C for 30 s, with a final extension step at 72°C for 5 m. To visualize DNA bands, a 2% agarose gel stained with SYBR™ Safe DNA Gel Stain (Thermo Fisher Scientific) was employed, and loaded alongside a 50 bp DNA Ladder. Electrophoresis was conducted in a 1X TAE buffer using the BlueGel™ electrophoresis system for 30 m.[86]

DNA Detection via LAMP Assays: Fresh LAMP reactions were prepared following a previously described LAMP protocol but adapted for QUASR detection [32]. In short, 10 µl homemade reactions contained of 1X Isothermal Buffer (20 mM Tris-HCl pH 8.8, 10 mM (NH4)2SO4, 50 mM KCl, 2 mM MgSO4, 0.1% Tween 20), 6 mM MgSO4, 1.4 mM of dNTPs, 0.16 mg/mL of locally-produced BstLF polymerase and a LAMP-QUASR 35S primer mix for the detection of the 35S promoter (1.6 µM FIP and BIP-FAM, 0.8 µM LF and LB, 0.2 µM F3 and B3, and 2.4 µM BIPc-Q; Table I). Reactions were incubated at 65 C for 35 minutes and then cooled down at 15 C for 15 minutes before being observed under a blue LED light using the acrylic or 3D-printed GMO Detective or a similar device. Nuclease-free water was used as a negative control and around 20000 copies of the 35S positive control.

For the lyophilization of the homemade GMO Detective reactions, we followed the protocol described by [46]. The reaction mix was prepared with 0.16 mg/mL *BstLF* (stored without glycerol at -80°C), 1.4 mM dNTPs, a 10X mix of LAMP 35S-Z primers and trehalose at 5% v/v. Each tube with 8 µl of this mix was sealed with parafilm and pre-frozen at -80°C for

10-20 minutes. These steps are important to avoid the content spilling over in the freeze-drying process due to abrupt pressure changes, and also to ensure a solid state of the mix that favors correct sublimation. The parafilm of each tube was perforated (with a sterile syringe) and the preparations were placed in a FreeZone 2.5L dessert freeze dryer (LabConco) at -84 °C and 0.04 mBar for 2- 3 hours, gradually increasing the temperature during the last 30 minutes. The tubes were closed and sealed in a vacuum in embossed plastic bags with a silica gel bag and an oxygen absorber. A pulse of argon gas was delivered inside to clear the air from the atmosphere in contact with the freeze-dried material. The bags were deposited in aluminum bags that were also sealed in a vacuum, allowing the preparations to be stored in the dark and at room temperature or 4 C for a period of 7 days to 2 months. Subsequently, each lyophilized mixture was rehydrated with DNA and a mixture containing 6 mM $MgSO_4$ and 1X Isothermal Buffer at a final volume of 10 µl per reaction. The reactions were incubated and their fluorescence was observed in a similar way to the fresh reactions. BstLF enzyme was expressed and purified following the protocol described in protocols.io [87], and diluted to a working concentration of 0.2 mg/mL. A fraction of the enzyme was stored in a buffer with glycerol at -20°C until use in LAMP reactions, while another fraction was stored at -80°C in a buffer without glycerol for use in a master mix of freeze-dried LAMP reactions.

Cell free reactions: Cell-free expression of the beta-galactosidase enzyme was produced incubating plasmid DNA (pT7-LacZ vector, 10nM) with 33.33% of cell-lysate from a BL21 DE3 strain deficient in the LacZ gene (https://www.addgene.org/99247/) and reaction buffer containing phosphoenol pyruvate as energy source [88]. Freeze-dried steps, when needed, were performed using a -80C benchtop lyophilizer following a protocol detailed in [89] for LacZ.

The colorimetric substrate for quantification of the β-galactosidase activity, o-nitrophenyl-β-D-galactopyranoside was purchased from Sigma-Aldrich (cat N1127) as well as the product o-nitrophenol (cat N19702). Both were diluted to 10 mM o-nitrophenol and 3 mg/ml ONPGal in saline buffer (PBS ,137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) as stock solutions (S7 Appendix).

Positive controls for COX1 and 35S were produced by commercial DNA synthesis and cloning into pL0R uLoop vectors [90] (S3 Appendix).

Table I. DNA sequences

Name	Sequence file	Reference
SARS-CoV-2 N gene vector IDT_10006625	https://benchling.com/s/seq-q0sxUo9s6cO1lgjsEu7G?m=slm-kIN0LjfxyYIMlOvRsVyX	[31]
pT7_N_amplicon	https://benchling.com/s/seq-y7ezWfUjm9NjpMmrIRsY?m=slm-dzV86r0Og7iosVEqnbSz	[31]

pT7-LacZ vector	https://benchling.com/s/seq-mVaLByGJR41kq99OFmp0?m=slm-zeAyOCaYQdWjUs1sIFvV	This work
Primer NT_Fw	CGAAATTAATACGACTCACTATAGGGGCAACGCGATGACGATGGATAG	[31]
Primer T7_Nter_Rv	ACTGATCAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTATGCTAGTTAGGCCTGAGTTGAGTCAG	[31]
2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT	[86]
2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG	[86]
2019-nCoV_N2-F	TTACAAACATTGGCCGCAAA	[86]
2019-nCoV_N2-R	GCGCGACATTCCGAAGAA	[86]
Primer 35S_FIP	AGGCATCTTCAACGATGGCCTTAAAGGAAGGTGGCTCCTACA	[91]
Primer 35S_BIP (5' FAM)	TGCCGACAGTGGTCCCAAAGTTGAAGACGTGGTTGGAACG	[91]
Primer 35S_F3	TGCCCAGCTATCTGTCACTT	[91]
Primer 35S_B3	TCCCTTACGTCAGTGGAGAT	[91]
Primer 35S_FLoop	TCCTTTATCGCAATGATG	[91]
Primer 35S_BLoop	AGCATCGTGGGAAAAGAAG	[91]
Primer 35S_BIPQ (3' Black Hole Quencher®-1 Or Iowa Black® FQ)	ACTGTCGGCA	[91]
Primer COX F3	TATGGGAGCCGTTTTTGC	[91]

Primer COX B3 (5’ FAM)	AACTGCTAAGRGCATTCC	[91]
Primer COX FIP	ATGGATTTGRCCTAAAGTTTCAGGGCAGGATTTCACTATTGGGT	[91]
Primer COX BIP	TGCATTTCTTAGGGCTTTCGGATCCRGCGTAAGCATCTG	[91]
Primer COX F-Loop	ATGTCCGACCAAAGATTTTACC	[91]
Primer COX B-Loop	GTATGCCACGTCGCATTCC	[91]
Primer COX FIPQ (3’ Black Hole Quencher®-1 orIowa Black® FQ)	YCAAATCCAT	[91]
COX-FIP-Q (Iowa Black FQ) *	YCAAATCCAT	This work
Primer COX FIP (Texas Red) **	ATGGATTTGRCCTAAAGTTTCAGGGCAGGATTTCACTATTGGGT	This work
35SZ-F3	AAGATGCCTCTGCCGACA	This work
35SZ-B3	CAGCGTGTCCTCTCCAAAT	This work
35SZ-FIP	ACGTGGTTGGAACGTCTTCTTCCCAAAGATGGACCCCCA	This work
35SZ-BIP (FAM)	ATCTCCACTGACGTAAGGGATGATAGAGGAAGGGTCTTGCGA	This work
35SZ-LF	TCCACGATGCTCCTCGTG	This work
35SZ-LB	ACGCACAATCCCACTATCCT	This work
35SZ-BIP-Q (Iowa Black FQ)	CAGTGGAGAT	This work
COX1 positive control	https://benchling.com/s/seq-109EuLvihD22XSvVEczf?m	This work

	=slm-73a6YZ62H7PlIG0X0mmd	
35S positive control vector	https://benchling.com/s/seq-wimfzH7J4KMBteXSejkK?m=slm-uIEwoaoNYJcLHEgg8bZo	This work

*BHQ quencher in the original COX-FIP-Q probe was replaced by Iowa Black FQ quencher (the sequence remains the same)
**FAM fluorophore in the original COX FIP primer was replaced by Texas Red (the sequence remains the same)

Hardware design and fabrication

All 3D printed parts were designed using the open-source software openSCAD, exported as STLs and printed in Ender 3 or Prusa MK6 3D printers. The openSCAD editable files of GMO device and RGBfluor are available in the git repositories [92] and [54], respectively.

Printed circuit boards were designed using the open-source software KiCAD 5. PCBs were manufactured in the JLC company and the components were acquired through the LCSC company.

Glossary

OSH: "any piece of hardware used for scientific investigations that can be obtained, assembled, used, studied, modified, shared, and sold by anyone. It includes standard lab equipment as well as auxiliary materials, such as sensors, biological reagents, analog and digital electronic components." [80]

OER: "Open Educational Resources (OER) are learning, teaching and research materials in any format and medium that reside in the public domain or are under copyright that have been released under an open license, that permit no-cost access, re-use, re-purpose, adaptation and redistribution by others." [93]

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Author Contributions

FF, JM, AC, designed research;
FF, JM, GA, AL, CSR supervised research;
AC, AA, VZ, APA, WA, DG, JA, FQ, IN, FN, TM, VF, MB, SV, MR, GA, FC, PC FF performed research;
SA, SR, designed the lab-in-a-box
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Competing interests:

The authors have declared that no competing interests exist.

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Supplementary information

Figure S1: Organization of the lab-box. a) the box consisted of a commercial plastic storage box, which was modified to hold a light on the top via a 3D printed holder. A light metallic tray was provided to run experiments at home. The box contained three smaller plastic boxes,

one per student, which can be used as a water bath for LAMP experiments. b) organization of the three smaller boxes inside the lab-in-a-box. c) The correct employment of the box was monitored by Zoom video calls (image courtesy of Josefina Lara).

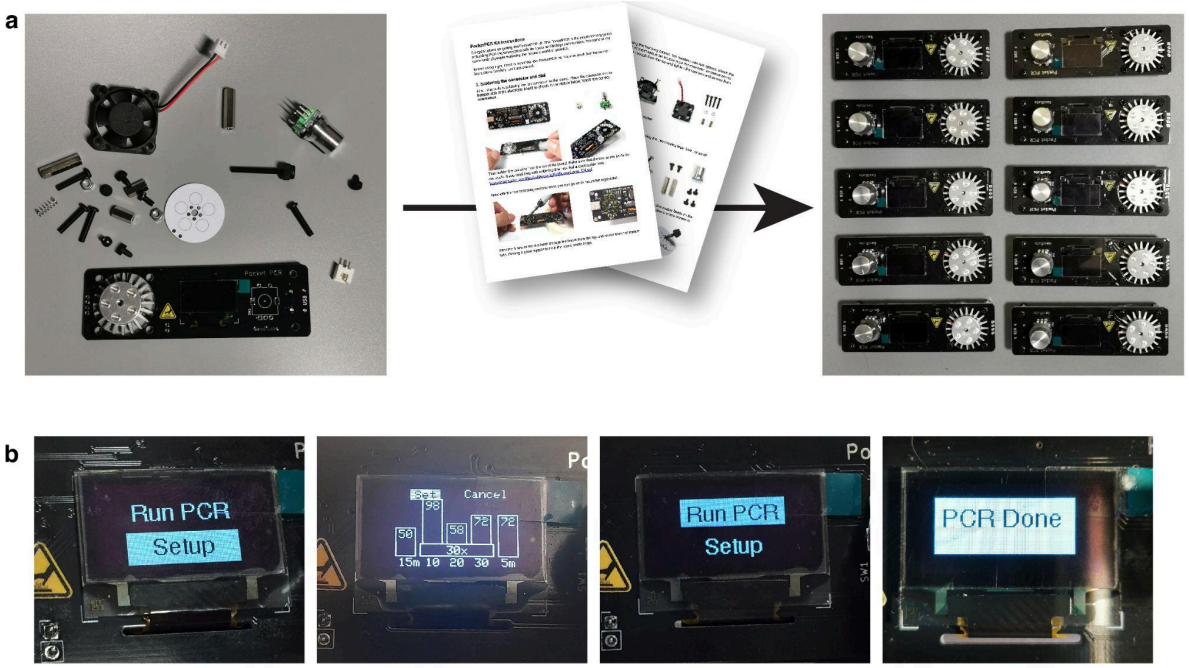

Figure S2: PocketPCR assembly and operation. a) PocketPCR is sold as a kit to be assembled with minimum soldering (it can also be obtained fully assembled). Clear instructions are provided by GaudiLabs. b) Sequential steps showing thermocycling setup. The main knob is rotated to choose values and pushed to confirm selections.

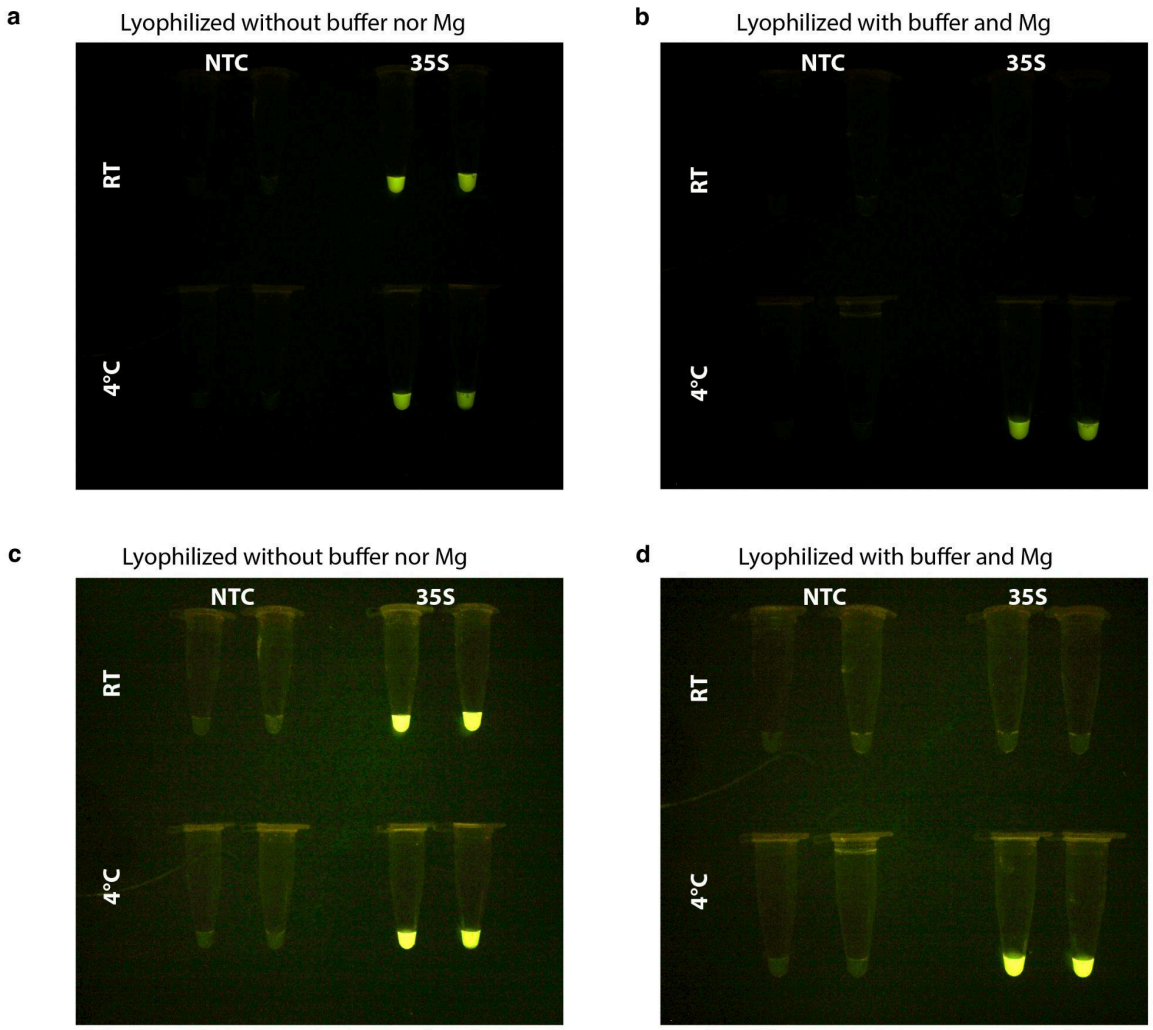

Figure S3: Lyophilization without magnesium nor reaction buffer provides longer storage capabilities. a) and c) Reactions lyophilized without buffer nor magnesium, rehydrated after two-month storage at room temperature (top) or 4°C (bottom). b) and d) Reactions lyophilized with buffer and magnesium, rehydrated after two-month storage at room temperature (top) or 4°C (bottom). Panel c and d are images with overexposure to show the negative tubes.

Figure S4: Comparison of acrylic and 3D printed GMO Detective device. a) Acrylic version of the GMO Detective device. b) 3D printed version designed in openSCAD to accommodate the same filters and PCB used in the original device. All editable files available in https://github.com/MakerLabCRI/GMODetective-Detector/blob/main/3D%20Model/STL/GMOdetective3D.stl

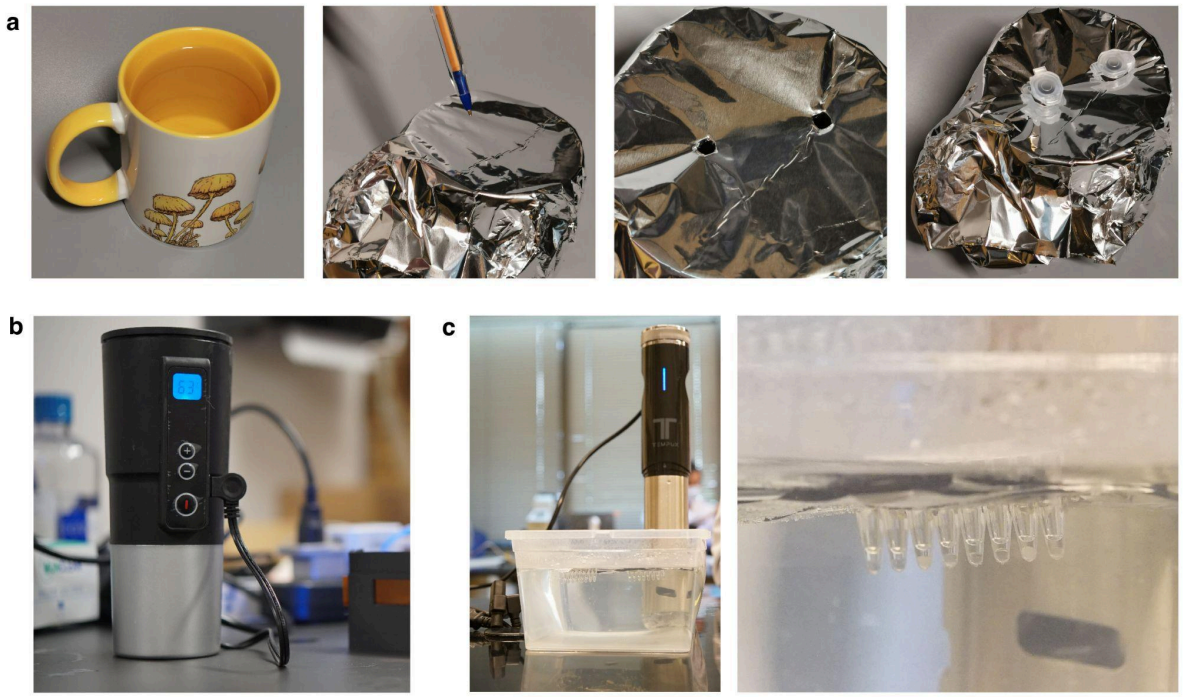

Figure S5: Heat incubation for sample and LAMP reactions. a) Step-by-step preparation of 100°C water incubation using aluminum foil for the initial 5 min heat treatment. b) Electronic coffee cup used for the 65°C incubation step. c) Sous vide heating device in a water bath used for the 65°C incubation step.

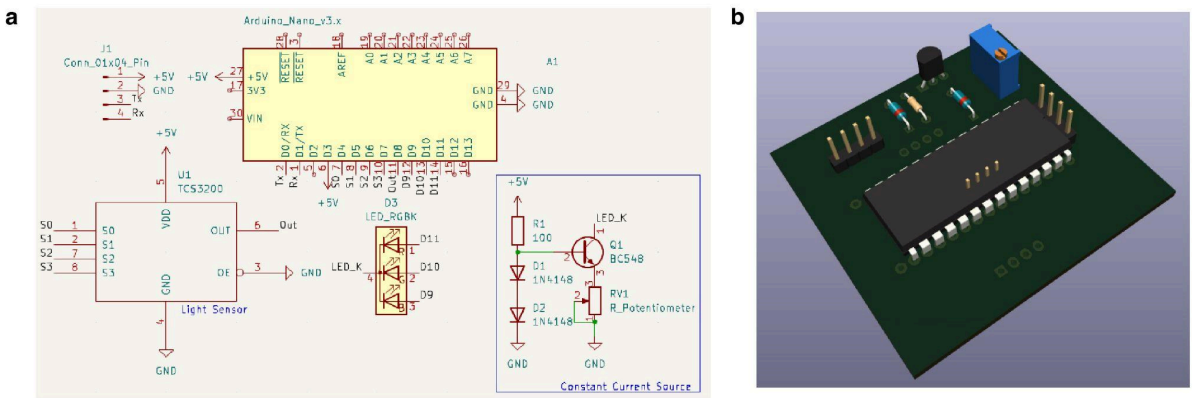

Figure S6: Colorimeter reGOSH. a) Schematic of the Arduino shield for the colorimeter reGOSH. b) 3D render of the colorimeter PCB. More details in https://gitlab.fcen.uncu.edu.ar/regosh/colorimetro-regosh

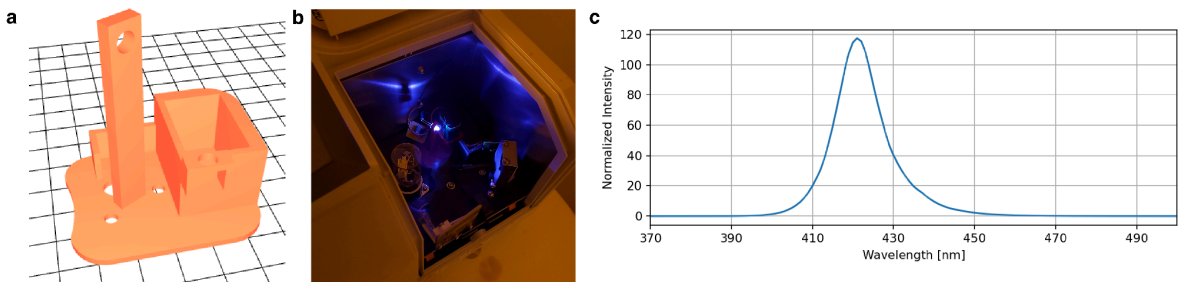

Figure S7: LED spectrum measurement. a) Schematic representation of the 3D printed LED adapter for the spectrometer's light source compartment. b) UV LED placed inside the spectrometer. c) Relative intensity spectrum of the UV LED.

S1 Appendix: student guidelines for using and sanitizing the lab-in-a-box
https://docs.google.com/document/d/1KMwxzWK4cWTPFVgZbOHRhUShHATnSlHd2MfOjHrskTM/edit

S2 Appendix: student instructions for micropipetting
https://docs.google.com/document/d/1mjkT8FLU9-LoPaSQ2xN7pbIX0ZF1hONYSzomao4EK0M/edit?usp=sharing

S3 Appendix: homemade GMO Detective
https://docs.google.com/document/d/10PBd-ULVOjwjJ4-2WYQs3YjBE437wTo92zYQj_dm2YQ/edit

S4 Appendix: RGBfluor instructions
https://docs.google.com/document/d/1etmeNLRWUVtNbVrLLukMpZDVyVb5YemQZKtyJJaN_Qc/edit#heading=h.x42dw0nlni7m

S5 Appendix: RGBfluor student guide
https://docs.google.com/document/d/1mPteHejz8g6PC-ZG_Bd16zuto2E1kiaeCLN4QVS88xs/edit?usp=sharing

S6 Appendix: student evaluation of the lab-in-a-box
https://docs.google.com/document/d/1XVAe3rLuFDaeI9_c9Jf2z-55CD4s-z8WyoBkV9ktg1o/edit?usp=sharing

S7 Appendix: Enzyme kinetics student guide
https://docs.google.com/document/d/1MFIiDmhkdflytR7mc_RqeJXvmucAFHjvXI8_bDFhOn0/edit

S1 Appendix: Box usage instructions

Receiving the box

When you receive the box, be careful and check it is the one corresponding to the practical that you must carry out (LAMP or RT-PCR) and student group. You can verify this information using the label on the box, which identifies what type of practical it corresponds to, the group number and the name of the students who should receive it. If you have any problem please contact the teaching team immediately.

You should keep the box in a cool place where it is not exposed to direct sunlight and **DON'T OPEN** it until the moment of carrying-out the practical.

What is inside the box?

You received a large box containing 3 individual smaller boxes inside and a metal tray. On the metal tray, there are elements that are common for the use of the 3 students, please, manipulate them very carefully.

The elements and reagents found inside the small boxes are for **personal use**. The material inside each small box is in sterile conditions and meant for each student's particular use. **ONLY OPEN THE SMALL BOX THAT CORRESPONDS TO YOU.**

Before sending the box to the next student, be sure to carefully clean and store each common item following the instructions in the manual.

Sterilization box

When you open the box, the first thing you should do is take out the gloves and put them on. Later, you will find a bottle that contains 70% ethanol and that will allow you to sterilize the large box **inside and out,** and also the small ones **only on the outside**. Apply as much 70% ethanol as you deem necessary on the surface of the boxes and use absorbent paper to distribute it over the surface. Let the ethanol dry by evaporation.

Once the practical activity is finished and after having completed the section **"Cleaning and storage of material"** found at the end of this manual, be sure to spray the entire surface of the box with 70% ethanol before storing it inside the box, following the instructions previously described.

The box as a workstation.

The large box will become each student's own workstation for the duration of the practical. Therefore, the first thing you should do is place it on a stable surface, avoiding

irregular and slippery surfaces, in order to avoid accidents. Once you have established your workplace, you must carefully remove the tray and one small work box (the other two small boxes can remain inside the large box). Place the large box horizontally, install the LED light on the corresponding bracket and connect it to the power source using the extension cord provided inside the box.

Position the tray at the front of the box so that it is illuminated by the LED light. You must develop the practical inside the tray, so that if a material spill occurs, it does not affect your home and is easy to clean.

Biosecurity Standards

You will carry out a practical activity at home, but you must take all the corresponding safety measures as if you were working in a laboratory, even being even **more cautious**. Therefore, you should, if possible, carry out the practical activity in an environment free of small children and pets.

Outfit:

- You should wear long pants, closed-toed shoes and avoid wearing loose clothing.
- Those students who have long hair must wear it up, ensuring that their face is completely uncovered. Those who have bangs should ensure that they do not fall over the forehead.
- You should not use pendants or rings.
- Avoid wearing contact lenses.
- The use of gloves and mask is mandatory.

Work conduct:

- Prior to the practical activity, you must have read and studied the corresponding protocol**.**
- **DON'T** handle objects in your home with gloves on, to avoid contamination of your living space or work materials.
- **DON'T** use gloves to handle your mobile device. If you need to use it to show something to your co-workers or to take a photograph, you must remove your gloves.
- **DON'T** touch your face with gloves on.
- **DON'T** eat or drink during the practical activity.
- You must follow the protocol delivered for each practical activity, without making any modifications to the detailed instructions.

IMPORTANT: Since we are in a pandemic, you will be asked to wear a mask during the practical activity (the mask is provided inside your individual work box). This measure will prevent work material from being contaminated in the probable case of SARS-CoV-2. If you present symptoms associated with the disease caused by SARS-CoV-2, we ask you to urgently notify the teaching staff.

Materials inside the box

Each box has all the elements and reagents necessary to carry out the practical experiences, you **MUST NOT** use any utensils from your home.. Below you will find a list of the common and personal materials and reagents in each box.

LAMP box

Common materials:

- Micropipettes:
  - P200.
  - P20.
- 70% ethanol.
- Plastic base
- Power-strip.
- Beaker.
- Water Heater or Thermometer.
- Printed circuit board (*PCB*) with LEDs and acrylics.
- USB cable - microUSB.
- Tube rack (2).
- Fine tip marker.

Personal materials (small box):

- Tip box.
- Bottle with tubes.
- Food coloring.
- Reaction strip.
- Problem sample (corn).
- Plant sample: Stems (2).
- Metallic paper.
- *Parafilm*.
- Reagents:
  - Nuclease-free H_2O (H).
  - Reaction buffer (R).
- Waste bag.

RT-PCR box

Common materials:

- Micropipettes:
  - P200.
  - P20.
- 70% ethanol.
- Plastic base
- Power strip.
- LED light.
- PocketPCR thermal cycler.
- USB-C adapter.
- 2A charger.
- blueGel electrophoresis chamber™.
- Spray ClearView™ (and its cloth).
- Tube rack.
- Fine tip marker.

Personal materials:

- Tip box.
- Bottle with tubes.
- Food coloring.
- Reagents (in parentheses is the label that you will see on the tube):
  - Nuclease-free H_2O (H).
  - 5X reaction buffer (B).
  - dNTPs (N).
  - DTT (DTT).
  - Primers (4).
    - Viral Forward (FN).
    - Viral Reverse (RN).
    - Plant Forward (FA).
    - Plant Reverse (RA).
  - Enzyme mix (E).
  - Genetic material (4).
    - Viral RNA (RV).
    - Viral cDNA (DV).
    - Plant RNA (RM)
    - Plant cDNA (DM).
  - 6X Load Buffer (BC).
  - Molecular weight standard 50bp (50).
  - Positive reaction controls (2).
    - Viral (+V)
    - Plant (+M)
- Mineral oil (AC).
- 2% agarose gel.
- 50mL TAE 1X.
- Waste bag

What to do with the waste?

During the practical instance you will produce a lot of waste plastic material (mainly tips and tubes), which you must store inside the zip-lock bag that you will find in its individual box. The waste bag corresponds to the same bag where the reagents are located. For this purpose, remove ALL tubes from the bag, put them in their rack, and begin using the bag as waste storage. Before adding waste to the bag, make sure each tube is properly closed to prevent spillage.

Once the practical instance is over, you must seal well the bag that will contain **ALL** of the plastic waste, spray it with 70% ethanol and leave it inside the individual box. The teaching team will be responsible for properly disposing of all waste produced during the practical activity when the boxes are collected after the third student.

Cleaning and storage of material

Store all material and waste in your personal box. Common materials must all remain inside the metal tray. Use the list of materials and reagents above to check that you do not forget any materials. The cleaning of the specific materials for each box can be found in the specific manuals for each of them.

Once all the materials are on the tray, wrap them with plastic wrap to prevent them from moving from the tray. Leave the remaining plastic wrap inside the box and close it. Now it is ready to send to the next student/back to the teaching team.

References

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S2 Appendix: Micropipetting instructions

Aim: Learn how to manipulate micropipettes correctly.

Before carrying out any practical activity, we must ensure that you know how to use micropipettes correctly and understand their volume range. This tutorial will teach you the basics of using micropipettes and will end with an activity checkup.

Before starting the corresponding LAMP or RT-PCR practical activity, you will need to upload a photograph of the pipetting practical to the task box (set up for this activity) on the CANVAS platform, this has the objective of verifying that you learned the correct handling of this delicate instrument.

1. Parts of a micropipette.

- Plunger (*Push button for Plunger*): part of the micropipette that, when pressed or released, will allow a volume of liquid to be taken and released. **IMPORTANT**: The plunger has two stops, the stop to use depends on the activity being carried out, and will be explained in detail below.
- Volume control wheel (*Thumb Wheel*): allows you to adjust the volume that the micropipette will take. **IMPORTANT**: each micropipette has a volume range in which it is functional, remember **NO** to use a volume outside that range (the micropipette loses calibration).
- Volume window (*Volumeter*): indicates the adjusted volume that the micropipette will take.
- Ejector button (*Tip Ejector Button*): button that, when pressed, will remove the tip of the micropipette through the movement of the ejector.
- Tip support (*Barrel Tip Holder*): place where the tip is adjusted to suck the desired volume.
- Tip (*Tip*): place where the volume of working liquid will be stored. **IMPORTANT**: NEVER use a micropipette without a tip, the liquid can enter the micropipette, contaminate it and cause it to decalibrate.

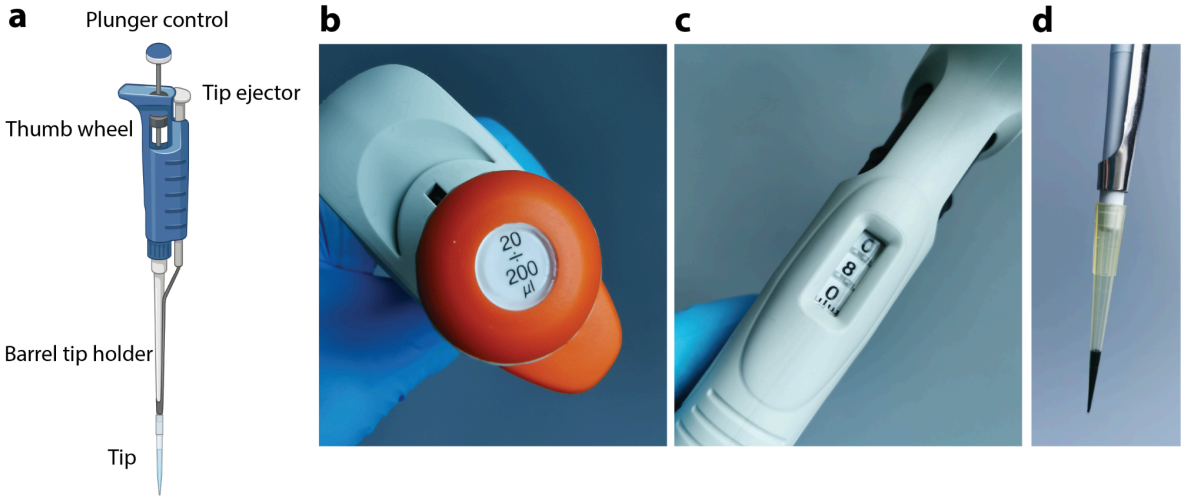

Figure 1: Parts of a micropipette. a) Schematic representation of a micropipette. b) Volume working range, here between 20 and 200 µL. c) volume window. d) tip containing a correct volume of liquid without bubbles.

2. Types of micropipettes.

The micropipette to use in the practicals will depend on the required volume. There are different micropipettes that work in limited ranges of volumes and require different tips depending on their size.

In this practical experience, you will have two micropipettes called: P200 and P20. The name of the micropipette is given by the maximum working volume that can be used with them:

- P200: working volume between 20 and 200µL (in some cases 50 to 200µL).
- P20: working volume between 2 and 20µL.

You will have a box of tips, which are suitable for P200 and P20. The tips should be changed each time a volume of a different liquid is used to avoid contamination problems between different vials.

The accuracy of the micropipette depends on its calibration, this must be done regularly (approximately every year). For greater accuracy when taking a volume with the micropipette, you should always choose the micropipette whose maximum allowable volume is closest to this volume. For example, if you want to take 20µl, it is preferable to use the P20 rather than the P200, this is because there is less space between the surface of the liquid and the plunger of the micropipette.

3. How to use a micropipette.

The proper way to hold a micropipette can be seen in Figure 2. This posture is comfortable for right- and left-handed people. The plunger is pressed with the thumb, while the rest of the fingers are in charge of holding the micropipette.

Figure 2: correct micropipette holding.

To suck volume for direct pipetting:

1. Adjust the workload with the wheel that allows you to control the volume.
2. Take one tip of appropriate size, verifying that it fits well in the tip support.
3. Press the plunger with the thumb up to the first stop.
4. Hold down until first stop (Figure 3) and immerse the tip in the liquid you want to suck. **CONSIDERATIONS**: Try to keep the pipette as vertical as possible or at a maximum angle of 40°. The tip of the pipette should be immersed a few millimeters into the liquid. DO NOT touch the bottom of the tube with the tip, as this prevents suction.
5. Slowly release the plunger to suck the liquid until it is no longer necessary to press it. **CONSIDERATIONS**: If the bottom of the tip does not contain liquid, but air, perform the procedure again. Air at the bottom of the tip indicates that it did not take in the proper volume.

b. To release volume:

1. Take the micropipette to the tube/paper on which the liquid will be deposited.
2. Touch the wall of the tube and slowly press the plunger until you reach the first stop, and then to the second stop (Figure 3).
3. **DO NOT** release the plunger until the micropipette moves from where it released the liquid (otherwise it will suck the liquid again).
4. Throw away the tip that has just been used in the corresponding waste, pressing the

ejector button.

Figure 3: Plunger positions for taking and releasing liquid.

4. Practical activity.

Now that you know the parts of a micropipette and the theory of how to suck and release a volume of liquid, it's time to put your knowledge into practice with a small activity.

The activity consists of carrying out two serial dilutions of a food coloring.

Dilution 1:

1. Label five 0.2mL tubes with the numbers 1 to 5.
2. In a total volume of 100µL, make a 1/5 dilution of the food coloring in tube #1.
3. Perform a 1/5 dilution of tube No. 1 for tube No. 2, obtaining a final volume of 100 µL.
4. Repeat the procedure until tube No. 5.

Dilution 2:

1. Label 4 0.2mL tubes with the letters A to D.
2. In a total volume of 100µL, make a 1/10 dilution of the food coloring in tube A.
3. Perform a 1/10 dilution of tube A for tube B obtaining a final volume of 100µL.
4. Repeat the procedure until tube D.

Arrange the tubes of both dilutions together according to the color gradient, write down the pattern of numbers and letters obtained according to this order and take a photograph like the one seen in Figure 4. The pattern obtained must match the one delivered by the equipment. teacher so that you can continue with the practical activity.

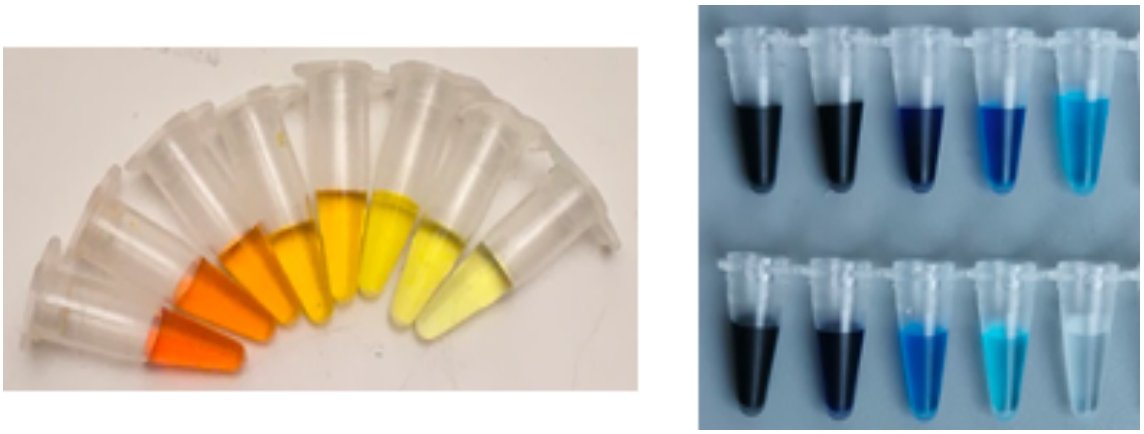

Figure 4: Serial dilution example.

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S3 Appendix: homemade GMO Detective

Based on the original GMO detective designed, produced and shipped from France [1], we made some modifications to primers as listed below:

Original 35S primer set

Name	Sequence 5'to3'	Modification
35S_FIP	aggcatcttcaacgatggccttaaaggaaggtggctcctaca	
35S_BIP	tgccgacagtggtcccaaagttgaagacgtggttggaacg	5' FAM
35S_F3	tgcccagctatctgtcactt	
35S_B3	tcccttacgtcagtggagat	
35S_FLoop	tcctttatcgcaatgatg	
35S_BLoop	agcatcgtgggaaaagaag	
35S_BIPQ	ACTGTCGGCA	3' Black Hole Quencher®-1 Or Iowa Black® FQ

Original COX1 primer set

Name	Sequence 5'to3'	Modification
COX F3	tatgggagccgtttttgc	
COX B3	aactgctaagrgcattcc	5' FAM
COX FIP	atggatttgrcctaaagtttcagggcaggatttcactattgggt	
COX BIP	tgcatttcttagggctttcggatccrgcgtaagcatctg	
COX F-Loop	atgtccgaccaaagattttacc	
COX B-Loop	gtatgccacgtcgcattcc	
COX FIPQ	YCAAATCCAT	3' Black Hole Quencher®-1 orIowa Black® FQ

Modified 35S primer set (modifications labeled in yellow)

Name	Sequence 5'to3'	Modification
35SZ-F3	aagatgcctctgccgaca	
35SZ-B3	cagcgtgtcctctccaaat	
35SZ-FIP	acgtggttggaacgtcttcttcccaaagatggaccccca	
35SZ-BIP	atctccactgacgtaagggatgatagaggaagggtcttgcga	5' FAM
35SZ-LF	tccacgatgctcctcgtg	
35SZ-LB	acgcacaatcccactatcct	
35SZ-BIP-Q	cagtggagat	3' Iowa Black FQ

Modified COX1 primer set (modifications labeled in yellow)

Name	Sequence 5'to3'	Modification
COX-F3	tatgggagccgtttttgc	
COX-B3	aactgctaagRgcattcc	
COX-FIP	atggatttgRcctaaagtttcagggcaggatttcactattgggt	5' Texas Red
COX-BIP	tgcatttcttagggctttcggatccRgcgtaagcatctg	
COX-LF	atgtccgaccaaagattttacc	
COX-LB	gtatgccacgtcgcattcc	
COX-FIP-Q	YCAAATCCAT	3' Iowa Black FQ

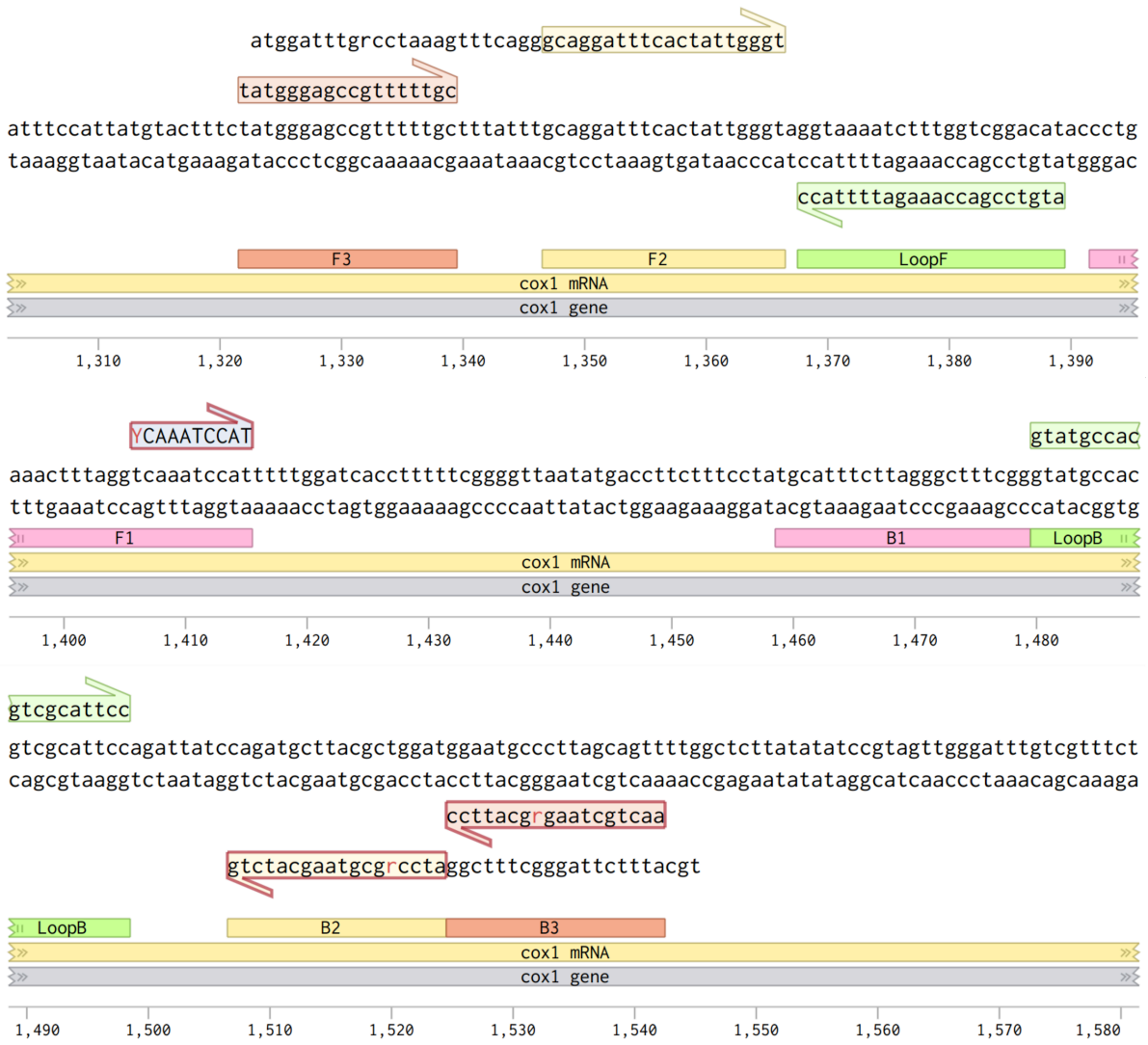
FAM: Fluorescein
Iowa Black FQ: Fluorescence quencher in the FAM range.

<u>QUASR probe diagram used:</u>

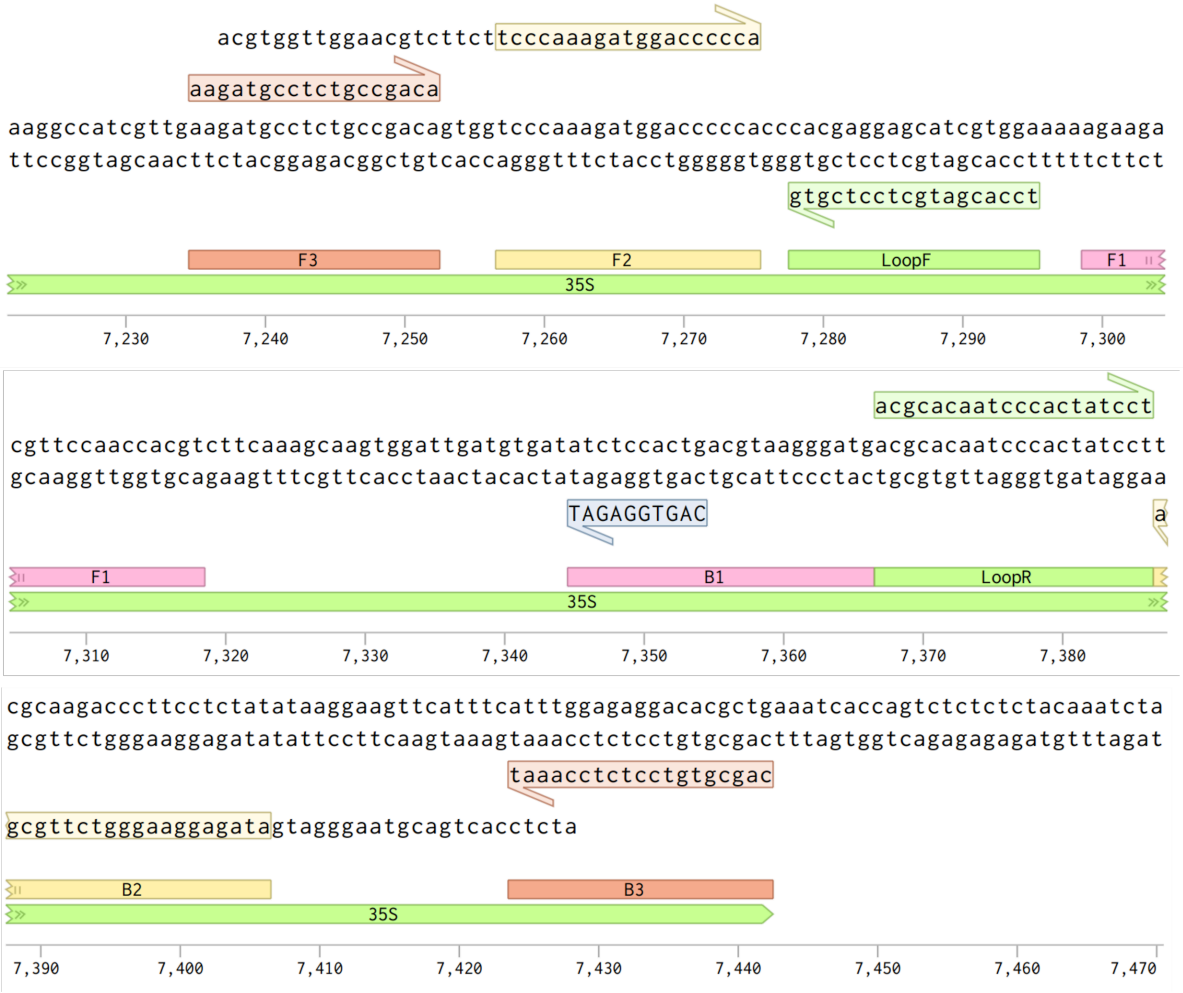
```
                    5'                                                  3'
35SZ-BIP      FAM  – ATCTCCACTGACGTAAGGGATGATAGAGGAAGGGTCTTGCGA
35SZ-BIP-Q    Iowa BFQ – TAGAGGTGAC
                    3'          5'

                    5'                                                  3'
COX-FIP       FAM  – ATGGATTTGRCCTAAAGTTTCAGGGCAGGATTTCACTATTGGGT
COX-FIP-Q     Iowa BFQ – TACCTAAACY
                    3'          5'
```

Annealing of LAMP primers for S.tuberosum mitochondrial cox1 gene [2]**:**



Annealing of LAMP primers for 35S CaMV [3]

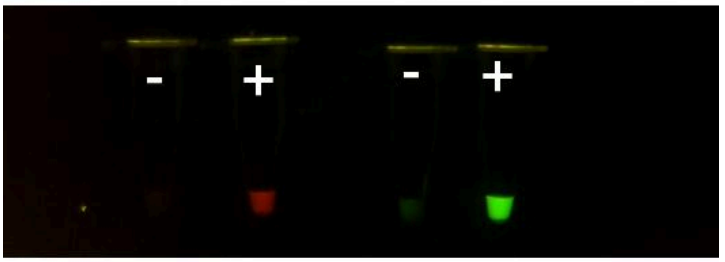


Primer quantities and purification methods at ordering

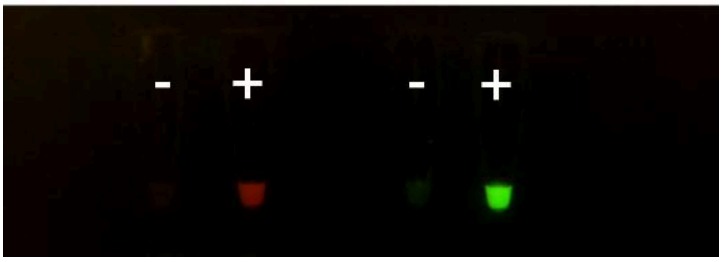
Name	**Sequence**	**Scale**	**Purification**		
COX-F3	tatgggagccgtttttgc	100nm	STD	100 nmole DNA oligo	Standard desalting
COX-B3	aactgctaagRgcattcc	100nm	STD	100 nmole DNA oligo	Standard desalting
COX-FIP	/5TexRd-XN/atggatttgRcctaaagtttcagggcaggatttcactattgggt	250nm	HPLC	250 nmole DNA oligo	**HPLC purification**

COX-BIP	tgcatttcttagggctttcggatc cRgcgtaagcatctg	250nm	STD	250 nmole DNA oligo	Standard desalting
COX-LF	atgtccgaccaaagattttacc	100nm	STD	100 nmole DNA oligo	Standard desalting
COX-LB	gtatgccacgtcgcattcc	100nm	STD	100 nmole DNA oligo	Standard desalting
COX-FIP-Q	YCAAATCCAT/3IAbRQ Sp/	250nm	HPLC	250 nmole DNA oligo	**HPLC purification**
35SZ-F3	aagatgcctctgccgaca	100nm	STD	100 nmole DNA oligo	Standard desalting
35SZ-B3	cagcgtgtcctctccaaat	100nm	STD	100 nmole DNA oligo	Standard desalting
35SZ-FIP	acgtggttggaacgtcttcttcc caaagatggaccccca	250nm	STD	250 nmole DNA oligo	Standard desalting
35SZ-BIP	/56-FAM/atctccactgacgt aagggatgatagaggaaggg tcttgcga	250nm	STD	250 nmole DNA oligo	Standard desalting
35SZ-LF	tccacgatgctcctcgtg	100nm	STD	100 nmole DNA oligo	Standard desalting
35SZ-LB	acgcacaatcccactatcct	100nm	STD	100 nmole DNA oligo	Standard desalting
35SZ-BIP-Q	C AGT GGA GAT/3IABkFQ/	250nm	HPLC	250 nmole DNA oligo	**HPLC purification**

Thermocycler



3D printer hot bed



Comparison of Texas Red COX1 (left) and FAM 35S (right) QUASR LAMP reactions run on a thermocycler (top) and 3D printer hot bed (bottom). The thermocycler was run at at 63°C while the heated bed was run at 65°C. Homemade enzymes and buffers were used in both. The photo was taken after 60 min of incubation in a FluoPi [4](at 200 ISO, 760000 µs exposure time) .

1. [No title]. [cited 22 Feb 2024]. Available: https://gmodetective.com/wp-content/uploads/2018/10/GMO-Detective-Wetware-V1.1.pdf?189db0&189db0

2. COX1_X83206 · Benchling. [cited 22 Feb 2024]. Available: https://benchling.com/s/seq-jsZWJHYQhWAf3DYHAdUo

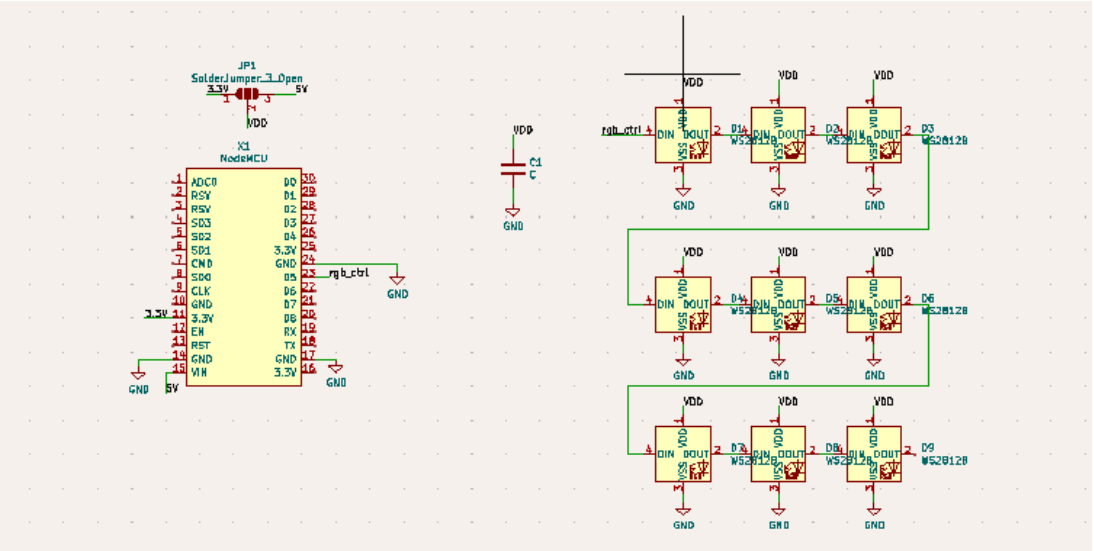
3. 35s_V00141 · Benchling. [cited 22 Feb 2024]. Available: https://benchling.com/s/seq-McxTSERY7eYYw9cOe7u9

4. Nuñez I, Matute T, Herrera R, Keymer J, Marzullo T, Rudge T, et al. Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering. PLoS One. 2017;12: e0187163.

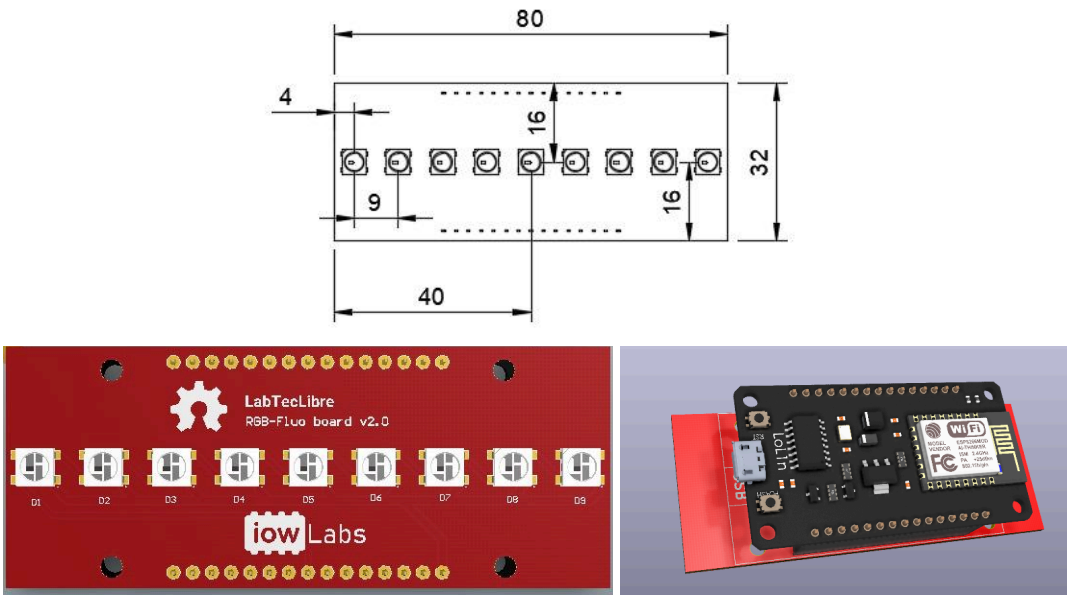
S4 Appendix: RGBfluor design and operation instructions

RGBfluor printed circuit board (PCB)

The PCB was designed to be compatible with the commercial NodeMCU v3 "Lolin" board based on the ESP8266 microcontroller. The PCB has a shield format and has 9 digital controlled WS2812B RGB LEDs.



Schematic of the Lolin (left) and the LEDs of RGBfluor PCB (right).



Schematic representation of the physical dimension and spatial arrangement of RGB LEDS in the RGBfluor PCB.

Bill of materials for the construction of the circuit is:

Item	Name	Quantity	Part number LCSC	Note
Capacitor	C1	1	C77386	MLCC SMD 0603 1uF.

RGB LED	D1-D9	9	C114586	WS2812B
Header	J1	2	C2897378	FH, 1 row x 15 pos. pitch 2.54.

The gerber files to replicate the board are available in the repository https://gitlab.com/FernanFederici/rgb_fluor under the path PCB/Gerber_Files. The board is simple to assemble and the components used can be assembled by hand soldering.

Firmware and software

The firmware was developed on platformIO with the Arduino framework. It implements a local software access point and mounts a local server on the ESP8266 board, which can be accessed from any device connected to the generated network. When the device is powered, the microcontroller will generate a local network with a specific name (by default RGB-Fluo 0X), which can be configured by programming the firmware.
The assembled network is discoverable by any device with the ability to connect via Wi-Fi protocol: PC, tablet, smartphone, and has no password. Once entered the network we can access to the web application by entering the following IP
http://192.168.4.1/

The code uses the following libraries to work (all of them available from the official Arduino library manager)

- ESP8266 WiFi
- me-no-dev/ESPAsyncTCP
- me-no-dev/ESPAsyncWebServer
- LittleFS
- fastled/FastLED

The ESP8266WiFi, ESPAsyncTCP and ESP Async WebServer libraries are used to manage WiFi communication, implement the access point and set up the local server. The LittleFS library implements a memory management (File system) for the microcontroller, allowing the html and css files from the web page to be stored in the flash memory of the board. The FastLED library allows you to control RGB LEDs. These LEDs can be controlled individually, however for the purposes of this application they are all controlled at the same time.

Compiling the code

To upload the code to the device, some preliminary steps are necessary. You have to mount an image in the memory of the microcontroller with the files index.html and syles.ccs that contain the web page found in the path

./firmware/data

You have to run the commands.

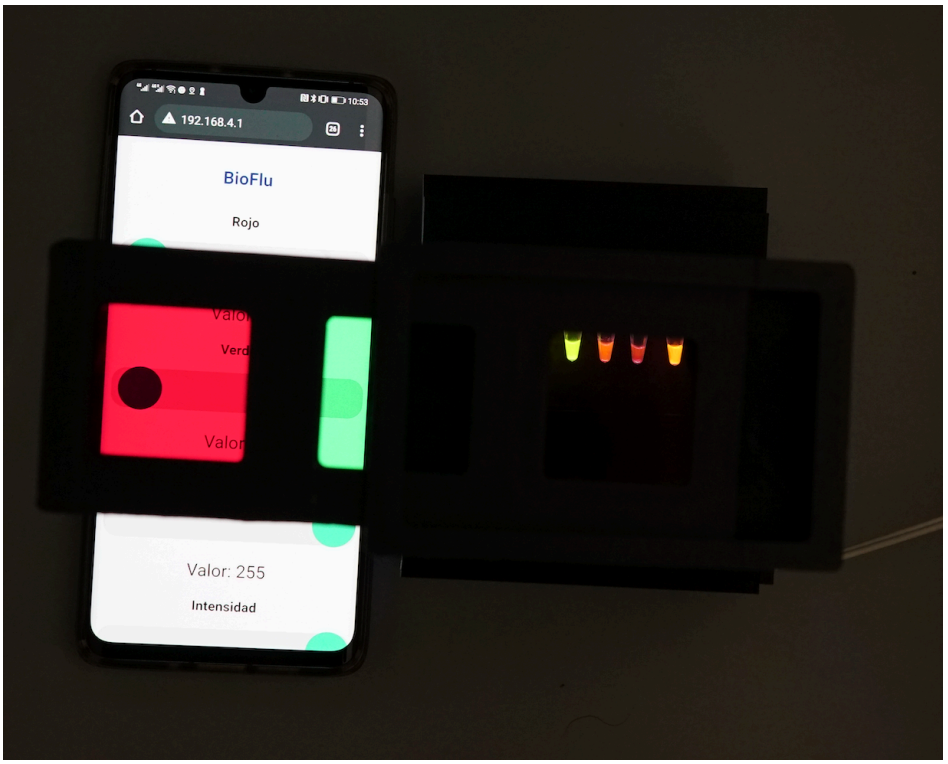
pio runs -t buildfs pio runs -t uploadfs

Once the image is uploaded we can load the firmware through the commands

Compile: run pio Load: pio run --target load

S5 Appendix: RGBfluor student guide

RGB Fluor



This document corresponds to the support material for the fluorescence practical of the Bio266e course.

Authors: Fernan Federici, Anibal Arce, Wladimir Araya, Domingo Gallardo, Isaac Nuñez, Tamara Matute.

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Practical learning objectives:

- To understand the organization of an optical system for the detection of a fluorescent signal; particularly the compatibility between illumination wavelength, optical properties of the emission filter and excitation/emission spectrum of the fluorescent molecules.

Task:

Applying the concepts learned in class on fluorescence microscopy, we ask you to take a photograph with your mobile phone of:

1- The best setup to photograph the protein **sfGFP**
2- The best setup to photograph the protein **mScarlet-I**
3- The best setup to photograph both proteins:**sfGFP and mScarlet-I simultaneously**.

(The idea is that the photograph has a dark background to increase the range between the signal and the background. That is, a photo where GFP is seen on an all-green background, for example, is not useful).

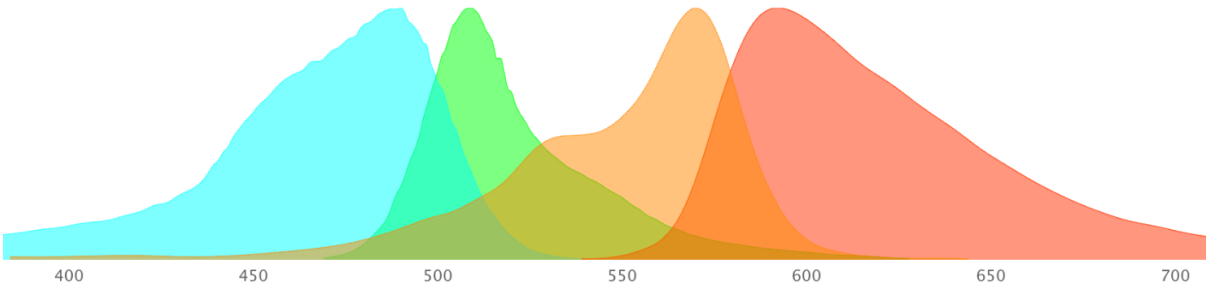
You must consider compatibility between:

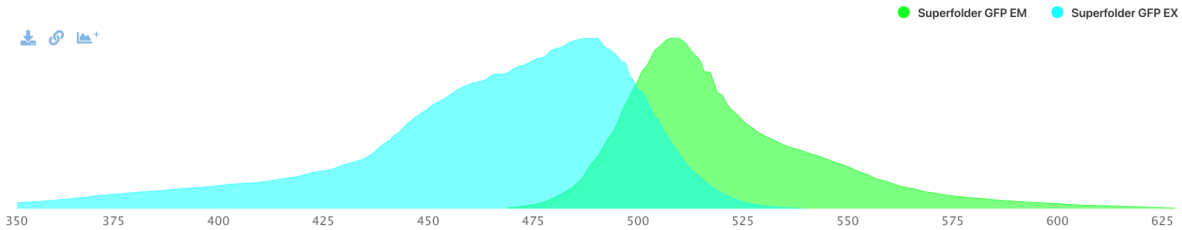
1. Excitation/emission spectrum of fluorescent molecules.
2. Optical properties of the emission filter
3. Lighting Wavelength

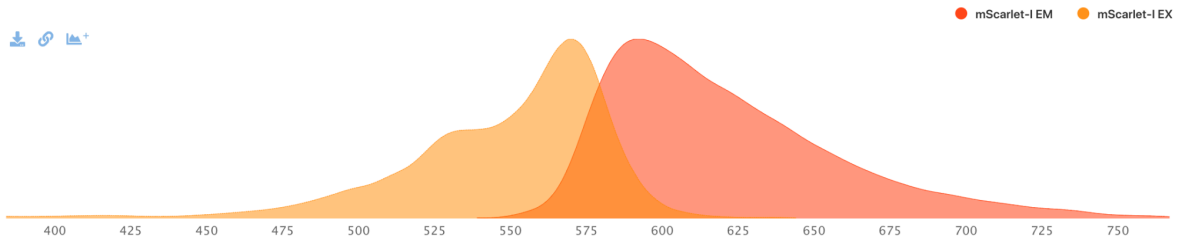
1) Excitation/emission spectrum of fluorescent molecules.

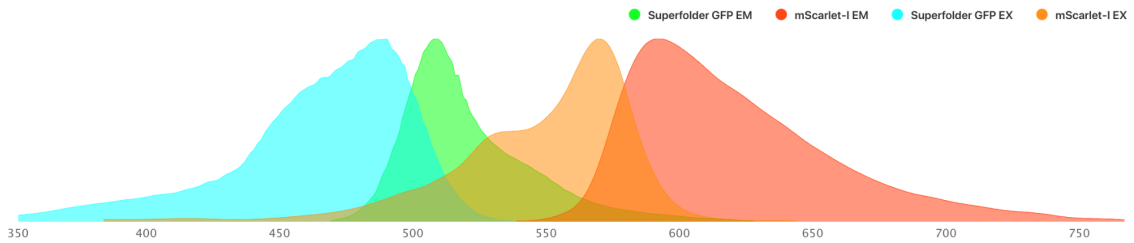
You can find the profiles of both fluorescent proteins in these links:
sfGFP: https://www.fpbase.org/protein/superfolder-gfp/
mScarlet-I: https://www.fpbase.org/protein/mscarlet-i/

Name	λ_{ex}	λ_{em}	Stokes
Superfolder GFP	485	510	25
mScarlet-I	569	593	24









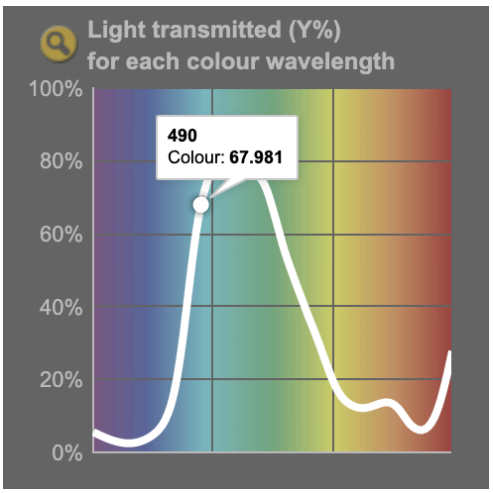
Attribute Comparison

Name	λ_{ex}	λ_{em}	Stokes	EC	QY	Brightness	pKa	Aggregation	Maturation	Lifetime	kDa
Superfolder GFP	485	510	25	83,300	0.65	54.15		wd	13.6		26.78
mScarlet-I	569	593	24	104,000	0.54	56.16	5.4	m	36.0	3.1	26.36

2) optical properties of emission filter

105: http://www.leefilters.com/lighting/colour-details.html#105
113: http://www.leefilters.com/lighting/colour-details.html#113&filter=cf
122: http://www.leefilters.com/lighting/colour-details.html#122

If you click on the links and move the cursor over the spectrogram you will be able to see the exact values:



These filters have very different optical properties that will allow you to observe sfGFP and mScarlet-I with different results.

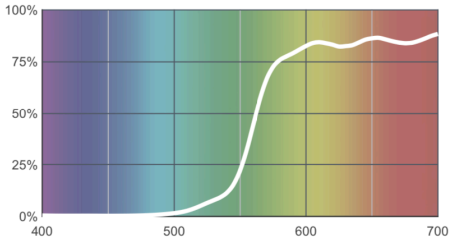
105 Orange

View on leefilters.com / Find a Dealer

Good for light entertainment and functions. Creates a good fire effect when used with 106 or 104.

	Source C 6774K	Tungsten 3200K
Transmission Y	41.3%	50%
x	0.563	0.59
y	0.428	0.406
Absorption	0.38	0.3

Light transmitted (Y%) for each colour wavelength (nm)



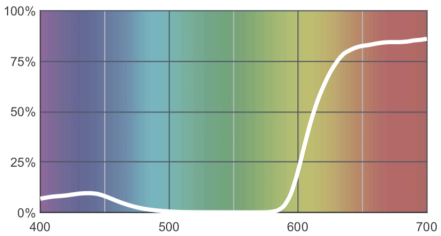
113 Magenta

View on leefilters.com / Find a Dealer

Very strong - used carefully for small areas on set.

	Source C 6774K	Tungsten 3200K
Transmission Y	10.9%	14.7%
x	0.563	0.662
y	0.217	0.29
Absorption	0.96	0.83

Light transmitted (Y%) for each colour wavelength (nm)



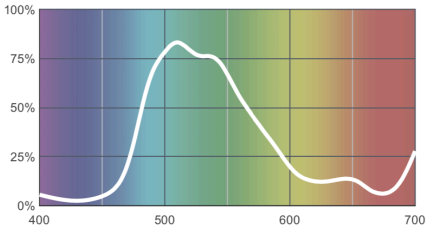
122 Fern Green

View on leefilters.com / Find a Dealer

Good for cycloramas and creates a great mood effect.

	Source C 6774K	Tungsten 3200K
Transmission Y	51.5%	45.7%
x	0.234	0.336
y	0.543	0.549
Absorption	0.29	0.34

Light transmitted (Y%) for each colour wavelength (nm)

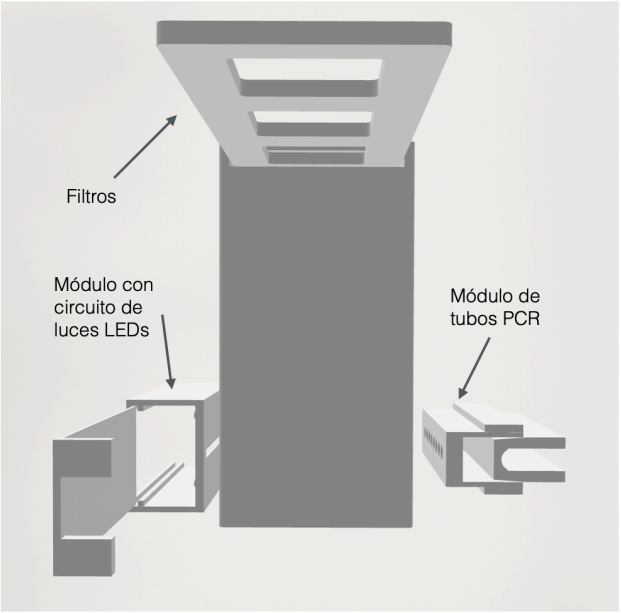


3) lighting wavelength

The lighting will be obtained through RGB LED lights, which have lighting Blue (465-467 nm), verde (522-525 nm) and red (620-625nm).

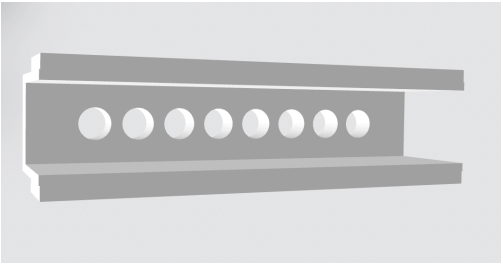
Instructions for use:

This device, called RGBfluor, is made up of a top part that houses the three lee filters (described above) and a bottom part that contains the lighting plate and fluorescent tubes. The modules at the bottom do not need to be manipulated. The interaction with the device will occur via 1) sliding the module with the three filters and 2) activating the corresponding lighting with your phone.

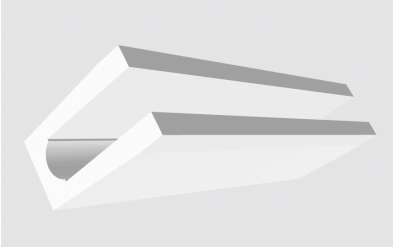


To control the LED lights, you must connect your phone to the WIFI signal called BioFluo1, 2, 3...depending on the device you have. Once your phone is connected, you must open a web page with the following IP:**192.168.4.1** and the following control interface will open:

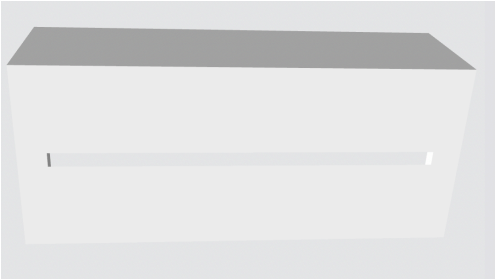
From this interface you can control the color of the excitation light of your samples.



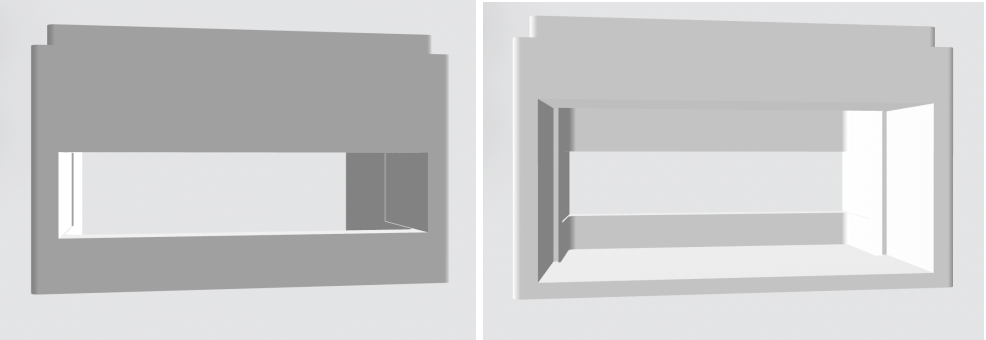
A) PCR tube container



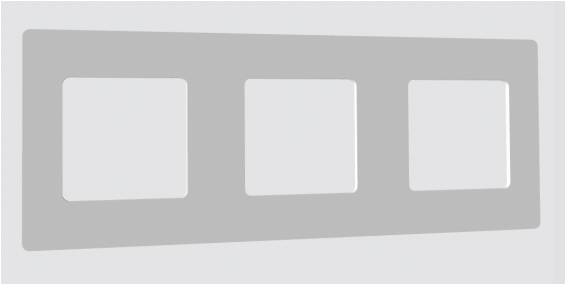
B) PCR tube holding cap



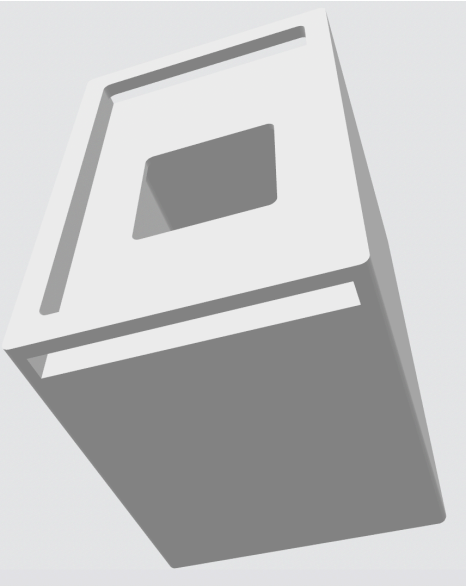
C) PCB circuit container with LED lights



D) Base of the RGB fluo, showing the two windows where, on the left, the PCR tube container is inserted (A and B) and the one on the right showing where the PCB container (C) is inserted.



E) Container of the three filters



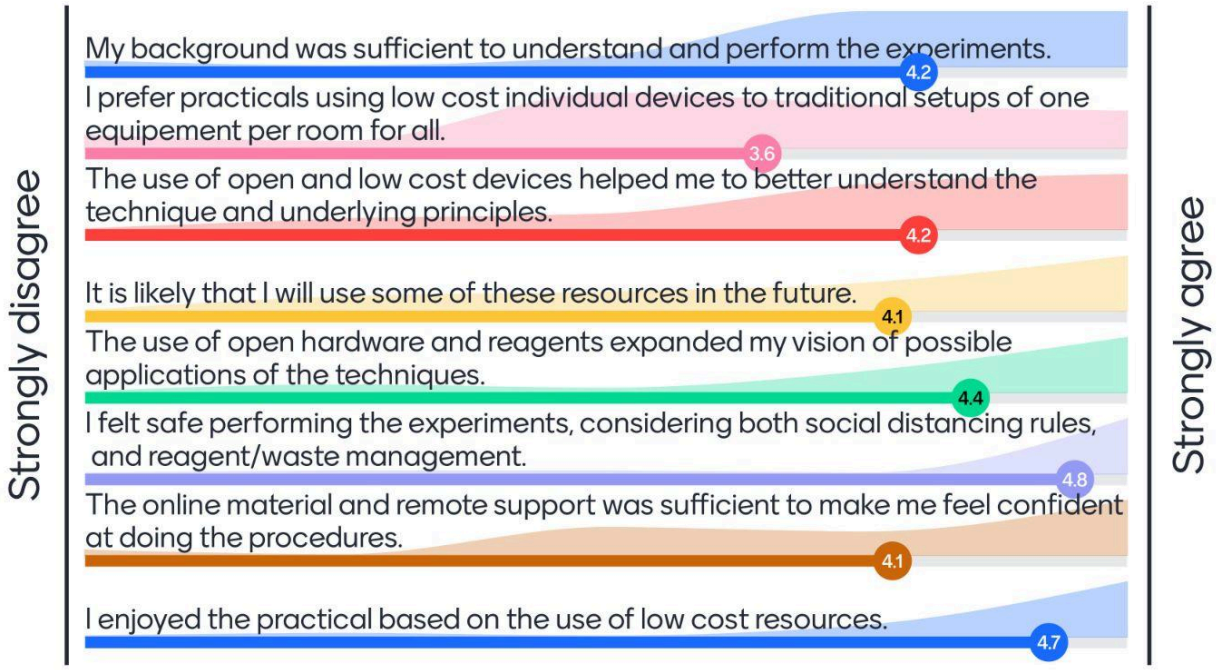
F) Top of the device

Links of interest:

- Complete list of fluorescent proteins:https://docs.google.com/spreadsheets/d/1RFIFfIIkxFzspQ8SHJcLsHKuf8X3u9uaLJn0-eQRJk8/edit#gid=354984915 (very useful in the future!)
- The best portal on fluorescent proteins:https://www.fpbase.org/
- Digital repository with design files of the open hardware device to be used:https://gitlab.com/FernanFederici/rgb_fluor
- Protein production protocol in “cell-free” systems:https://www.protocols.io/view/preparation-of-cell-free-rnapt7-reactions-kz2cx8e

(Please leave us your comments where you think the document needs improvements.)

S6 appendix: student evaluation



Spanish translation used for the anonymous interview:

1. Mi experiencia fue suficiente para comprender y realizar los experimentos / My background was sufficient to understand and perform the experiments.
2. Prefiero las prácticas con dispositivos individuales de bajo costo a las configuraciones tradicionales de un equipo por habitación para todos / I prefer practicals using low cost individual devices to traditional setups of one equipement per room for all.
3. El uso de dispositivos abiertos y de bajo costo me ayudó a comprender mejor la técnica y los principios subyacentes. / The use of open and low cost devices helped me to better understand the technique and underlying principles.
4. Es probable que utilice algunos de estos recursos en el futuro / It is likely that I will use some of these resources in the future.
5. El uso de reactivos y hardware abierto amplió mi visión de las posibles aplicaciones de las técnicas / The use of open hardware and reagents expanded my vision of possible applications of the techniques.
6. Me sentí seguro realizando los experimentos, considerando tanto las reglas de distanciamiento social como la gestión de reactivos / residuos / I felt safe performing the experiments, considering both social distancing rules, and reagent/waste management.

7. El material en línea y el soporte remoto fueron suficientes para hacerme sentir seguro al realizar los procedimientos / The online material and remote support was sufficient to make me feel confident at doing the procedures.
8. Disfruté de la práctica basada en el uso de recursos de bajo costo / I enjoyed the practical based on the use of low cost resources.

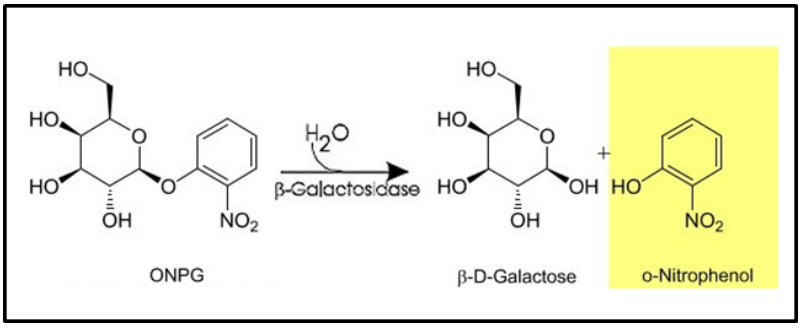
Interview performed by 25 students in mentimeter.com

S7 Appendix: Student`s guide fo practical on enzyme kinetics

In this practical exercise, we will use β-galactosidase enzyme from E. *coli*, an enzyme that degrades β-galactoside disaccharides to monosaccharides, including lactose to glucose and galactose. The substrate used in this activity will be a synthetic substrate called **o-nitrophenyl-β-D-galactopyranoside (oNPGal)**. oNPGal is used because the assay method is simpler than using the natural substrate lactose, as one of the reaction products, **o-nitrophenol (oNP)**, absorbs at 420 nm and can be quantified by absorbance (e.g., with a colorimeter, spectrophotometer or by image analysis).

During this practical experiment, a characterization of *E. coli* β-galactosidase enzyme produced by a cell-free transcription-translation reaction will be carried out. The lysate containing the enzyme has been lyophilized for its transport at room temperature. In the first step, students must hydrate the enzyme with 1X PBS buffer to then quantify its activity against different substrate concentrations in order to characterize its kinetic parameters (Vmax and Km). Calibration curves of the product oNP will be used to determine the amount of product produced in the reactions.

1. Calibration curve for the quantification of the reaction product, o-nitrophenol (oNP)



A series of oNP dilutions was previously prepared by the teaching assistants as indicated in the table below from a freshly prepared stock of oNP and using 1X PBS buffer. This curve represents the oNP calibration curve, conducted under the same buffer conditions as the reactions that will be performed to measure β-galactosidase activity. This curve will be used to interpolate the absorbance values obtained experimentally, to transform these values into units of concentration of the reaction product.

Each student must calculate the μM concentration of oNP (and fill out the last column of the table). Also, each student should measure the absorbance at 420 nm of each dilution using any of the different methods of this laboratory (spectrophotometer, colorimeter or quality images for posterior processing) and fill out the Absorbance at 420 nm column of the table.

o-nitrophenol 10 mM (µl)	Water (µl)	PBS pH 7.4 (µl)	Na_2CO_3 1M pH 10 (µl)	Absorbance at 420 nm	[oNP] µM
0	400	100	500		
1	399	100	500		
3	397	100	500		
5	395	100	500		
10	390	100	500		
15	385	100	500		
20	380	100	500		
25	375	100	500		
30	370	100	500		
45	355	100	500		
60	340	100	500		

Note: The total volume of the reaction mixture plus sodium carbonate (Na_2CO_3) is constant and equal to 1 ml. Absorbances should be read from the most diluted to the most concentrated (Na_2CO_3 is an alkaline buffer, which will halt enzymatic activity).

2. **To obtain the saturation curve for β-galactosidase, follow these steps:**

1. Rehydration of lyophilized enzyme:

Perform a quick spin-down to ensure the enzyme (should look like a white powder) is at the bottom of the tube and will not be lost when opening the cap. Add 1 ml of saline buffer (PBS) 1X to the tube containing the lyophilized enzyme. Vortex for 40 seconds to ensure homogeneous resuspension of the enzyme. Keep on ice.

2. Preparation of substrate dilutions

Prepare 9 substrate dilutions from the concentrated oNPGal stock (3 mg/ml), following the table below (including a substrate-free blank). Use water, substrate, and phosphate buffer as indicated in the table below. Remember that enzyme addition will start the reaction, so enzyme will be added as the last reagent, when all tubes are ready and timing starts. When adding the enzyme to the tubes, invert the tubes 10 times to initiate the reaction.

3. Incubation

Incubate the tubes for 13 minutes at 37°C to allow the reaction to occur.

4. Reaction termination

Add 0.5 ml of Na_2CO_3 1M pH 12 to each tube to stop the reaction. Invert the tubes (closed) 5-10 times to mix well.

5. Determination of product concentration:

Measure absorbance at 420 nm of the product reaction by the same methods used for the calibration curve. Use the previously obtained calibration curve to infer the product concentration in each sample. Fill the table bellow with the absorbance values.

Water (µl)	oNPGal 3 mg/ml (µl)	PBS pH 7.4 (µl)	Resuspended enzyme (µl)	Na_2CO_3 1M pH 12 (µl)	Absorbance 420 nm
300	0	100	100	500	
295	5	100	100	500	
294	6	100	100	500	
292	8	100	100	500	
287	13	100	100	500	
275	25	100	100	500	
250	50	100	100	500	
200	100	100	100	500	
175	125	100	100	500	

3. Estimation of Km y Vmáx

1. Prepare a table with the following data:

Substrate concentration µM	Absorbance 420 nm	[Product] µM	Initial velocity (Vo) µM/min	1/[Substrate] (1/µM)	1/Vo (1/min)

2. Represent the data using the Lineweaver Burk (double reciprocal plot). The x-axis is the reciprocal of the substrate concentration, or 1 / [S], and the y-axis is the reciprocal of the reaction velocity, or 1 / V. Interception in the x axes represents 1/Km; slope represents Km/Vmax; interception with the y axis represents 1/Vmax